

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

000723

DATE: January 30, 1979

SUBJECT: Benlate Registration#352-354; Caswell#75A (benomyl)

FROM: William Dykstra, Ph.D
Toxicology Branch/HED TS-769

WWD 11/31/79

TO: Henry Jacoby
Product Manager#21

& RD, TS-767

Action Type: Miscellaneous Data

Registrant: E.I. Du Pont de Nemours & Co.

Recommendations:

1. The submitted studies can be added to the registrant data file as supplementary studies. The study with benomyl was terminated at 44 weeks and the study with MBC was terminated at 51 weeks due to systemic infection, morbidity, and mortality from ear tag irritation. No nutritional, clinical, hematological or pathological evidence of toxicity could be attributed to the compounds. These studies are being repeated and are in progress. Further toxicological conclusions regarding these compounds are contingent on the final reports.

Review:

1. Long-Term Feeding Study in Mice with 2-Benzimidazolecarbamic acid, Methyl Ester (INE-965); Report on Early Termination - 44 Weeks (Haskell Laboratory Report No. 548-78, Medical Research Project No. 2757, Oct. 6, 1978, Accession No. 236766.

Test Material: 2-Benzimidazolecarbamic acid, methyl ester (99% purity); INE-965, 320 Chr-CD-1 mice were used in the experiment. The test material was fed to 80/sex/group at levels of 0, 500, 1500 and 5000 - 7500 ppm for 44 weeks. Parameters monitored were food consumption, weight gain, behavior and appearance, mortality, hematology and pathology. Appropriate statistical analyses were performed. The termination of the study, with concurrence by EPA TOX personnel, was necessitated by (1) a high morbidity and mortality in control and test mice due to an overwhelming systemic bacterial (staphylococcal) infection and (2) the likelihood that any further histological or clinical pathological evaluation of the mice would be impossible due to the intercurrent disease or its sequelae. The route of entry for the bacteria appeared to be at the site of local irritation produced by the metal ear tags used for identification.

• Results: After male and female mice were fed INE-965 for 9 weeks, it was noted that mice fed 5,000 ppm showed an increase rate of body weight gain when compared to the controls. As of 10 weeks on test, the 5000 ppm dietary level was raised to 7,500 ppm. At termination of the study, there were no meaningful differences among control and test groups with respect to weight gain. There were no meaningful differences among control and test groups with respect to food consumption data. The first appearance of ear irritation occurred approximately 7 weeks after the initiation of the study in the vicinity of the ear tag and consisted of swelling and redness that progressed in severity until a portion or all of the ear became necrotic and sloughed off. Accompanying this was a creeping wet pustular dermatitis that spread down the side of the head, neck and over the shoulder. Systemic signs were wet and stained perineal area, diarrhea, labored breathing, weakness, dehydration, extreme weight loss and death. The incidence was evenly distributed among control and test groups. These clinical signs were not attributed to feeding of the compound. A summary of the mortality data as of 44 weeks on test is presented below:

<u>Mortality</u>	<u>Male</u>	<u>Female</u>
control	19/80	5/80
500 ppm	23/80	11/80
1500 ppm	13/80	9/80
5000-7500 ppm	14/80	15/80

The mortality was attributed to the systemic infection. There were no meaningful differences among the control and test groups with respect to the hematological parameters evaluated. At termination, the mice were sacrificed without pathological examination and no necropsy sheets were filled. All mice that died or were killed in extremis prior to termination of the test were examined grossly at necropsy and select tissues were subsequently evaluated histologically. At necropsy, the advanced autolysis present in mice found dead was remarkable.

This rapid rate of autolysis after death accompanied by red tinged abdominal and thoracic walls and a general dark appearance to all the visceral organs suggested a septicemia with an accompanying high fever. Proteus mirabilis, Klebsiella pneumoniae and Staphylococcus aureus were isolated from liver, kidney, spleen and blood of selected sick mice. S. aureus was the most frequent isolate obtained. This organism can produce α -toxin which is debilitating and can be necrotizing, hemolytic, leucocidal and damage platelets.

• Conclusion: The chronic feeding study in mice was terminated after 44 weeks necessitated by (1) a high morbidity and mortality in control and test mice due to an overwhelming systemic bacterial infection attributed to ear tag infection. No nutritional, clinical, hematological or pathological evidence of toxicity could be attributed to compound.

Classification: Supplementary Data

• (a) Study terminated at 44 weeks.

2. Long-term Feeding Study in Mice with methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate (INT-1991) Report on early termination - 51 weeks (Haskell Laboratory Report #539-78; Medical Research Project #2703, Oct. 13, 1973) Accession No. 236765.

Test Material: INT-1991-336 (99% purity)

320 CHR-CD-1 mice were used in the experiment. The test material was fed to 80/sex/group at levels of 0, 500, 1500 and 5000 - 7500 ppm for 51 weeks. Parameters monitored were food consumption, weight gain, behavior and appearance, mortality, hematology and pathology. Appropriate statistical analyses were performed. The termination of the study, with concurrence by EPA TOX personnel, was necessitated by a (1) high morbidity and mortality in control and test mice due to an overwhelming systemic bacterial (Staphylococcal) infection and (2) the likelihood that any further histological or clinical pathological evaluation of the mice would be impossible due to the intercurrent disease or its sequelae.

The route of entry appeared to be at the site of local irritation produced by metal ear tags used for identification.

Results: After male and female mice were fed INT-1991 for 22 weeks, it was noted that the mice fed 5,000 ppm showed only a 4% decrease in rate of body weight gain when compared to the controls. As of 23 weeks on test, the 5000 ppm dietary level was raised to 7500 ppm. At the termination of the study, male and female mice fed 7500 ppm exhibited a 9% decrease in rate of body weight gain to that of controls. There were no other meaningful differences among the control and other test groups with respect to weight gain. There were no other meaningful differences among the control and test groups with respect to the food consumption data. The first appearance of ear irritation occurred approximately 13-14 weeks after the initiation of the study in the vicinity of the ear tag and consisted of swelling and redness that progressed in severity until a portion or all of the ear became necrotic and sloughed off.

Accompanying this was a creeping wet pustular dermatitis that spread down the side of the head, neck and over the shoulder. Systemic signs were wet and stained perineal area, diarrhea, labored breathing, weakness, dehydration, extreme weight loss and death. The incidence was evenly distributed among control and test groups. These clinical signs were not attributed to feeding of the compound. A summary of the mortality data as of 51 weeks on test is presented below:

<u>Mortality</u>	<u>Male</u>	<u>Female</u>
Control	23/80	11/80
500 ppm	17/80	7/80
1500 ppm	20/80	8/80
5000 - 7500 ppm	18/80	9/80

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There were no meaningful differences among the control and test groups with respect to hematological parameters evaluated. At termination, the mice were sacrificed without pathological examination and no necropsy sheets were filled. All mice that died or were killed in extremis prior to termination of the test were examined grossly at necropsy and select tissues were subsequently evaluated histologically. At necropsy, the advanced autolysis present in mice found dead was remarkable. This rapid rate of autolysis after death accompanied by red tinged abdominal and thoracic walls and a general dark appearance to all the visceral organs suggested a septicemia with an accompanying high fever. Proteus mirabilis, Klebsiella pneumoniae and Staphylococcus aureus were isolated from liver, spleen, kidney and blood of selected sick mice. S. aureus was the most frequent isolate obtained. This organism can produce α -toxin which is debilitating and can be necrotizing, hemolytic, leucocidal and damage platelets.

Conclusions: The chronic feeding study in mice was terminated after 51 weeks necessitated by (1) a high morbidity and mortality in control and test mice due to an overwhelming systemic bacterial infection attributed to ear tag irritation. Other than the 9% decrease in rate of body weight gain observed in male and female mice fed 7500 ppm, there was no nutritional, clinical, hematological or pathological evidence of toxicity which could be attributed to compound.

Classification: Supplementary Data

(a) Study terminated at 51 weeks.

TQX/HED:th:RD Initial

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