Environmental Impact Analysis Report

ALBAMIX®

Date: May 1, 1979

Applicant: The Upjohn Company, Agricultural Division

Address: Kalamazoo, Michigan 49001

A. PROPOSED ACTION

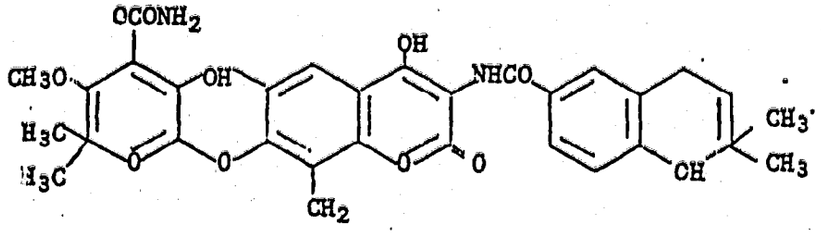
1. Proposed Action

It is proposed that the medicated premix, Albamix, containing novobiocin, 25 grams per pound, be distributed for addition to duck grower feeds at the rate of 14 pounds (350 grams of novobiocin) per ton of feed. The medicated feed is to be given for five to seven days as the sole ration to growing ducks for the control of infectious serositis and fowl cholera caused by *Pasteurella anatipestifer* and *Pasteurella multocida*, respectively, susceptible to novobiocin. Albamix is currently approved for use in chickens, turkeys, and mink for the treatment of staphylococcal and pasteurella infections susceptible to novobiocin.

2. Chemical and Physical Properties of Albamix

Albamix is a medicated feed premix containing novobiocin mixture incorporated into a soybean mill feed carrier at the rate of 25 grams of novobiocin base per pound. The active ingredient, novobiocin mixture, contains the free acid form of the antibiotic in amorphous fractions from novobiocin (*Streptomyces niveus*) fermentation. Novobiocin mixture is light to dark brown in color and has a characteristic odor. It contains not more than 5% moisture and has a pH of 4.0 to 7.5.

The free acid of novobiocin has a molecular weight of 618. It is insoluble in water, and has an octanol/water partitioning coefficient of 100%.[1,2,a*] Novobiocin has the following chemical formula: $C_{31}H_{36}N_2O_{11}$. The structural formula is as follows:



Albamix is a dry, feed additive premix intended for incorporation into complete duck feeds. One percent U.S.P. mineral oil is added to the premix as an antidusting agent.

3. Pharmacological/Toxicological Properties of Novobiocin

a. Pharmacology

Novobiocin has antibacterial activity against both gram-positive and gram-negative bacteria including the staphylococcal, streptococcal, diplococcal, pasteurella, proteus and pseudmonas species. When given orally it is rapidly absorbed from the gastro-intestinal tract of man and lower animals. It is widely distributed throughout body tissues and fluids with the exception of the cerebrospinal fluid. The antibiotic tends to concentrate in the liver and bile and is excreted in the feces and urine.

b. Toxicity

(1) Human Toxicity[3]

Human toxicity data on novobiocin indicate that the principal organs affected (in the following order of

*Numerical references are Upjohn reports and are attached to this document.
Alphabetical references are published works in the public domain.

significance) were: skin, digestive system, hematopoietic system, and liver. Symptoms generally do not occur following one oral dose but only after multiple dosing. The prinicpal changes were diffuse maculopapular rash (∿7%), loosening of stools (∿1.5%), eosinophilia (∿1.2%), and hyperbilirubinemia (∿.58%). These clinical findings generally occurred around the sixth to eighth day of treatment but sometimes as early as two days and were completely reversible following cessation of treatment. The stool softening generally did not interfere with therapy. The elevated icterus index and indirect serum bilirubin levels were not associated with other positive tests for impaired hepatic function. Skin rashes were probably due to sensitization but were often seen in patients with prior sensitivity histories or in combination with penicillin. The hematology alteration was considered to be associated with the skin rashes.

(2) Animal Acute and Subacute Toxicity[3]

Acute intraperitoneal toxicity studies in animals indicated that the drug was more toxic to guinea pigs (I.P. LD_{50} = 11.5 mg/kg) than to mice (I.P. LD_{50} = 300 mg/kg). The drug was less toxic to mice by the oral route (oral LD_{50} = 962 mg/kg). The drug was less toxic to the rat than to the mouse by the oral route (oral LD_{50} = 3200 mg/kg). Although the dog is not the most sensitive species relative to toxicity, blood absorption studies suggest that the dog and man react similarly.

Four groups of three beagles treated orally at 0, 30, 100 and 300 mg/kg/day for 60 days had significant changes at the high dose level. Phenolsulonphthalein decreased in three and Bromsulphalein retained in two high dose (300 mg/kg) animals. Two also had decreased hematocrit, hemoglobin, and leucocyte counts.

Tests with groups of ten rats, fed at the rate of 0, 50, 100, 200 and 385 mg/kg/day for 56 days, showed that all levels were non-toxic but there was a slight increase in female liver weights at the high dose.

Rats given massive doses (1.2 gm/kg) of novobiocin had weight loss and diarrhea in two days; 40% of the animals died after five days and the liver had foci of necrosis.

Four groups of three cats were injected subcutaneously for 70 days at 0, 10, 30, 56 and 100 mg/kg/day. Cats given 100 mg/kg became moribund after three weeks; diarrhea occurred in two cats receiving 56 mg and one cat given 30 mg/kg. Other than irritation at the site of injection, no gross or microscopic pathology was observed.

With the exception of the skin rashes, the organs affected in the animal tests were similar to the organs affected in man. The available data in both man and animal would suggest that the compound is relatively non-toxic and showed no irreversible changes.

4. Purpose and Benefits of Albamix for Ducks

The objective of distributing the medicated premix, Albamix, for addition to duck grower feeds, is to assist in the control of infectious serositis caused by *Pasteurella anatipestifer* and fowl cholera caused by *Pasteurella multocida*. These are two serious, infectious diseases of growing ducks which inflict significant morbidity and mortality on this class of poultry. Dr. W. F. Dean, Director of the Duck Research Laboratory, Cornell University, Long Island, New York, estimates the incidence of infectious serositis to be 50% of the growing ducks on Long Island (a major duck producing area) and 33% of the growing ducks in other production areas of the United States.

The duck producing industry will benefit and is anxious to have Albamix available for the control of these diseases. There are no drugs currently approved for use in feed for the control of infectious serositis or fowl cholera in this species. Without Albamix (novobiocin) medicated feed there will continue to be substantial losses, through both morbidity and mortality, to the duck growers, with the potential for a subsequent increase in the cost of duckling to the consumer.

5. Potential Market Penetration, Handling, Storage, and Distribution for Albamix

There are approximately 13 million ducks produced annually in the United States. The following table, provided by Dr. W. F. Dean, Director, Cornell University Duck Research Laboratory on Long Island, gives the geographic distribution, the type of rearing facilities, and the waste disposal practices for the production of ducks nationwide.

Commercial Duck Production in the United States

Location	Approx Annual Duck Production	Dry Lot or Wet Lot	Disposal of Litter	Treatment of Waste Water
Long Island (NY)	4 Million	3 Million Wet Lot 1 Million Dry Lot	Nursery Land	Secondary Treatment[1]
Wisconsin	4 Million	Dry Lot	Farmland	Secondary Treatment
Indiana	3 Million	Dry Lot	Farmland	No Discharge
Virginia	1 Million	Semi-Dry Lot	Farmland	Presettling and extended retention time
Massachusetts	½ Million	Dry Lot	Farmland	No Discharge
Ohio	½ Million	Dry Lot	Farmland	No Discharge
Calif	½ Million	Dry Lot	Nursery-Farmland	No Discharge
Missouri	½ Million	Dry Lot	Farmland	No Discharge

[1] Treatment by aeration followed by settling to remove solids and chlorination.

Of the ducks produced each year, approximately five million may have or will be exposed to infectious serositis and/or fowl cholera. It is estimated that approximately 50%, or 2.5 million birds, would be treated if effective medications were available. It is anticipated that Albamix will be used on approximately 10% (250,000) of the birds needing medication. This will result in a market penetration of 10%.

It is calculated that growing ducklings on medicated feed containing Albamix (350 grams of novobiocin per ton) will consume, on the average, 105 milligrams of antibiotic per day. If 250,000 ducks are treated for seven days with feeds containing Albamix at the recommended level, 183.75 kilograms of novobiocin will be used during a 12-month period. This is approximately 1/20th of the novobiocin activity, as Albamix, that is currently being used annually in turkeys, chickens, and mink.

All of the novobiocin and Albamix will be manufactured in Upjohn production facilities in Kalamazoo, Michigan. Depending on demand, it may be stored in Upjohn Regional Distribution Centers in Kalamazoo, Atlanta, Dallas, Kansas City, Los Angeles, Minneapolis, and Washington, D.C. From the distribution centers, it will be shipped to feed manufacturers who produce medicated feeds for the duck growers on Long Island, in Wisconsin, Indiana, Virginia, California, Massachusetts, Missouri, and Ohio.

6. The Environments Affected by the Manufacture, Distribution, Consumption, and Disposal of Albamix

a. Primary Environment

The primary environment potentially affected by the use of Albamix in ducks will be the ducklings slaughtered for human consumption sometime after having been treated with the product. Edible tissues of duckling (muscle, kidney, liver, skin-fat) have been found free of detectable amounts of novobiocin two days after the end of a 21-day experimental treatment period. Ducks affected with infectious serositis (*P.*

anatipestifer) and fowl cholera (*P. multocida*) are treated for seven days with Albamix. They are usually young birds that have not reached market weight, so a two day post-treatment withdrawal period prior to slaughter does not create undue hardship for the duck producer. There should be no novobiocin residues in marketed ducks.

Added protection to the consumer from inadvertent novobiocin residues is the small amount of duckling in the American diet. There is an annual U.S. production of 13 million ducks compared to 3.4 billion broilers and 140 million turkeys.

b. Secondary Environment

The secondary environment affected by the use of Albamix in ducks will be the ground and pond water where ducklings are produced in "wet lots" and the ground and ground water where ducklings are produced in "dry lots" (see table, page 5).

The "wet lot" system of husbandry provides ponds for the birds for drinking and swimming. This system is used to produce approximately three million ducks and is confined primarily to Long Island, New York. The effluent from the ponds is subjected to settling and secondary treatment so that it meets New York and Federal requirements before flowing into the fresh water streams which empty into Long Island Sound. It is estimated that 75,000 to 90,000 birds raised in this environment could be treated with Albamix.

Approximately ten million ducks are produced in "dry Lots" where the manure, or solid wastes, are spread on farm or nursery lands. The manure is usually spread at the rate of one to two tons per acre and is blended by tillage with the top six to eight inches of soil. In Wisconsin and Virginia waste water from duck growing operations is subjected to secondary treatment or retention in lagoons for aerobic microbiological degradation. It is estimated that approximately 170,000 ducks raised in dry lots could be treated with Albamix.

B. ENVIRONMENTAL IMPACT OF THE PROPOSED ACTION

1. Introduction Into the Environment

The distribution of Albamix for use in medicated duck feeds for the control of infectious serositis and fowl cholera will introduce approximately 200 kg of novobiocin per year into the environment. This will occur as the result of:

a. Manufacturing novobiocin by the fermentation process.

b. Manufacturing the medicated premix, Albamix, and the medicated duck feed containing the premix.

c. Utilizing Albamix medicated feed for 250,000 infected ducks as their sole ration for seven days, and the excretion of a portion of the novobiocin in the feces.

As detailed in subsequent sections of this report, the 200 kg of novobiocin utilized in this manner will have no significant effect on the environment.

2. Environmental Impact of the Manufacturing Process

a. Impact of novobiocin manufacturing (see attached statement, Appendix I).

b. Impact of Albamix manufacturing (see attached statement, Appendix II).

3. Environmental Fate

a. Tissue Residues of Novobiocin

For Albamix (novobiocin) to be effective in combating infectious serositis (*P. anatipestifer*) and fowl cholera (*P. multocida*) in ducks, the antibiotic must be consumed in the daily ration, absorbed from the gastro-intestinal tract, and carried to the affected tissues by blood, lymph, and other body fluids. In the process, the edible tissues will be

perfused with novobiocin. To assure that the food-producing ducklings would be free of antibiotic residues at the post-treatment slaughter time, two experiments were conducted as follows:

(1) Report No. 002-9760-25, September 15, 1972,[4] gives the results of a study in which ducks were treated with novobiocin, 350 gm/ton of feed for 21 days (three times the recommended seven day treatment period). Using an assay sensitive to 0.1 ppm, novobiocin was detected in kidney, liver, muscle, and skin-fat samples while the birds were on treatment. Samples of similar tissues obtained at three, four, and five days post-treatment were free of the antibiotic as measured by the same assay procedure.

(2) Report No. 9670-002-27, October 7, 1975,[5] gives the results of a second novobiocin tissue residue study in ducks. Birds were treated with the antibiotic at 350 gm per ton of feed for 21 days. Utilizing assay methodology sensitive to 0.1 ppm, novobiocin was detected in kidney, liver, muscle and skin-fat while the ducks were on treatment. The antibiotic was not detected in any similar tissues on days two, three, and four post-treatment.

Data from these two studies support a post-treatment drug withdrawal period of two days prior to slaughter of Albamix treated ducks for human consumption.

b. Excretion and Environmental Persistence of Novobiocin

To determine excretion patterns of novobiocin following oral administration of Albamix to ducks, and the persistence of the antibiotic in pond waters, a cooperative study was conducted with the Cornell University Duck Research Laboratory.[6]

(1) A commercial flock of ducks, housed with access to a swimming pond, was given Albamix (novobiocin) 350 gm/ton in its feed for seven days. Trace amounts of the antibiotic were detected in the pond water for the first four days immediately following treatment but none was detected for three additional days of sampling. In accordance with standard practice, the swimming pond water was subjected to secondary treatment consisting of a settling pond, aeration and chlorination. The effluent water from the secondary treatment contained no detectable levels of novobiocin during day one through seven of the post-treatment sampling period.

(2) Another part of the study was conducted to determine the rate of excretion of novobiocin given orally to ducks. Following the administration of a single 105 mg oral dose, 33.1% of the novobiocin was recovered in the feces during the five days immediately post-treatment. The average concentration in the feces ranged from a high of 38.03 mcg/ml six hours after administration to an estimated low of 0.01 mcg/ml at the end of the fifth day. The excretion curve indicated that no detectable novobiocin would be found on the sixth day.

c. Effects of Novobiocin on Soil Organisms, Algae, and Fish

Three studies were conducted by the Wisconsin Alumni Research Foundation of Madison, Wisconsin to determine the effects of novobiocin on soil organisms, algae, and fish.

(1) Soils containing 0, 20 and 40 ppm of novobiocin, inoculated with *Pseudomonas fluorescens* and *Aspergillus niger* and incubated at 30°C for two and seven days, found these organisms increasing at the same rate in all three samples. Novobiocin at these levels had no adverse effect on the growth of these organisms.[7]

(2) Soil containing 0, 20 and 40 ppm of novobiocin, inoculated with *Chorella pyrenoidosa* and incubated at 22°C for two and seven days found this organisms growing at the same rate in all three samples. Novobiocin at 20 and 40 ppm had no adverse effect upon the organism.[7] In addition to the WARF studies, the 1971 annual report of the Ministry of Agriculture for Northern Ireland, United Kingdom,[b] contained a study showing that a combination of novobiocin and tetracycline, used to produce a bacteria-free culture, was found to have no adverse effect on the growth patterns of two additional species of bloom-forming blue-green algae.

(3) Water containing 1, 10, 100 and 1000 ppm of novobiocin at temperature of 72°F caused no mortality of bluegill sunfish held in the water for 96 hours.[8]

d. Potential for Novobiocin Accumulation in the Environment

Applying the information concerning excretion and persistence of novobiocin from the Cornell University study to production standards in the duck industry provides the theoretical potential for accumulation of the antibiotic in the environment.

(1) Potential Novobiocin in the Soil

Ducks are grown to market weight of five pounds in approximately seven weeks. During this time they will consume 20 pounds of feed and excrete 15 pounds of manure. If they are treated during the growing process with Albamix, it will be incorporated into the feed at the rate of 350 grams per ton. During the seven day treatment period each duck will consume approximately four pounds of feed containing 735 mg of novobiocin, and excrete about two pounds of feces. Of the 735 mg consumed, 33% or 242.5 mg will be excreted. Manure containing novobiocin at the rate of 242.5 mg per two pounds will contain 242.5 grams per

ton. The stability of novobiocin in feces is unknown, but the characteristics of the antibiotic indicate that the temperature and moisture in this medium would expedite its complete degradation in a relative short time.[9,c,d] This is corroborated by its rapid disappearance from pond water.[6]

If poultry manure containing novobiocin is spread on farmland, the high nitrogen content limits application to one or two tons per acre, not to exceed three tons.[e] At the maximum, three tons per acre, 727.5 grams of novobiocin (242.5 grams/ton of manure) per acre would theoretically be applied to farmland. With normal farming practices, this 727.5 grams would be blended into the top eight inches of soil. Eight inches of top soil in an acre weighs 1,212,000 kg.[f] This mixture would result in a maximum novobiocin concentration of 600 mcg/kg (ppb).

Of the estimated 200 kg of novobiocin which may be used annually in ducks, 33% or 66 kg would be excreted in the feces. At the rate of 242.5 grams per ton of manure, there would be 273 tons of manure containing the antibiotic. If this were spread at the rate of one ton per acre, 273 acres of land would be affected. Inasmuch as the four major duck producing states have much more farmland available (more than 50 million acres[g]), it appears impossible that the level of novobiocin would attain 727.5 grams (3 tons of manure) per acre, and unlikely that it would reach 242.5 grams (1 ton of manure) per acre on this land area.

The maximum potential concentration of novobiocin in the soil, from spreading three tons to the acre of duck manure, 600 ppb, is very much lower than the 20 and 40 ppm found to have no adverse effects on soil or water organisms.[7,b,c]

(2) Potential Novobiocin in Ground Water

The average annual rainfall in the two major "dry lot" producing states, Indiana and Wisconsin, is approximately 34 inches. New York and Virginia average approximately 41 inches per year.[h] Using the lower average, 34 acre inches of rainfall weigh approximately 3,480,440 kilograms. Therefore, 242.5 grams of novobiocin (242.5 grams/ton/acre) leached out of one acre of soil by the 3,480,440 kilograms of water results in a potential concentration of 69.68 mcg/kg (ppb) in the water. Actual concentration would be less due to degradation of novobiocin.

The estimated potential concentration of novobiocin in ground water (70 ppb) is more than 500 times lower than the highest level tested (40 ppm) and found to have no adverse effect on soil or water organisms.[7] The level of 1000 ppm in water which was found to have no effect on bluegill sunfish[8] is about 15,000 times higher than the estimated potential level of novobiocin in ground water.

e. Conclusions Relative to Environmental Fate

Novobiocin residues in the edible tissues of ducks treated with Albamix in the feed are not detectable at a level of 0.1 ppm on the second day post-treatment. Approximately 33% of the antibiotic is excreted in the feces and trace amounts may be found in the ground or ground water during and for 4-5 days post-treatment. While minute amounts of novobiocin may be spread in duck manure on farmland, the rapid degradation in manure, soil, and water is such that it would not accumulate to higher levels in the environment. Since novobiocin is produced by an organism derived from the soil and is degraded therein, the therapeutic use of relatively small amounts (200 kg) in duck feeds would not be of biological significance in the ecosystem.

4. Environmental Effects

a. Novobiocin Tolerance

To establish a tolerance of 0.1 ppm of novobiocin in milk, four chronic toxicity studies of the antibiotic in laboratory animals have been conducted. Reports of these studies are attached and summaries of the findings are presented here.

(1) One Year Novobiocin Tolerance Study in Dogs[10]

Novobiocin was administered orally via gelatin capsules to three groups of dogs (eight males and eight females each) at the rates of 0, 1.0, and 2.0 mg/kg/day for one year. Measurements to evaluate tolerance to the drug treatment included clinical observations, body weights, food consumption, blood chemistry, hematology, urinalysis and gross and microscopic observations.

There were no adverse effects from novobiocin treatment in this study. Analysis of the data did not detect differences that could be attributed to treatment.

(2) Three Generation Reproductive Study in Rats[11]

Novobiocin was administered orally via diet to three groups of rats at the rates of 0, 10 and 20 ppm or approximately 0, 1.0 and 2.0 mg/kg/day, respectively, from weaning until maturity, mating, two pregnancies and lactations for each of three successive generations. The first generation contained 66 females and 33 males in each dose group, to accommodate the animal needs for the lifetime rat study. The second and third generations contained 20 females and 10 males each. Dams and sires of the third generation were treated at 20 times the above doses (0, 20 and 40 mg/kg/day) starting two weeks prior to breeding for females and six weeks for males for the F_{3b} litters, to be used in a subsequent teratology study.

Measurements to evaluate drug effects included clinical observations, food consumption, and body weights of dams and sires and reproductive measurements of the litters (from 10 to 16 parameters).

Analyses of the data from the study indicated there were no adverse treatment effects to the respective dams and sires and resulting offspring of two litters for each of three successive generations. Treatment group means, although statistically significantly different in a few cases, were considered not to be related to novobioicin treatment.

(3) Novobiocin Teratology Study in Rats[12]

Novobiocin was administered in the diet to three groups of rats at the rates of 0, 20 and 40 mg/kg/day from six weeks before mating until mating for sires, and from two weeks before mating through gestation day 20 for dams. The dams were killed and the pups delivered via Caesarean section. Observations were made for number of live and dead fetuses, number of fetuses per uterine horn, fetal body weights, sex, number of resorption sites, and number of gross abnormalities. Reproductive performance and gross examination of the dams and visceral and skeletal examination of the pups gave no evidence of adverse effects from drug treatment.

(4) Lifetime Novobiocin Feeding Study in Rats[13] (on-going)

Novobiocin was administered in the diet to three groups of male and female rats at the rates of 0, 1 and 2 mg/kg/day. Clinical observations, weekly food consumption, body weights, terminal clinical pathology, organ weights, gross and microscopic observations are or will be used for evaluating drug effects. Examination of body weights, food consumption, clinical observations, and gross pathology shows no evidence of adverse drug effects from novobiocin treatment.

(5) Novobiocin Safety in Turkeys[14]

Turkey poults were maintained for 35 days on a ration containing 350 grams per ton of novobiocin. There were no adverse reactions and the birds had satisfactory feed consumption, weight gains, and feed conversion coefficients when compared to contemporatory controls.

b. Potential for Environmental Disruption

The use o. Albamix (novobiocin) as described in Part A.1., Proposed Action, will not be disruptive of the environment, as determined by the data presented in Part B.3., Environmental Fate. Chronic toxicity studies described in Part B.4.a. indicate that the short-term use of novobiocin in ducks for the control of pasteurella infections will not adversely affect the reproduction, growth, development, or behavior of this species or environmental organisms which may be exposed to the antibiotic as a result of this use.

5. Other Information

We are not aware of other information which indicates that the distribution of Albamix (novobiocin) for use in ducks would be detrimental to the environment. As stated in Part A.5., the potential market for Albamix in ducks, a minor species, is extremely limited. It is estimated that initially the product would be used on about 250,000 birds, or 2% of the total population. This would require only 200 kg of novobiocin.

Although there are concentrations of ducks in three geographic locations, Long Island, Indiana, and Wisconsin, the areas are widely separated. This fact would further contribute to a lack of novobiocin in the environment.

The entire duck population is small, 13 million, compared to a turkey population of 141 million and a chicken (broiler and layer)

population currently estimated at 3.65 billion. Albamix has been used to treat pasteurella and staphylococcal infections in chickens and turkeys for 18 years without reports of adverse environmental effects. These users require approximately 4000 kg annually, or 20 times the amount estimated for use in ducks.

6. Summary

It is proposed that Albamix, a medicated feed premix containing the antibiotic, novobiocin, be distributed for use in the control of certain infectious diseases in ducks caused by pasteurella organisms. The product has been used for 18 years for the control of infectious diseases in chickens, turkeys, and mink, caused by pasteurella and staphylococcal organisms.

It is estimated that 200 kilograms of novobiocin will be manufactured and distributed for the described purpose. The manufacturing will have essentially no effect on the environment since it is an extremely small amount compared with the total output of the fermentation plant.

Approximately one-third of the administered novobiocin will be excreted by the ducks. The calculated potential concentration of the unmetabolized antibiotic in ground water and soil is too low to result in any antimicrobial activity in these media. Produced by an organism originally isolated from the soil, the physical/chemical characteristics of novobiocin indicate that it will undergo rapid biological degradation in the environment.

Since introduction in 1961, Albamix has been used to treat millions of turkeys and chickens with no observed or substantiated adverse effects on feedmill operators, turkey or chicken producers, birds, or the environment. The use of the antibiotic has salvaged many sick birds, resulting in a more profitable operation for the producers and eventually lower cost of turkey and chicken meat for the consumer. This same pattern of productiveness and conservation is predicted with the use of Albamix in ducks.

CERTIFICATION

The undersigned applicant/petitioner certifies that the information in this Environmental Impact Analysis Report is true, accurate, and complete to the best of his knowledge.

May 7, 1979
Date

Gordon G. Stocking, D.V.M.
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Agricultural Division
The Upjohn Company