BB-694

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RDCoberly
11/21/66

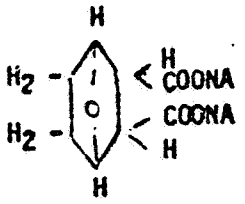
421

Chemical and Physical Data Sheet

Name: Endothal

Chemical Name: 7-Oxabicyclo-[2.2.1]-heptane-2,3-dicarboxylic acid

Structure:



State: Solid

Solubility: Soluble in water

Use: Herbicide

Company: Pennsalt Chemicals Corporation

Disodium Endothal

Data Review as of November 18, 1966

1. Acute Rat Oral - LD_{50} = 38 mg/kg/day
2. Acute Rabbit Dermal - LD_{50} = ~15% - irritant
3. Acute Guinea Pig Inhalation (Endothal Acid) - respiratory irritant
   LD_{50} => 0.05 mg/L - effect level =< 0.05 mg/L
4. Acute Rat Inhalation - parenchymatous changes in liver and kidneys
5. Acute Dog I.V. - LD_{50} =< 5 mg/kg - heart appears to be target organ
6. Acute Rabbit I.V. - LD_{50} =< 10 mg/kg - heart appears to be target organ
7. Acute Eye Irritation (Rabbit) - irritation was transient
8. Subacute Oral Dose Range (Dog) - MLD ≅ 20 mg/kg
9. Subacute Rat Feeding
10. Subacute Rabbit Dermal (21 day) (19.6% Endothal Acid) - Irritant
11. Subacute Guinea Pig Inhalation (Endothal Acid) - respiratory irritant
12. Subacute Sheep Feeding (5 days)
13. Subacute Sheep Feeding (6 weeks)
14. Subacute Sheep Grazing
15. Chronic Rat Feeding (2 years)
16. Chronic Dog Feeding (2 years)
17. Three Generation Rat Reproduction - high level effected health NEL 100 ppm
18. Metabolic Study I.P. (Rats) - Excreted at 48-72 hrs.
19. Human Skin Irritation - 1.0% and 4.0% produced light to moderate erythema
20. Metabolism in Fish
21. Fish Toxicity - LD_{10} => 40 ppm
22. Precautions in Handling

Di-N,N-dimethylcocoamine Endothal (TD-47)

1. Acute Rat Oral - LD_{50} = 205 mg/kg
2. Acute Rabbit Oral - MLD = 23-46 mg/kg
3. Acute Rabbit Dermal - conc. skin irritant
   1% solu. No irritation
   conc. MLD = 46-70 mg/kg
4. Acute Eye Irritation - 1% solution irritant
5. Acute Inhalation Rat - 25% dilution - fatal
6. Subacute Dog Feeding (16 weeks) - Effective level => 2.55 mg/kg

Endothal

Acute Rat Oral

Several groups of ten animals each received from 0.12-0.29 cc of the test solution as a 3% aqueous solution. Animals were fasted for 18 hours prior to treatment.

Results - LD_{50} equals 38 mg/kg. Reference is made to an FDA acute study in which the material was found to have an LD_{50} of 35 mg/kg.

Summary - the results of this study definitely places this material, as an economic poison, in the highly toxic class to man.

Dose Range Findings in Dogs (Oral) (Number of dosages not given)

A total of nine male mongrel dogs were utilized for this study. A single animal was tested per level of 50, 40, 30, 20, 10, 5, 3, 2, and 1 mg/kg/day. The test material was administered daily to each animal by capsule.

Results - The dosage range 20-50 mg/kg/day caused death. Death occurred at the 50 mg/kg/day level in three days whereas death occurred at eleven days in the 20 mg/kg/day level. Animals receiving ten mg/kg/day or less survived six weeks at which time they were sacrificed. Gross signs of toxicity were not observed in the dogs which survived or which succumbed with the exception of emesis in the 40 mg/kg/day level. Hemalogical determinations and biological test (blood glucose, urea nitrogen, thymol turbidity) were performed on the five lower dosage levels and found to be within normal limits.

Pathologic changes - The stomach walls of the highest four dosage levels were congested and edematous with occasional erosion and hemorrhages. Edema in the

upper intestines and multiple superficial ulcerations were rather constant findings. Other observations were normal.

Summary - Results of this study clearly indicate that the chemical must be considered a GI irritant.

5.6.b Acute Rat Feeding (28 days)

Four groups of albino rats consisting of 20 male and 20 female animals each were used at the dosage levels of 1% (10000 ppm) and 0.1% (1000 ppm).

Results - The 1% dietary level produced 100% deaths in the male animals by day 6 and 85% deaths in the female animals by day 7. Prior to death, the rats showed convulsions, ataxia, and irritation of the nose and eyes. Necropsies performed on these animals showed necrosis of the mucous membrane of the stomach, massive hemorrhage in the stomach, and large amounts of the blood in the intestines.

The three surviving female rats lost from 12 to 25% of their original body weights. A microscopic examination of representative tissue revealed slight degeneration of the liver parenchyma in three rats and focal areas of hemorrhage in the kidneys in two rats. These three female rats were sacrificed after seven days of feeding.

The female rats of the 0.1% level showed a very slight increase of body weight over that of the corresponding control. The male animals at this level gained considerably more weight than the corresponding male control rats. Some irritation of the nose and eyes was observed during the first two weeks. No other adverse effects were noted. Necropsy were unremarkable.

Comments - The dosage level (1% and 0.1%) seemed rather high when one compares them to the acute LD_{50} value of 38 mg/kg/day. As an estimate the 1% level and the 0.1% are equal to 800 and 80 mg/kg/day respectively. This is an estimate because the data presented did not list the food consumption of the test animals.

Another interesting point is the fact that the male animals of the 0.1% level gained 61% more weight than the corresponding control animals. Due to missing food consumption data and the related food utilization data, I cannot offer a concrete reason for this excessive increase in weight.

Sheep Grazing Experiments.

Groups of ewe lambs were turned into lots treated with 8 lbs. endothal per acre. Body weight changes, general appearance and certain hemological studies were utilized in evaluating the effects of the treatments on the animals.

Results - The sheep disliked the treated grass. As a result the animals ate less than normal and lost weight. Overall condition of the animals was good. No significant changes were noted in the differential leucocyte count, total erythrocyte count, erythrocyte morphology, and hemoglobin levels.

Subacute Sheep Feeding

18 ewe lambs were separated into groups of three and treated at levels of 0.0, 0.5, 1.0, 5.0, 10.0, and 20.0 grams for five days. The test material was added to the daily rations.

Results - the animals generally refused to eat the treated hay. No harmful effects were noted.

Summary - This study indicates that sheep would not voluntarily consume toxic quantities of treated material in the fields.

Subacute Sheep Feeding (6 weeks)

Six ewe lambs were fed hay from an area treated with endothal the previous fall at the rate of 8 lbs per acre.

Results - No significant differences were observed between the test and the control animals.

Summary - Sheep can and will consume hay treated the previous fall with endothal without adverse effects.

Chronic Rat Feeding Study (2 years)

Ten male and ten female animals were used per dietary level of 0.0, 0.01, 0.03, 0.10, and 0.25%. These percent levels are approximately equal to 0, 100, 300, 1000, and 2500 parts per million.

Results - Survival and body weight change of the test animals were comparable to the corresponding control animals. No toxic or pharmacologic effects were observed in any test animals during the study.

The body weight gains of the two highest levels were less than the average control body weight gain. However, this difference was statistically not significant.

Hemological studies consisting of total erythrocytes and leucocyte counts and hemoglobin determinations appeared to be within normal limits.

Both test and control animals showed degenerative changes of the miocardium and tubular tissue of the kidney, hyperemic liver, gastritis and gastroenteritis. These findings could not be absolutely related to the feeding of the test compound as each of the findings were observed in both the test and control animals.

Benign tumors were exhibited by all groups of female rats including the control and test groups. Most of these tumors were adenomas and fibroadenomas with a wide variability of location.

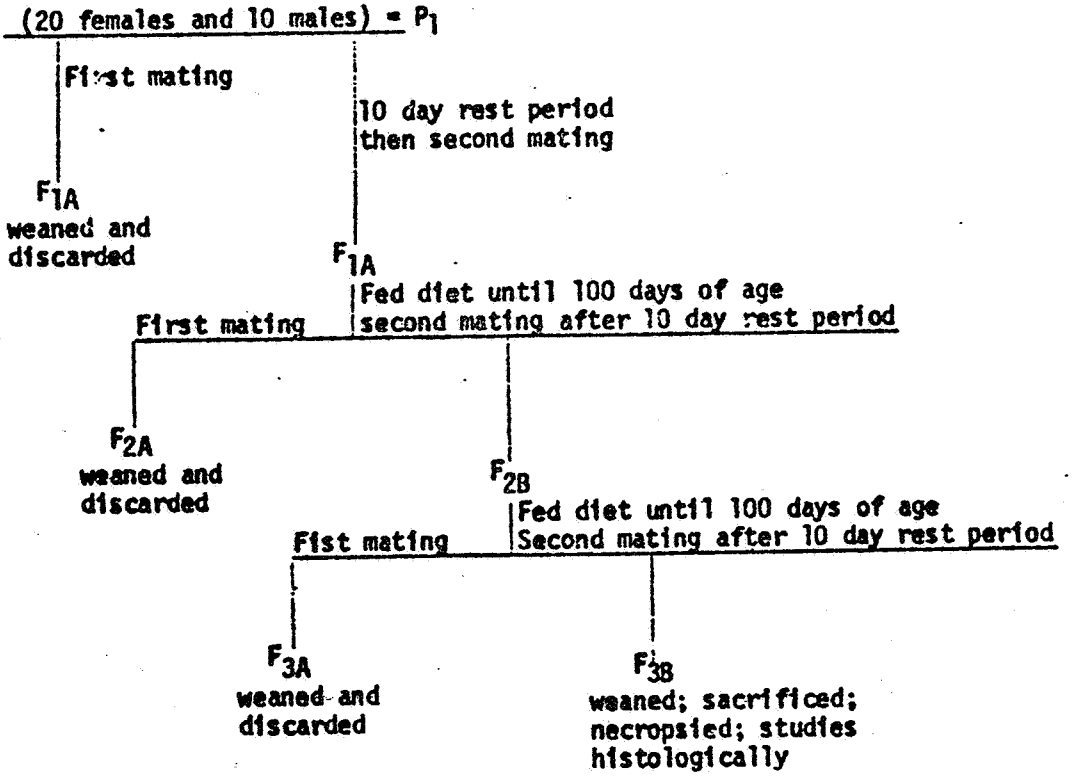
Summary - It is apparent from this study that the feeding of the test material at high levels does not produce readily noticeable adverse effects other than the already established findings of gastritis and gastroenteritis.

Three-generation Rat Reproduction Study

Ten male and 20 female animals were utilized per level of 0, 100, 300, and 2500 ppm. The corneal generations (P_1) were maintained on the appropriate diet until 100 days of age at which time mating was permitted. During the mating period four females and two males were placed together in a breeding cage. The females were examined for copulation plugs twice daily for six days. When a plug was observed, the female was removed from the breeding cage. If these females did not prove to be pregnant they were returned to their respective breeding cage and the procedure repeated. If no copulation plug was observed the female was left with the male for the total mating period of 21 days or until visible signs of pregnancy were noticeable. Aborted fetus were examined grossly for possible teratogenic effects and preserved in 10% neutral formalin.

The aforementioned procedure and the remaining procedures follow the outline as shown below:

Schematic Outline



Results - The appearance and behavior of the test animals from the 100 ppm and 300 ppm dosage levels were generally normal for both parental animals and their offsprings and comparable to the control group. Parent animals and their offsprings of the high test levels, 2500 ppm exhibited signs of toxicity of varying degrees throughout the study, and due to the high mortality of the F_{2B} litters this dosage level was discontinued.

The number of actual prenancies, averaged gains of females through gestation, average number of pups per litter were essentially the same for the 100 ppm and 300 ppm test animals and comparable to the controls for each of the three generations. These values for the 2500 ppm level were much lower.

One pup of the F_{1A} litter of the 100 ppm level possessed spina bifida and two additional rear hind limbs. No other teratogenic effects were found among the remaining pups for any of the test group or the control.

No signs of resorptions or abortions were observed in any of the females during both litters of each generation for any of the test groups or controls. The weaning weights of the survivors were considerably lower for the 2500 ppm offspring for litters of the P_1 and P_2 parents as compared to the control. The weaning weight for the survivors of the 300 ppm offspring were slightly lower in most cases as compared to the controls, however, the weaning weight appeared to be within normal limits.

A dense whitish mass which was detected at weaning within the eyeball of three animals of one litter (F_{2B}) of the 300 ppm test level, all of the survivors of one litter (F_{2B}) of the 2500 ppm test level and one animal of the control

group (F_{3A}) showed upon histopathological examination an amorphous material within the aqueous humor which was non-specific and could not be identified.

Gross and microscopic examination of tissues from representative weanling animals from the control and 300 ppm test level of the final (F_{3B}) litter showed similar findings which indicated that the ingestion of a test material at this level did not produce any significant alterations.

Summary per generation - the P_1 control animals showed no adverse effects during the pre-mating feeding period. Eighteen of 20 mated animals gave birth to normal F_{1A} litters and 17 of 19 mated animals gave birth to normal F_{1B} litters. Both litters displayed normal viability at birth. Cannabalism was noted in seven mothers of the F_{1B} generation. Necropsy of the sacrificed P_1 parents showed no abnormalities.

The P_1 100 ppm animals showed no adverse effects during the pre-mating feeding period. Twenty of 20 mated animals bore litters for the F_{1A} generation and 19 of 20 animals gave birth for the F_{1B} generation. Both litters displayed normal viability at birth. Cannabalism was observed in the parent females of the F_{1B} generation. Necropsy of the sacrificed P_1 parents showed no abnormalities.

The P_1 300 ppm animals showed no adverse effects during the pre-mating feeding period. Eighteen of 20 mated animals bore litters for the F_{1A} generation. Eighteen of 19 animals gave birth for the F_{1B} generation. Both litters displayed a normal viability at birth. Cannabalism was observed in six mothers. Necropsy of the sacrificed parents showed no abnormalities.

The P_1 2500 ppm animals showed unthriftiness and poor acceptance of diet during the pre-mating period. Fifteen of 20 mated animals gave birth to litters for the F_{1A} generation and 14 of 17 gave birth for the F_{1B} generation. Toxic signs were observed in the majority of offspring of the F_{1A} litters while adverse effects to a lesser degree occurred in the F_{1B} generation. Necropsy of the sacrificed P_1 parents showed kidney discoloration of varying degrees (olive to dark brown) with slight mottling in some cases, accompanied by pale adrenals.

The P_2 control animals showed normal appearance and behavior during the pre-mating season. Eighteen of the 19 mated animals gave birth to normal F_{2A} litters. Nineteen of 19 mated animals gave birth to F_{2B} litters. One animal of the F_{2B} litter showed a pedunculated, attached to the dorsal medial surface of the head. Other pups from this litter showed spare hair growth at weaning. Necropsy of the sacrificed P_2 parents showed no gross abnormalities.

The P_2 100 ppm animals showed normal appearance and behavior during the pre-mating period. Food consumption data for this group were comparable to the food consumption of the control animals. Twenty mated animals bore normal litters for the F_{2A} generation and 20 animals mated bore normal litters for the F_{2B} generation. Gross changes were not observed in any of the offspring. Both litters displayed normal viability at birth. Necropsy of the sacrificed P_2 parents showed no abnormalities.

The P_2 300 ppm animals showed normal appearance and behavior during the pre-mating feeding period. Food consumption data was comparable to the control animals. Twenty of 20 mated animals bore normal litters of the F_{2A} generation

and 19 of 20 animals gave birth for the F_{2B} generation. Both litters displayed normal viabilities at birth. Cannabalism was noted in two females (F_{2B}). Three rats from one litter (F_{2B}) at weaning showed an amorphous material within the aqueous humor. This was not specific and could not be identified upon histological examination. Necropsy of the sacrificed P_2 parents showed no gross abnormalities.

The P_2 2500 ppm animals were less healthy as a group during the pre-mating feeding period. Body weights were considerably reduced at the time of mating. Food consumption data for this group were comparable to the control animals. Nine of ten mated animals produced litters for the F_{2A} generation and four of nine mated animals produced litters for the F_{2B} generations.

Both F_{2A} and F_{2B} were, in general, less healthy than the corresponding control litters. Weakness and emaciation were observed in many of the F_{2B} offspring of which only seven survived through weaning. All of the survivors of one F_{2B} litter showed an amorphous material within the aqueous humor. Only one weanling offspring survived for more than one week after weaning. No gross changes were observed in any of the remaining offspring from birth to weaning. Necropsy of the P_2 parents showed abnormalities in the region of the ovaries of one animal, otherwise the findings were not remarkable and were comparable with the controls. Due to almost total weanling death, the 2500 ppm level was discontinued at this point.

The P_3 control animals showed normal appearance during pre-mating season. Fifteen of 19 mated animals gave birth to F_{3A} litters and 18 of 18 mated animals gave birth to F_{3B} litters. Both litters displayed normal viability

at birth. Cannabalism was noted in four females. One male offspring of the F_{3A} generation showed an opague mask over the one eye at weaning. No gross abnormalities were detected in the remaining pups. Gross necropsy of the sacrificed F_{3B} offspring and the P_3 parents showed no abnormalities.

The P_3 100 ppm animals showed no adverse effects during the pre-mating period. Nineteen of 20 mated animals bore normal litters for the F_{3A}. Twenty of 20 animals gave birth to normal F_{3B} litters. Both litters displayed normal viability at birth. No gross abnormalities were detected in any of the offspring. Gross necropsy of the sacrificed F_{3B} offspring and of the P_3 parents shows no abnormalities.

The P_3 300 ppm animals showed no adverse effects during the pre-mating feeding period. Nineteen of 20 mated animals bore normal litters for the F_{3A} and F_{3B} generation. Both litters displayed normal viability at birth. Poor care of the litters was demonstrated by mothers in each of the F_{3A} and F_{3B} litters. No gross abnormalities were detected in any of the offspring. The mortality rate of these litters was higher than the corresponding control. Poorer quality of survivors was evident. Gross necropsy of the sacrificed F_{3B} offspring and the P_3 parents showed no abnormalities.

An examination of the brain, spinal cord, pituitary, thyroid, heart, lungs, liver, kidneys, adrenals, spleen, stomach, small intestine, large intestine, pancreas, urinary bladder, ovary, testes, bone marrow, and unusual lesions from ten males and ten females F_{3B} offspring of group 1 (control) and group 3 (300 ppm) did not show any histopathologic changes.

Summary - This study definitely shows that the test material has a chronic physiological effect on the rodents. This effect is noticeable at the 300 ppm level and is outstanding at the 2500 ppm level.

The data concerning the number of animals bred and the resulting pregnancies indicate the test material does not have an effect on fertility. In considering this statement, one must keep in mind the reduced number of pups which may have been due to the general poor health of the animals in the two higher dosage levels.

The two teratogenic effects noted in the F_{1A} offspring of the 100 ppm level was not observed in the two higher levels. I do not believe a concrete reason can be given for these observations.

In general, it appears the no-effect chronic level of the test material is less than 300 ppm in the rat.

Two-Year Chronic Dog Feeding

Three male and three female pure-bred beagle dogs were used per level of 100, 300, and 800 ppm. These levels are equivalent to approximately 2.5, 7.5, and 20 mg/kg/day. The 800 ppm level was increased to 1000 ppm (25 mg/kg/day) at the end of the 19th month; then increased to 1300 ppm (32.5 mg/kg/day) at the end of the 20th month; then increased to 1600 ppm (40 mg/kg/day) at the end of the 21st month; then increased to 2000 ppm (50 mg/kg/day) at the end of the 22nd month.

Observations included general behavior and appearance, food consumption, hematology studies, urinalyses, liver function study, and at necropsy a microscopic examination of brain, spinal cord, femoral nerve, skeletal muscle, pituitary, thyroid, parathyroid, adrenal, lung, heart, aorta, spleen, hymus, bone marrow, submaxillary salivary gland, stomach, small intestine, secum, colon, pancreas, liver, gall bladder, kidney, testes or ovary, prostate or uterus, mesenteric, and cervical lymph nodes.

Results - No gross signs of toxicity and/or pharmacological effects were observed in any test animals during the course of study. One death which occurred at the end of the 12th month in the 300 ppm level was attributed to pneumonia and not ingestion of the compound.

Body weight gain and food consumption of the test animals were generally within normal limits. One high level dog showed a net loss of 1 kg.

Hematological, liver function, and urine analyses values for the test dogs generally remained within normal limits and were comparable to those of the control dogs.

Necropsy findings showed the dogs with a high level generally exhibited congested and/or hemorrhagic stomachs. Other significant pathological changes which could be attributed to test material were not observed.

Microscopic examination of tissues from the 800 ppm level generally showed a slight increase in activity of mucosus glands in the fundus; the liver examination showed in general slight hyperemia and/or proliferation of bile

ducts through the liver. One dog of the high level also showed parenchymatous liver cell alterations.

Summary - The data from this study seems to indicate that there is some effect at the high dosage level. It should be noted that this dosage level was increased gradually from 800 ppm to 2000 ppm. The major effect of the test material appears to be a general irritation of parts of the GI tract.

Intraperitoneal Administration of C^{14} Tagged Endothal (Rat)

Albino rats were injected intraperitoneally with 3.5-27.5 microcuries of Endothal tagged with C^{14} at the one or two positions on the ring. The test material was dissolved in water so that one ML equals five microcuries or 1.038 mg. The animals were placed in metabolism cages for the collection of expired air, feces and urine.

Results - It appears that endothal is almost completely excreted from the body within 48 and 72 hours. It also indicates that within the first 12 hour period, approximately 83% of the material has already been excreted. The insignificant excretion of radioactive CO_2 indicates that if the compound was metabolized, the process did not involve opening the range structure.

Acute Rabbit Dermal

Powdered sodium endothal and 1%, 10% and 20% aqueous solution of the compound were applied for three consecutive days, seven hours a day to the shaved abraded and unabraded abdominal skin using 8 groups of 3 rabbits each.

Results - The 1% level produced minimal indiscrete lesions on the abraded skin. The 10% and 20% levels produced lesions varying only in severity. At the higher concentration the lesions increased in size, frequently merging to large patches and assuming a purplish mottled appearance which is indicative of ecchymosis or hemorrhagic necrosis. Microscopic examination of the skin tissues revealed changes ranging from superficial excoriation to hemorrhagic and necrosis.

Three rabbits of the 20% endothal solution and one of the three rabbits which received the powdered endothal to the abraded skin secumbed on the second exposure day.

Summary - From the aforementioned data, it appears that the test material is a skin irritant.

Subacute Rabbit Dermal (21 days) (Formulation with 19.6% Endothal Acid)

Five male and five female rabbits were used per level of 20 mg/kg and 50 mg/kg of Herbicide 282 (dimethyltridecylamine - 50%, and endothal acid - 19.6%). Application was made 15 times over a 21 day period. A control group was tested with aqueous ethylene glycol.

Six deaths were noted at the 50 mg/kg level and were attributed directly to the absorption of the test material. The hemological findings of the test animals were in general comparable to the control animals. Signs of general irritation were noted in both levels of the test animals with the reactions being more intense in the 50 mg/kg group. These toxic signs consisted of erythema, edema, eschar, exfoliation, fissuring, prostration and scarring.

Microscopic examination established that Herbicide 282 produced a definite desquamate change in the epidermal - dermal complex of the test rabbits.

Two animals of the high level showed a slight to moderate congestion of the liver. One animal of the same level showed interstitual congestion of the kidney.

Summary - Data from this study again clearly indicates the test material is a dermal irritant. Also that the chemical has the ability to be absorbed through the skin in sufficient quantity to cause histopathologic changes in the liver and kidneys. Product labelling must compensate by adequate warnings.

Eye Irritation (Rabbit)

A small quantity of 1% or 4% sodium endothal was placed into the eye of several rabbits.

Results - Slight to marked but transit irritation was produced in the eyes of rabbits. Signs of irritation disappeared in two to ten days.

Acute Rat Inhalation

Four groups consisting of 16 female and 16 male rats were exposed to mists of water, 8.3% solution of ammonia and sulfate, a 2.5% solution of disodium-endothal, and a solution containing 1.67% disodium endothal and 8.33% of ammonia sulfate. Exposure period was for one hour. Air flow into the exposure chamber was regulated at 0.2 mg of solid per liter of air inhaled by the test animals.

Results - No rats died during the exposure. The animals which were sacrificed immediately after the exposure period were considered normal. At ten weeks post exposure, the rats which were exposed to the mix containing disodium endothal and ammonium sulfate showed a somewhat higher incidence of slight parenchymateous changes in the liver and the kidneys.

Summary - It appears as though the combination of disodium endothal and ammonium sulfate was responsible for this degenerative change in the liver and kidneys. As either one alone produced the same effect, a potentiation study between these two materials would either strengthen or dilute this theory.

Acute Guinea Pig Inhalation

20 guinea pigs were subjected to a six hour inhalation of an endothal acid aerosol at an air concentration of approximately 0.05 mg/ML.

Results - Animals sacrificed immediately after exposure and at 24 hours post-exposure show pulmonary congestion, occasional hemorrhage and mild kidney congestion. Seven days after exposure no residual signs of tissue damage were apparent. Signs of respiratory irritation as well as eye irritation were also observed during the exposure period.

Comments - Cautionary statements must be contained on labels of this product warning the user against the dangers of inhalation.

Subacute Guinea Pig Inhalation Study

The remaining guinea pigs of the acute inhalation study were reexposed to the same aerial concentration for a total of four such exposures.

Results - Results of this experiment were similar to the results of the acute study.

Acute Intravenous Dog Study

Study No. 1

Two dogs received 10 mg of sodium endothal per kg of body weight intravenously in 1.0% solution.

Results - After about one hour both animals exhibited intermittent retching, vomiting, and repeated expulsion of semi-liquid fecal material. The animals grew progressively weaker without showing any typical neurological signs and died in asphyxial convulsions 7-8 hours post treatment.

Study No. 2

Two dogs received 5 mg of sodium endothal per kg of body weight intravenously in 1.0% solution.

Results - Both animals showed progressive weakness and essentially the same picture as the two dogs of the 10 mg/level. The progress of the condition was arrested in one animal after about 4 or 5 hours with a slow return to normal. The condition of the other animal worsened, with death occurring at 8-10 hours post treatment.

Study No. 3

Four male dogs weighing 8.5 to 9.5 kg were anesthetized with I.V. injections of phenobarbital sodium. Sodium endothal in a 1% solution was injected in an amount equal to 10 mg/kg of body weight. Continuous recordings of blood pressure and respiration were made and electrocardiograms were taken at frequent

intervals. The first dog was sacrificed for autopsy after 90 minutes; at this time dogs known as 2, 3, and 4 received a second injection of 40, 40, and 50 cc of the anesthetic solution respectively (I calculated this to be approximately 45 mg/kg). Dog 2 was observed for 15 minutes following the second injection and then sacrificed for autopsy.

Results - Respiration for dogs No. 3 and 4 remained practically unchanged until the blood pressure had fallen to about 25 mm of mercury when respiration suddenly failed. Electrocardiographic changes were indicative of myocardial insufficiency. Both animals died 28 minutes following the second injection. Necropsy findings showed marked dilation and flabbiness of the heart with severe passive venous congestion of the liver and other organs. The heart appeared to be the primary organ of failure.

Neurologic signs were absent and necropsy findings tended to exclude primary peripheral circulatory failure.

Electrocardiographic Evaluation of Disodium Endothall

Electrocardiographic evaluation of the tracings done on twenty-two test and control dogs shows evidence of T-wave changes suggesting altered ventricular repolarization in four dogs of which three were test animals and one a control. An additional control dog had T-wave changes at the beginning and end of the study. Also, electrocardiograms of fourteen other dogs taken at the termination of the study showed T-wave alterations in six animal subjects, including control and test animals.

No heart blocks were evident--however, a sinus irregularity was present in a majority of tracings.

Conclusions:

1. Altered T-waves in both control and test dogs suggesting abnormal repolarization.
2. Sinus arrhythmia present.
3. Electrocardiographic effect cannot be assessed by these results.

solution respectively (I calculated this to be approximately 45 mg/kg). Dog 2 was observed for 15 minutes following the second injection and then sacrificed for autopsy.

Results - Respiration for dogs No. 3 and 4 remained practically unchanged until the blood pressure had fallen to about 25 mm of mercury when respiration suddenly failed. Electrocardiographic changes were indicative of myocardial insufficiency. Both animals died 90 minutes following the second injection. Necropsy findings showed marked dialation and flabbiness of the heart with severe passive venous congestion of the liver and other organs. The heart appeared to be the primary organ of failure.

Neurologic signs were absent and necropsy findings tended to exclude primary peripheral circulatory failure.

Acute Rabbit I. V.

Study I. Four rabbits received 40 mg of disodium endothal per kg of body weight intravenously as a 1.0% solution in water.

Results - Death occurred in 90-120 minutes following a period of progressive weakness. Occasional asphyxial convulsions were observed. No other neurological signs were apparent.

Study II. Three rabbits received 13 mg of disodium endothal per kg by intravenous administration.

Results - The observed signs were quantitatively similar with those of study I. Death occurred in three to five hours. Necropsy examination of the rabbits revealed marked dialation and flabbiness of the heart. So this data indicates that the heart is the target organ. I am surprised that passive congestion was not noted in the kidneys and liver.

Metabolism in fish of C^{14} label in endothal

The radioendothal used in this study was ring labelled and had a specific activity of of 0.44 microcuries per mg. Cold fish were exposed to 6 and 12 ppm of radioendothal in a 3 liter aquarium for 5.5 days at room temperature. Fingerling silver salmon were exposed to 2.5 and 5. ppm radioendothal in 7 liters of water for 3 days at 1° C. The fate of the radioactivity arising from the radioendothal was calculated using two procedures which are listed in the main report. These procedures will not be listed here.

Results - It was concluded that due to extensive breakdown of this chemical and incorporation of radioactivity into normal constituents of the organisms, as were represented by different fractions, it is doubtful that any endothal remains in the fish.

Toxicity of Disodium endothal to Fish

Disodium endothal was relatively non-toxic to fishes. The 10% level of mortality was greater than 40 ppm for 96 hour exposure for red-fin shiver, blunt-nosed minnow, red shiner, and black bullhead. Extended bioassay tests and higher concentrations revealed minimal lethal concentrations of 80-100 ppm. These higher concentrations caused injury to the gills and other external tissues and dehydration by osmosis.

Primary Skin Irritation (Man)

Aqueous solutions of sodium endothal at 1% and 4% concentrations produced light to moderate erythema of the skin.

Sensitivity study in Rabbits

In a preliminary study, the protocol of which is not included in the report, it was concluded that sensitization was not produced. This was based on the fact that the cutaneous intraocular and intravenous administration of endothal to previously exposed rabbits after an interval of four weeks, produced no accentuated skin lesions and no altered systemic reactions.

Summary - As I stated, the protocol of this study was not included, thus I do not know the number of animals nor the concentration of the chemical as applied. Thus, I am forced to accept the results as listed in this report from the company.

Precautions in handling endothal submitted by the company

1. Continuous contact with unbroken skin may cause reddening or blistering in 12 or more hours. Daily baths and change of clothing should be standard practice while handling endothal in any form.
2. Contact with abraded skin produces an immediate burning sensation. An individual so affected will voluntarily wash the affected area. Since danger of intoxication by absorption is present, large amounts of water should be used.
3. Endothal dust, when enhaled, irritates the membranes of the nose and throat accompanied by considerable mucous formation. A respirator should always be worn when handling finely divided solid endothal formulations.
4. In direct contact with the eye, endothal solutions can cause severe burning of the tissue. Standard eye protection devices should be worn when handling endothal. In case of accidental contact with the eye, it is essential to wash out immediately with liberal amounts of water. If this treatment has been ineffective and pain develops, accompanied by reddening and watering of the eye and blurring of the vision, a physician should be consulted.
5. If endothal is swallowed, vomiting should be avoided. Ice pellets will prevent vomiting. Give two tablespoonfuls of amphojel and half a glass of cold water and call a physician immediately. If amphojel is not available, give cold milk or cold water.

NOTE: I believe the use of amphojel is solely for the purpose of coating the sotmach against the irritant properties of the test material. As the heart is to be the target organ, I feel some information pertaining to this effect should be listed on the label.

Toxicity studies on Di-N,N-dimethylcocoamine endothal (TD-47)

Acute Rat Oral

LD_{50} = 205.83 mg/kg with limits of error of 82-122%.

Acute Rabbit Oral

MLD = 23.39-46.78 mg/kg

Acute Rabbit Dermal

The compound produced severe skin irritation when applied to the intact and abraded skin of rabbits with a maximum average score of 8.0.

A 1% water dilution of the compound applied to intact and abraded skin produced no visible signs of irritation.

The MLD of the undiluted material by skin absorption was in the range of 46.78-70.17 mg/kg.

Acute Eye Study (Rabbits)

The test material produced severe ocular damage with a maximum average score of 110.

A 1% water solution of the test material produced slight swelling of the eyelids accompanied by slight redness and a minimal amount of discharge. Complete recovery was within 48 hours.

Acute Rat Inhalation

Rats exposed to a concentrated atmosphere of a 25% water dilution of the preparation succumbed within 24 hours after exposure.

Subacute Dog Feeding Studies

Three groups of pure-bred beagles consisting of three males and three females were fed di-N,N-dimethylcocoamine endothal for 16 weeks at dietary levels of 36, 72, and 108 ppm.

Results - The groups of animals ingested an overall daily average of 3.03, 6.26, and 9.17 mg/kg which is equivalent to 0.84, 1.74, and 2.55 mg/kg of endothal acid respectively.

All animals survived the experimental period. Weight changes of the test dogs did not vary significantly from those of the control dogs. No significant hematological changes were evident. Urinalyses did not reveal significant abnormalities.

Bromsulphalein excretion tests revealed no signs of hepatic involvement. Serial electrocardiograms revealed no specific abnormalities.

Organ weights (liver, kidneys, spleen, heart, testes, ovaries, adrenals, stomach, small intestines, colons, secum, thyroid) of experimental animals did not differ markedly from the control animals.

Gross histopathological examination of the sacrificed animals revealed no significant alterations associated with the ingestion of the test material at any of the three dosage levels tested.

Summary - From the aforementioned data I can safely assume that the maximum no-effect level was not reached in this study. The order of toxicity for this disodium endothal derivative appears to be in the same area as the current compound.

Di-potassium Endothal

Acute Rat Oral

Groups of six male rats were tested at dosage levels of 50, 88, 125, 163, and 200 mg/kg. The animals were fasted overnight prior to the administration of the test material. Gross effects and mortality were noted for 14 days post treatment.

Results - LD_{50} equals 125 mg/kg. (Range of 99-157 mg/kg)

No signs of toxicity were noted in the 50 mg/kg level. Diarrhea, weakness, and depression were observed in the majority of animals at the three upper dosage levels on the day of dosage. Survivors appeared normal within five days.

Gross necropsy of the animals which succumbed showed congested adrenals and congestion and hemorrhaging of the gastro-intestinal tract. The linigg of the stomach appeared bleached and the stomach contained copious amounts of mucous and sloughed mucous membrane mixed with food particals.

Gross necropsy of the surviving animals at 14 days revealed no remarkable findings.