ENVIRONMENTAL IMPACT ANALYSIS REPORT

ROFENAID®-40 Medicated Premix
(Active Drug Ingredients are Sulfadimethoxine and Ormetoprim)
for Control of Certain Duck Diseases

A. Date: January 31, 1983

B. Name of Applicant/Petitioner: Hoffmann-La Roche Inc.

C. Address: Nutley, New Jersey 07110

D. Environmental Information:

Summary:

The applicant has filed a supplement to approved New Animal Drug Application 40-209V providing for the use of ROFENAID®-40 premix for the manufacture of medicated duck feeds for the prevention and therapy of certain infectious disease of ducks. The continuous use of the medicated feed is restricted to a maximum of two weeks time for prophylaxis and five days for therapy. The duck species is considered by FDA to be a minor meat producing species. Present approved uses are for the use of ROFENAID®-40 in poultry feeds for disease control in chickens and turkeys.

1. Describe the proposed action.

The present supplemental application provides for use of ROFENAID®-40 at a concentration of 0.04% in feed as an aid in the prevention of bacterial infections caused by Salmonella spp. (salmonellosis) in ducks up to 2 weeks of age, as an aid in the treatment of coccidiosis, and for control of bacterial infections caused by Pasteurella multocida (fowl cholera) in breeder ducks with a treatment time of seven days, and at a concentration of 0.08% in feed for seven days for the control of bacterial infections caused by Escherichia coli (colibacillosis), P. multocida (fowl cholera), P. anatipestifer (P.A. infection) and Salmonella spp. (salmonellosis) in ducks.

The environment will be affected by this action in two ways:

a) through the excretion of ROFENAID®-40 components (sulfadimethoxine and ormetoprim) by the treated ducks, and

b) through the unavoidable but controlled discharge of some pollutants into the ecosphere during ROFENAID®-40 manufacture.

D. (cont'd.)

2. Discuss the probable impact of the proposed action on the environment, including primary and secondary consequences

The present supplemental application provides for use of ROFENAID®-40 at a concentration of 0.04% in feed as an aid in the prevention of bacterial infections caused by Salmonella spp. (salmonellosis) in ducks up to 2 weeks of age, as an aid in the treatment of coccidiosis, for the control of bacterial infections caused by Pasteurella multocida (fowl cholera) in breeder ducks, and at a concentration of 0.08% in feed for control of bacterial infections caused by Escherichia coli (colibacillosis), P. multocida (fowl cholera), P. anatipestifer (P.A. infection) and Salmonella spp. (salmonellosis) in ducks.

ROFENAID®-40 at a concentration of 0.02% in feed is presently approved as an aid in the prevention of coccidiosis caused by Eimeria tenella, E. necatrix, E. acervulina, E. brunetti, E. mivati and E. maxima, and bacterial infections due to H. gallinarum (infectious coryza), E. coli (colibacillosis), P. multocida (fowl cholera) in broiler and replacement chickens, and at a concentration of 0.01% in feed as an aid in the prevention of coccidiosis caused by E. adenoeides, E. gallopavonis and E. meleagrimitis, and bacterial infections due to P. multocida (fowl cholera) in turkeys.

The animal efficacy to include in vitro activity, in vivo battery and floor-pen trials as well as field trials under commercial conditions is summarized in the F.O.I. statement. These summaries include information concerning the prophylactic activity and its field experience as well as the therapeutic activity with the corresponding field experience. At this point in time, five million ducks plus have been treated with ROFENAID®-40 with no adverse reports concerning drug efficacy, effect on the environment, or drug residues in the tissues.

ROFENAID®-40 is a broad spectrum antibacterial and anticoccidial premix containing sulfadimethoxine and ormetoprim used in the preparation of medicated feeds. Each of these drugs exhibits both coccidiostatic and antibacterial efficacy alone. However, when they are combined in a pound of premix at a ratio of 113.5 gm (25%) of sulfadimethoxine and 68.1 gm (15%) of ormetoprim, a greater and broader degree of efficacy at a lower dosage is observed.

The mode-of-action of the combination is that of a potentiated sulfonamide. Sulfadimethoxine, a sulfonamide, has been widely used in the treatment of a variety of infectious diseases in humans and in domestic animals. It possesses a broad spectrum of antibacterial and anticoccidial activity. Rapidly absorbed into the bloodstream after administration, it is quickly dispersed into body tissues, and therapeutic blood levels are well sustained. The drug is rapidly cleared by the kidneys, minimizing the hazards of kidney damage.

D. 2. (cont'd.)

Ormetoprim, a pyrimidine, when used alone possesses some antibacterial and coccidiostatic properties. However, when used in combination with sulfadimethoxine, its primary function is to potentiate the activity of the sulfadimethoxine against pathogenic Eimeria species and against a wide variety of bacteria.

The combination of sulfadimethoxine and ormetoprim at a 5:3 ratio (ROFENAID®-40) is a potentiated sulfonamide which affords a lower use level, enhanced sulfonamide activity, and a decrease in the emergence of drug-resistant organisms. It provides an increased chemotherapeutic index and a broader spectrum of antibacterial activity when compared to non-potentiated sulfonamides.

The mechanism by which non-potentiated sulfonamides suppress bacterial growth is well understood. Folic acid (pteroylglutamic acid) is a vitamin for man and animals, but is not required by many bacteria because they are able to synthesize their own folic acid. One of the steps of the bacterial synthesis of folic acid involves the incorporation of *p*-aminobenzoic acid into the molecule. This step is blocked in the presence of sulfonamides by competitive inhibition.

Sulfonamides do not have this effect in man and animals because these species do not synthesize folic acid but depend on dietary sources of the vitamin. The biologically active form of folic acid is its reduction product tetrahydrofolic acid, which is an important coenzyme in one-carbon metabolism. Tetrahydrofolic acid is required for the biosynthesis of amino acids, purines and pyrimidines for protein as well as in nucleic acid metabolism. The pyrimidine potentiator inhibits one step in the enzymatic reduction of folic acid to tetrahydrofolic acid, thereby rendering ineffective any folic acid remaining in the bacterial cell and potentiating the effect of the sulfonamide.

The net effect is that less drug is required for the same antibacterial activity using the potentiated drug than the non-potentiated sulfonamide. ROFENAID®-40 has been an approved and used product in turkeys and chickens since 1970 for disease control. The use of Rofenaid in 1982 has been split with 51% used in turkeys, 47% usage in chickens, and 2% used in ducks. Hence, the primary geographic areas are the turkey and chicken raising areas.

The duck raising industry is a highly sophisticated and limited industry restricted to those areas where the geographic and environmental conditions (water availability and soil drainage conditions) facilitate duck rearing and management. Additionally, these areas are restricted by the need for ready access to markets where ducks are a traditional part of the various ethnic diets.

D. 2. (cont'd.)

Of necessity then, the turkey, chicken and duck raising geographic areas tend to overlap to a major degree, especially in the midwest and mid-Atlantic areas, and the Long Island area remains the major duck growing area (about one-half of the total ducks raised are raised on Long Island).

In this connection, it may be of interest to note the relative size of the three segments of the poultry industry and their consumption of Rofenaid. There are approximately 150 million turkeys raised annually and their growing period is in the order of 20-24 weeks; four billion chickens with a growing period of 7-8 weeks, and there are approximately 12 million ducks raised annually with a growing period of 7-8 weeks.

The total usage of Rofenaid in 1982 was 72,730 kg of the 40% premix representing 29,092 kg of drug substance. The turkey industry used 14,837 kg of Rofenaid (51% of the Rofenaid drug total) to treat 10 million of the 150 million turkeys grown in 1982. The broiler chicken industry used 13,673 kg of Rofenaid drug substance (or 47% of this total) to treat 150 million of the four billion broilers grown in 1982. The duck industry used 582 kg of Rofenaid drug substance (or 2% of this total) to treat 450,000 of the 12 million ducks raised in 1982.

Thus, it is clear that the duck industry, while important by itself as a source of meat consumed by humans, is relatively insignificant as related to the rest of the poultry industry which represents the current major use of Rofenaid medicated feeds.

The basic utility of the use of Rofenaid in ducks and how this will effect the environment is evaluated in the following sequences and revolves around the basic duck industry itself. Basically, the duck industry is a very small industry which raises approximately 12 million ducks a year; the ducks are divided in locate with approximately one-half the ducks raised on Long Island, New York and the other half raised in Wisconsin and Indiana.

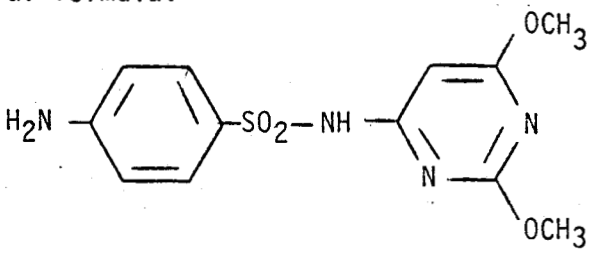
D. 2. (cont'd.)

The normal age for ducks when they go to market is approximately 7 weeks of age. During this term, each duck consumes approximately 20-lb of feed. Medicated feed will be utilized as a course of therapy for a period of time up to 2 weeks in length at dosages no greater than 0.08% active drug in the feed. Shorter periods of drug usage will be most common because of cost factors and a high degree of efficacy in most flock situations. Total drug consumed will vary with the age of the bird and feed intake at that age, with the majority of drug usage occurring during the younger ages (first 2 weeks of life).

ROFENAID®-40 contains a combination of five parts sulfadimethoxine and three parts ormetoprim (5:3 ratio). The chemical data for both compounds is listed by compound in the following two sections.

Sulfadimethoxine, Ro 4-0517, is a white crystalline powder with the chemical name, N'-(2,6 Dimethoxy-4-pyrimidinyl) sulfanilamide. Its empirical formula is $C_{12}H_{14}O_4N_4S$; its molecular weight is 310.3.

Structural formula:



The solubilities in the following table are given in gm per 100 ml at 25°C., unless otherwise specified:

Water	0.005%
95% Ethanol	0.5% cold, 4.0% hot
Chloroform	0.1%
Ether	0.1%
Petroleum Ether	0.1%
2N Hydrochloride	2.0%
Acetone	5.0%
Sodium Salt pH 9.3	0.5 gm/ml
pH 8.6	0.1 gm/ml
pH 8.1	0.05 gm/ml

The pH of a saturated aqueous solution is 6.3.
The melting point is 199.4°C. corrected, via the U.S.P. method.
The ultraviolet spectrum exhibits a maximum at 272 nm and a minimum at 234-236 nm in U.S.P. 95% ethanol, with the $E^{1\%}_{1cm}$ = 707.
Sulfadimethoxine is stable in water.

D. 2. (cont'd.)

The compound is known to undergo three principal color reactions, vis:

1. Bratton-Marshall reaction

2. With ferricyanide in aqueous potassium hydroxide, a reddish-brown color is produced

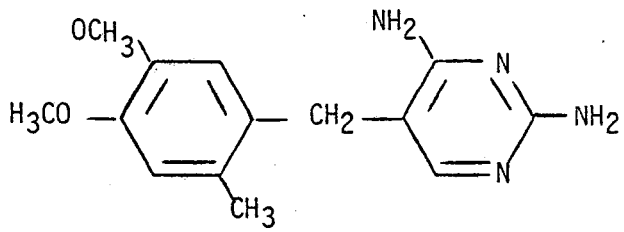
3. With cupric sulfate in aqueous sodium hydroxide, a yellow precipitate is produced.

No degradation of the compound could be detected when a 1 mg percent solution in 0.01 N NaOH of Ro 4-0517 was irradiated for 24 hours with high intensity long wave (360 nm) ultraviolet light.

Sulfadimethoxine is stable in the dry form as evidenced by its excellent stability in the ROFENAID®-40 premix for over 24 months at 72°F.

Ormetoprim, Ro 5-9754, is a white crystalline powder with the chemical name of 2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl) pyrimidine. Its empirical formula is $C_{14}H_{18}N_4O_2$; its molecular weight is 274.3.

Structural formula:



The solubilities in the following table are given in gm per 100 ml at 25°C.

Water	0.02	Petroleum Ether: (b.p. 30-60°C.)	Insoluble
95% Ethanol	0.81		
3A Alcohol	0.28	Benzene	0.03
Methanol	0.46	Dimethylacetamide	0.30
Isopropanol	0.14	Propylene Glycol	0.70
Chloroform	2.06	Benzyl Alcohol	4.30
Ethyl Ether	0.02	Acetone	0.03

The pH of a 1% aqueous suspension is 7.9.
The melting point is 232.8°-233.3°C. (U.S.P. XVI, Class I)
The ultraviolet spectrum exhibits a maximum at 275-279 nm in acidified 3A alcohol (0.01N HCl) with an $E^{1\%}_{1cm}$ of 274.
Ormetoprim is stable in water.

D. 2. (cont'd.)

The compound undergoes oxidative cleavage in alkaline permanganate to yield 3,5-dimethoxy-o-toluic acid which is fluorescent with excitation and emission maxima at 305 and 345 nm, respectively. Thus the above reaction forms the basis for the regulatory assay of ormetoprim in edible tissues.

No degradation could be detected when a 1 mg percent solution in 0.01N HCl of Ro 5-9754 was irradiated for 24 hours with high intensity long wave (360 nm) ultraviolet.

Ormetoprim is stable in the dry form as evidenced by its excellent stability in the ROFENAID®-40 premix for over 24 months at 72°F.

The safety of ROFENAID®-40 to ducks has been evaluated and reported in the animal safety section of the F.O.I. statement. The animal safety summary states that ROFENAID®-40 at 0.04% or 0.08% has been fed to over 1.8 million ducks under commercial growing conditions for the periods of time recommended for prevention or control of disease, without a single report of untoward effects as measured by mortality, morbidity, weight gain, feed efficiency and downgrading at federal inspection.

The toxicity of ROFENAID®-40 as a combination and each of its components has been evaluated using the array of animal models listed below:

Acute Toxicity

1. Acute oral toxicity in chicks (single oral dose via capsule in 6-day old chicks w/a 14-day observation period)

   a. The LD_{50} for sulfadimethoxine is established to be greater than 15,000 mg/kg body weight

   b. The LD_{50} for ormetoprim alone has been shown to be 700 ± 30 mg/kg

   c. The LD_{50} for Rofenaid is 1575 ± 100 mg/kg

2. Acute oral toxicity in turkeys (single oral dose via capsule in 2-week old poults w/a 14-day observation period)

   a. The LD_{50} for sulfadimethoxine is established to be 1750 ± 200 mg/kg body weight

   b. The LD_{50} for ormetoprim alone has been shown to be 400 ± 40 mg/kg

   c. The LD_{50} for Rofenaid is 930 ± 45 mg/kg

D. 2. (cont'd.)

3. Acute oral toxicity in mice: (single oral dose via suspension in 5% gum acacia w/a 72-hr observation period)

   a. The LD_{50} for sulfadimethoxine is established at greater than 4000 mg/kg body weight

   b. The LD_{50} for ormetoprim alone is at 1495 ± 56 mg/kg

   c. The LD_{50} for Rofenaid is established at 2440 ± 153 mg/kg

4. Acute oral toxicity in rats: (single oral dose via suspension in 5% gum acacia w/a 5-day observation period)

   a. The LD_{50} for Rofenaid is 2275 ± 115 mg/kg body weight

5. Acute oral toxicity in rabbits: (single oral dose via suspension in 5% gum acacia w/a 5-day observation period)

   a. The LD_{50} for Rofenaid is 1270 ± 118 mg/kg body weight

Tolerance Toxicity

6. Toxicity in rats:

   Rats were given sulfadimethoxine plus ormetoprim continuously in the diet at dosages up to 100 mg sulfadimethoxine + 60 mg ormetoprim per kg body weight per day for 13 weeks. No drug related signs of toxicity were noted except for a slight depression of body weight gains in the group receiving the highest dosage.

7. Toxicity in dogs:

   In a 13-week study, the tolerated oral daily dose (in gelatin capsules) was 75 mg/kg sulfadimethoxine + 45 mg/kg ormetoprim, or 45 mg/kg ormetoprim by itself.

In summary, the toxicity data indicate that both sulfadimethoxine and ormetoprim alone and in combination are relatively non-toxic with toxic effect concentrations in orders of magnitude greater than any Rofenaid concentrations that will be encountered under any use conditions.

These toxicity data were used by the FDA, and as provided by 21CFR §556.490 and §556.640, tolerances of 0.1 parts per million (ppm) have been established for ormetoprim in the edible tissues of chickens and turkeys, and for sulfadimethoxine in the edible tissues of chickens, turkeys and cattle. Practicable regulatory analytical methods for determination of tissue residues of ormetoprim and sulfadimethoxine have been published and are on file in the Food Additives Analytical Manual on display in the Public Records and Document Center, Food and Drug Administration, Rockville, MD.

D. 2. (cont'd.)

Three residue studies with ROFENAID®-40, using more than 400 ducks, have been conducted. These studies involved administration of ROFENAID®-40 in the feed at concentrations of 0.02% and 0.04% for eight weeks, at 0.02%, 0.04% and 0.08% for six weeks, and at 0.08% for three weeks. The results of the three studies showed that with all treatment regimens, the tissue residues of sulfadimethoxine and ormetoprim had decreased below the tolerance levels within five days of drug withdrawal, and supported assignment of a five day drug withdrawal time for ROFENAID®-40 administered to ducks at concentrations of up to 0.08% in the feed.

These data adequately indicate that there is no bioaccumulation in any of these tissues. These data should also adequately cover the concerns on bioaccumulation in wild flying birds. The data that have been submitted as part of other applications on ROFENAID®-40 for the chicken and turkey indicate as well that no bioaccumulation would occur for wild flying birds.

The use of ROFENAID®-40 in the duck industry will impact on the environment when the excreta from the treated ducks enters the environment. Analytical methods suitable for assay of sulfadimethoxine and ormetoprim in excreta, soil and excreta mixtures were developed based on the regulatory methods, validated and are included as Appendix A.

In order to provide a basis for evaluating the environmental fate of sulfadimethoxine and ormetoprim in the duck industry, the concentrations of both were determined in excreta from ducks receiving the maximum treatment of 0.08% ROFENAID®-40 in their feed.

Fresh fecal material from ducks maintained on feed medicated with ROFENAID®-40 at the 0.08% level was assayed for ormetoprim via the regulatory tissue assay procedure for that drug. Triplicate samples indicated a content of 30.1 ppm with a standard deviation of ± 2.3.

Total sulfadimethoxine was assayed via a modified procedure reported in Appendix A for both unconjugated and conjugated drug. Triplicate assays yielded 34.3 ppm with a standard deviation of ± 0.3.

Knowing this maximum quantity for unit feces, the next consideration is how the duck was raised over its lifetime, the interaction of its fecal output, and its entry into the overall environment.

D. 2. (cont'd.)

Basically, the growers use the following regimen:

The starting building contains straw litter, and it is usually over a dirt or concrete floor. This building is used to house the ducks for their first 2 weeks; at that point, they are allowed to run out of doors. A typical yard is sandy; in some of the older units, they slope down to the water or stream. In other operations, the stream has been replaced with a concrete paddling water pond. The operation on Long Island typically involves the movement of ducks from building to building; and in many instances, the hatchery is located on one side of the farm and the processing plant is located on the other extreme end of the farm.

Therefore, there is a progression from the hatchery to the processing plant in movement of these birds. This is pretty typical on a Long Island operation where they have an indoor-outdoor type of operation. The mid-west is different inasmuch as most of the ducks there are raised in total confinement.

The disposition of the fecal material during this growth cycle and the eventual fate of this fecal material is the primary question on the environmental impact of a quantity of drug in this particular fecal material. As noted above in treated animals (0.08% active drug in feed), the fecal material will have 30-35 ppm initial concentrations of sulfadimethoxine and ormetoprim. The fecal material is then handled in contact with the straw litter in the first 2 weeks of the growth cycle with the sandy soil and stream or paddling lagoons for the remaining growth period. The straw is moved from the building and is then utilized by nurseries, gardeners or is allowed to stand. The end fate of the fecal material associated with the straw is for fertilizing use. For wire raised birds, a wash is used to remove the feces from the wire and the wash goes through a settling process to meet the State and Federal requirements.

The State and Federal requirements are instrumental in dictating the fate of the feces itself and consequently any drug involved with it. Since fecal material has to be treated to decrease the bacterial count and to decrease the oxygen demand of this fecal material to a prescribed level as dictated by the State, these steps have to be included in the consideration of the fate of any of these compounds. The evaluation of the duck feces, therefore, centers around the following areas:

(1) The stability of the compounds in the fecal material itself and on standing in contact with feces-water and feces-soil

D. 2. (cont'd)

(2) On aerobic oxidation conditions to simulate the aeration step of the waste water processing treatment

(3) The consequent leaching of these compounds through various types of soil to simulate rainfall on the exposed fecal material

(4) The concentrations of sulfadimethoxine and ormetoprim that would be found in practice on a working duck farm using ROFENAID®-40

(5) The effect of the compounds on plant types that could be grown in fields fertilized with the duck manure

(6) The basic evaluation of the toxicity of the compounds themselves to standard aquatic test species, bluegill, water flea and algae

The stability of sulfadimethoxine and ormetoprim in duck-derived environmental samples at elevated temperature and humidity was determined using fecal material obtained from ducks maintained on unmedicated feed at a Long Island duck farm.

Individual 10 g fecal samples, soil-feces in a 20:1 ratio, and water-feces in a 20:1 ratio were fortified at a 10 ppm concentration of sulfadimethoxine or ormetoprim in glass vials and placed in an environmental chamber maintained at 37°C and 95% relative humidity, equipped with visible and ultraviolet light to simulate sunlight.

Loamy soil and tap water were used. Duplicate assays were done for all samplings. The results are shown graphically in the next three pages for feces, feces-soil and feces-water.

Examination of the data shows that after two days, the quantity of sulfadimethoxine in the feces and soil-feces dropped to less than 6% of the initial values and to less than 2% at 20 days with zero remaining after 40 days. In the water-feces mixture which was basically anaerobic, the value was 82% remaining after two days, 59% after six days, less than 2% at 20 days and zero after 40 days.

These data indicate that the sulfadimethoxine, upon standing, is decreased effectively in feces and in water-feces mixtures under anaerobic conditions and when mixed with the soil.

Ormetoprim shows less of a decrease under these conditions with approximately 60-64% remaining after two days in the feces and soil-feces, 50% remaining after 20 days, and it remains essentially constant after that point. In the case of water-feces mixture, 89% remains after six days, and as with the others after 20 days, the value essentially stays constant at approximately 50% of the initial. In the case of ormetoprim, the presence of ormetoprim at the 55-day interval was verified by the fluorescence spectra of the oxidation obtained and its comparison to the standard.

The aerobic oxidation step in the waste treatment process has been evaluated and the original reports submitted to NADA 49-209V on March 16, 1979.

Ambient air was passed through a 20:1 tap water:duck feces mixture at 25°C after duplicate mixtures were initially fortified with 5 ppm of sulfadimethoxine and ormetoprim, assayed in duplicate, and sampled 12 times over the next 40 days. The assays were reported as percent of zero time concentrations and are listed as follows:

Aerobic Oxidation of Sulfadimethoxine and Ormetoprim

Time Interval (Days)	% of Zero-Day Concentration	
	Sulfadimethoxine	Ormetoprim
1	92.3	93.6
2	77.3	84.3
5	71.0	74.6
9	52.3	34.3
12	37.7	9.6
13	5.8	8.0
14	4.8	8.5
15	9.5	8.3
19	9.9	2.9
22	8.0	2.6
27	7.6	0
40	8.5	0

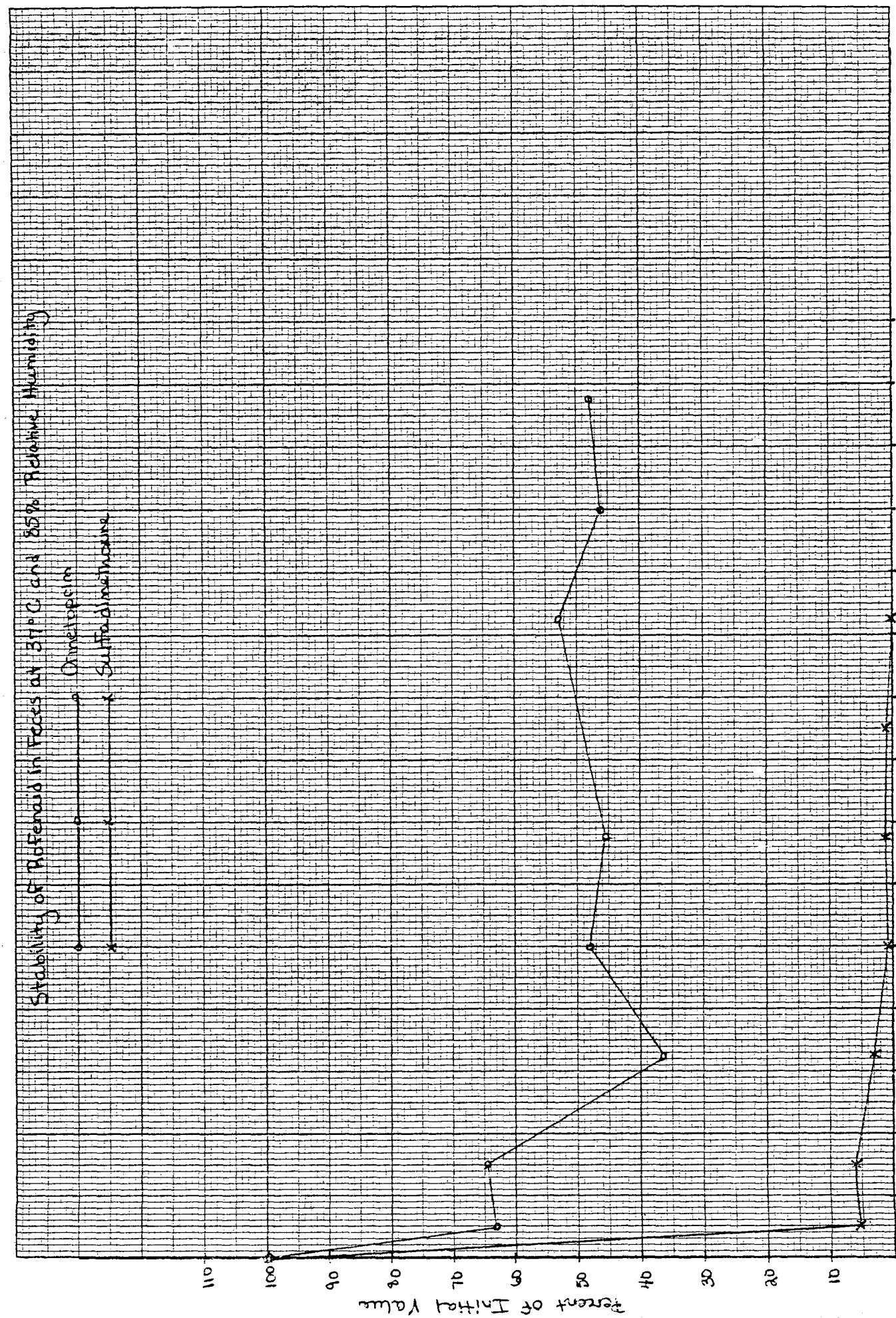
Inspection of the aerobic oxidation data indicates that both sulfadimethoxine and ormetoprim are extensively decreased in the feces-water mixture under these conditions. This long-term study indicates that the ormetoprim which indicated stability under anaerobic conditions, is unstable under aerobic conditions and shows a steady decrease, with less than 10% of the initial material remaining after 12 days; after 27 days, the value goes to zero remaining.

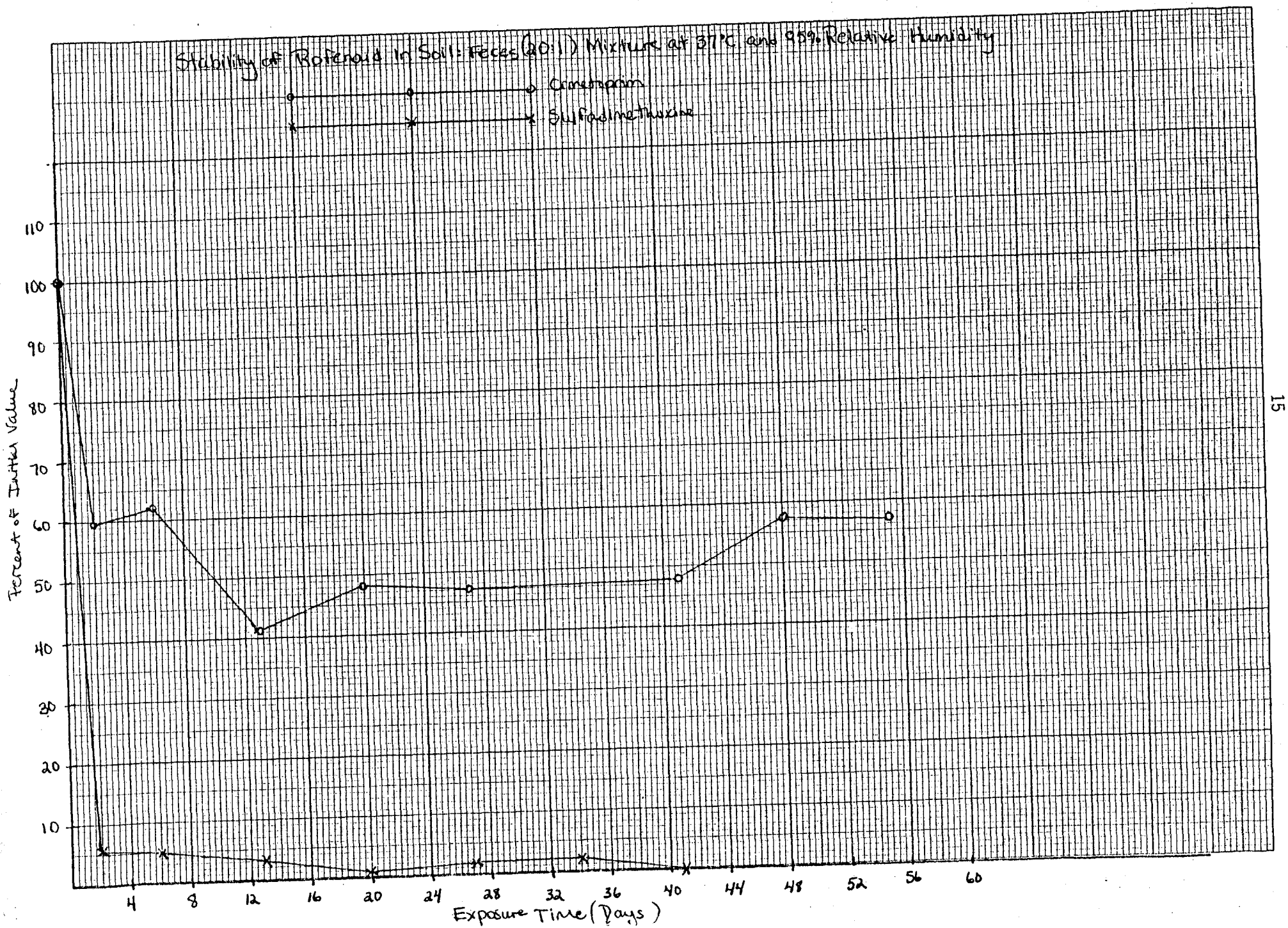
Sulfadimethoxine under aerobic conditions shows the similar rapid decrease and then a leveling effect after 13 days with the quantity of sulfadimethoxine remaining essentially constant at about 10% under aerobic conditions after that point. In summary, the aeration step utilized in water treatment will result in a massive decrease of the concentration of sulfadimethoxine and ormetoprim in the feces-water mixture.

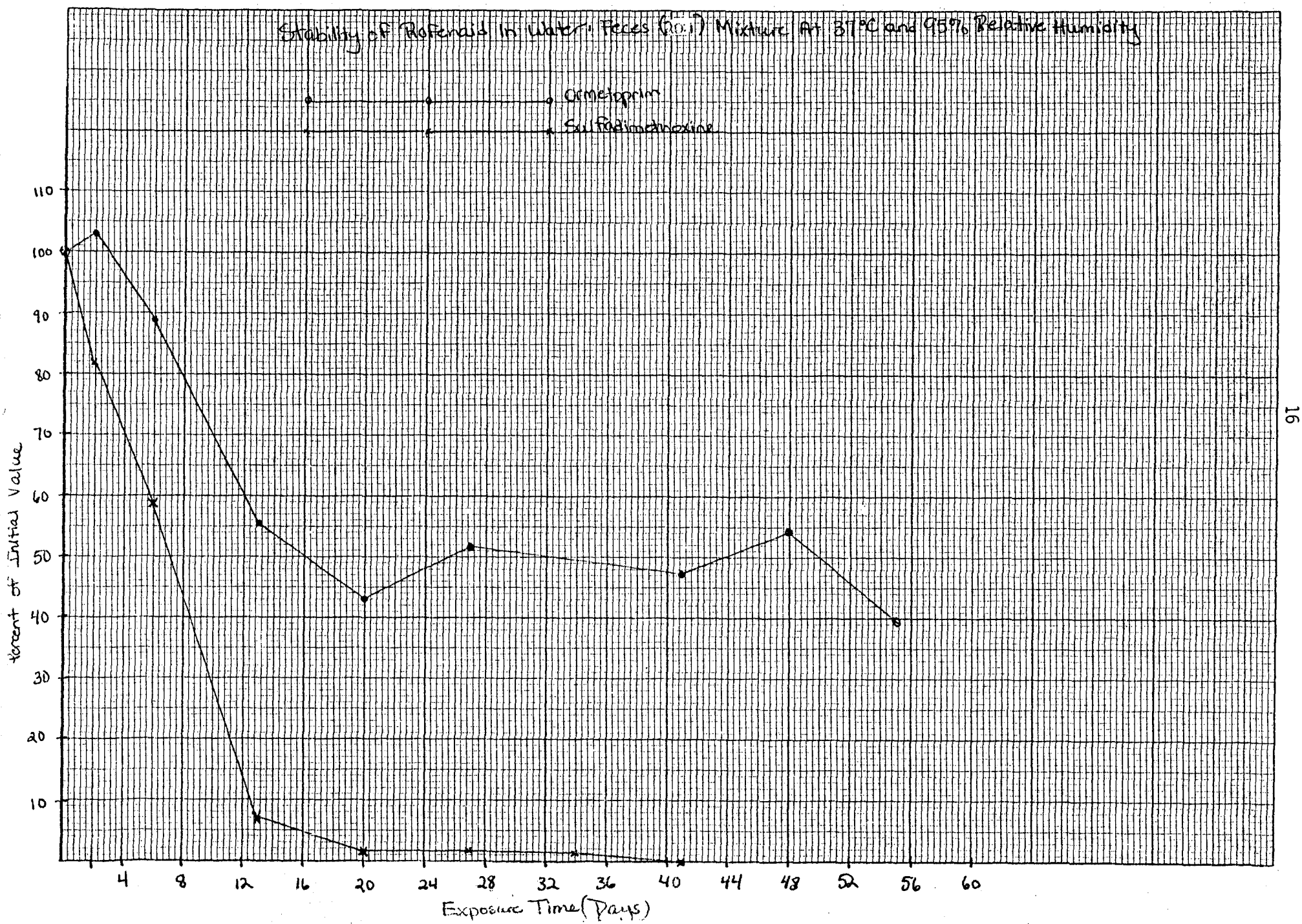
Translocation of sulfadimethoxine and ormetoprim in soil for simulating the effect of rain washing the sulfadimethoxine and ormetoprim from the feces into the soil was evaluated utilizing three different types of soil (see p. 17).

Three agricultural soils, classified as loamy sand, loam and sandy clay loam, were evaluated individually, each in triplicate, by placing the soil sample into a 20 mm in diameter column to a height of 5"; 172 ml of a 5 ppm solution of sulfadimethoxine or ormetoprim was passed through the column. This volume is equivalent to 20" of rain passing through the soil.

The effluent water was collected, 1-inch at a time and assayed. Subsequently the column was divided into 5 segments which were individually assayed. The results are presented on tables I and II located on pages 19 and 20 with the total of each compound applied to the column of 858 mcg.







This 5-inch quantity of soil was adequate in the case of sulfadimethoxine to adsorb anywhere from 125-475 mg, depending upon the type of soil. The material is definitely adsorbed and not degraded and can be extracted from the soil's surface by a pH adjustment and organic solvent extraction. With ormetoprim, it was adsorbed on the column of soil with most of it concentrated at the bottom of the column.

The data indicate that in the process of washing or passing the material through these columns, the fines with the larger surface area per unit volume have migrated to the bottom of the column; and in the case of ormetoprim, is the explanation for the concentration at the bottom inch of the soil column.

For ormetoprim, a second experiment was evaluated to determine what happens when an additional 20 inches of tap water is forced into the column after the first 20 inches as noted. This second 20 inches of water did not elute any ormetoprim from the soil column, and the adsorption basically has to be considered irreversible in terms of an aqueous system. Ormetoprim was recovered from the column by a pH adjustment, followed by an organic solvent extraction so that the total material was recovered.

These data can be summarized to indicate that sulfadimethoxine is adsorbed on the surface of the various types of soil and can range from 25 mg per inch of soil as the lowest case to 95 mg per inch as the highest. In the case of ormetoprim, the adsorption was complete and total, with the total adsorption capacity greater than the sample load of 900 mg.

This binding capacity can also be determined in terms of mg/cu. ft. as shown below:

Soil (% Clay)	Sulfa-dimethoxine (mg/cu. ft.)	Ormetoprim (mg/cu. ft.)
Loamy Sand (8)	103	345
Loam (16)	224	345
Sandy Clay Loam (24)	275	345

A summary at this point is in order to tie together the model studies that have been done to simulate the various routes of handling that can occur in actual practice. In summarizing these various routes, it is obvious that the amount of available sulfadiamethoxine and ormetoprim remaining in the environment after any of the waste routes taken in actual practice is very small, if not zero. The routes noted indicate extensive decrease and/or irreversible adsorption. To verify these laboratory data, samples were taken from an actual working duck farm where ducks were on 0.08% ROFENAID®-40 in the feed for at least two weeks.

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August 16, 1973

Dr. A. MacDonald
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Roche Laboratories
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Nutley, N. J. 07110

SOIL TEST REPORT

Lab. No.	Serial No.	EXCHANGEABLE CATIONS Magnesium	Potassium	Calcium	Sodium	Sample No.
		ME/100g				
P 3023	ES 6347	.56	.15	1.00	.16	1-Pasture 1 (Sandy)
P 3024	ES 6348	1.99	.59	4.87	.15	2-Hayfield by
P 3025	ES 6349	1.89	.61	3.85	.20	3-Santh Hayfield (25A) no end

		pH.	Phosphorus ppm	Cation exchange Capacity ME/100g	Organic Matter	Sample No.
P 3023	ES 6347	6.3	63	2.75	1.30%	1
P 3024	ES 6348	5.7	191	12.95	5.06%	2
P 3025	ES 6349	4.7	39	13.90	2.57%	3

		MECHANICAL ANALYSIS Sand	Silt	Clay	Total Nitrogen	Sample No.
P 3023	ES 6347	89%	3%	8%	.048%	1
P 3024	ES 6348	49%	35%	16%	.178%	2
P 3025	ES 6349	62%	14%	24%	.089%	3

		SOIL TEXTURE	
P 3023	ES 6347	Loamy Sand	1
P 3024	ES 6348	Loam	2
P 3025	ES 6349	Sandy Clay Loam	3

Table I

Recovery of Sulfadimethoxine from Soil Columns (mcg)

Segment # (Top to Bottom)	Loamy Sand Soil			Loam Soil			Sandy Clay Loam Soil		
	Col I	Col II	Col III	Col I	Col II	Col III	Col I	Col II	Col III
1	70.1	28.4	32.4	102.9	111.9	122.0	47.5	59.1	90.3
2	54.6	33.7	41.9	45.4	57.3	99.4	34.7	74.1	79.9
3	50.6	30.9	42.3	44.5	47.9	89.6	57.6	53.6	78.0
4	17.6	27.8	17.6	42.4	41.3	40.2	56.9	51.5	63.1
5	8.5	4.8	12.8	52.3	52.2	39.0	163.1	179.3	167.5
Total	201.4	125.6	147.0	287.5	310.6	390.2	359.8	417.6	478.8
Amount translocated through column	653.0	679.1	740.6	508.7	537.6	463.4	417.5	482.9	435.3
Total recovered	854.4	804.7	887.6	796.2	848.2	853.6	777.3	900.5	914.1
% of applied sample	99.5	93.8	103.4	92.9	98.9	99.6	90.6	104.9	106.6

Table II

Recovery of Ormetoprim from Soil Columns (mcg)

Segment # (Top to Bottom)	Loamy Sand Soil			Loam Soil			Sandy Clay Loam Soil		
	Col I	Col II	Col III	Col I	Col II	Col III	Col I	Col II	Col III
1	4.6	1.2	6.1	8.0	78.2	3.4	1.8	7.1	1.0
2	2.3	2.0	1.2	3.4	63.1	0.9	2.3	2.2	0.8
3	10.5	15.0	5.9	29.2	27.3	0.9	1.2	0.8	0
4	45.1	84.9	35.5	45.7	26.0	91.3	1.6	2.8	0
5	825.2	734.8	825.0	707.1	633.8	811.7	877.8	838.2	877.9
Total	887.7	838.0	873.7	793.4	828.4	908.2	884.7	851.1	888.7
Amount translocated through column	0	0	0	0	0	0	0	0	0
Total recovered	887.7	838.0	873.7	793.4	828.4	908.2	884.7	851.1	888.7
% of applied sample	103.5	97.7	101.8	92.5	96.6	105.8	103.1	99.2	103.6

In order to determine what environmental concentrations of sulfadimethoxine and ormetoprim would be encountered in actual use of ROFENAID®-40 for ducks, samples were taken from a Long Island, New York, duck farm.

The farm operates with a population of approximately 40,000 ducks raised for a period of 7 weeks 3 days. It's an in-and-out operation with birds placed each week.

The unit had been on Rofenaid®-40 at an equilibrium level equating to that which would be accomplished on the usual commercial Rofenaid®-40 for approximately three months. We would have expected an equilibrium to have developed,as the sludge removal is accomplished once a week.

The freshly voided samples from birds that have been on 0.08% ROFENAID®-40 were assayed and reported earlier as having 34 ppm and 30 ppm of sulfadimethoxine and ormetoprim, respectively. The birds were maintained on ROFENAID®-40 at 0.04% for the first 2 weeks and have received in almost all cases, at least one 5-7 day treatment at 0.08% ROFENAID®-40 once during their growing period.

The assay procedures reported in Appendix A were used to assay each sample in triplicate.

The samples analyzed from the duck farm are described below along with their respective values of sulfadimethoxine and ormetoprim.

1) East Duck Run. This sample represents the water coming largely from the young birds on wire. The fecal material is flushed into a conduit for eventual transmission to the first lagoon (indicated by sample 3).

Sulfadimethoxine	0.14 ppm
Ormetoprim	0.0 "

2) West Pond. This sample was taken from the pond where birds may swim. It is initially derived from sping water. It contains the output of the East Duck Run (sample 1) plus the water from enviscerating and dressing plant.

Sulfadimethoxine	0.08 ppm
Ormetoprim	0.0 "

3) Sludge from the First Lagoon. This lagoon is the area in which the ducks swim and contains the water from locations indicated by the above samples.

Sulfadimethoxine	0.74 ppm
Ormetoprim	0.23 "

4) North Settling Bed. This sample is the sludge taken from the bottom of the North Settling Road. This is normally removed once a week.

Sulfadimethoxine	0.32 ppm
Ormetoprim	0.13 "

5) South Settling Bed. Sludge normally removed once a week.

Sulfadimethoxine	0.34 ppm
Ormetoprim	0.17 "

6) Effluent. This is the effluent following chlorination which is then pumped into the normal Long Island Sound inlet water.

Sulfadimethoxine	0.0 ppm
Ormetoprim	0.1 "

7) Surface Sample. A large duck run containing a high percentage of fecal material.

Sulfadimethoxine	0.0 ppm
Ormetoprim	0.0 "

8) Sample of the Sandy Soil from 3-12" Below the Surface of the Runs. Sample was taken immediately under sample 7. Ducks are currently using this run.

Sulfadimethoxine	0.0 ppm
Ormetoprim	0.0 "

9) Fallow Subsoil Sample Comparable from a Pen Which Had Not Been Used for Ducks for Several Weeks.

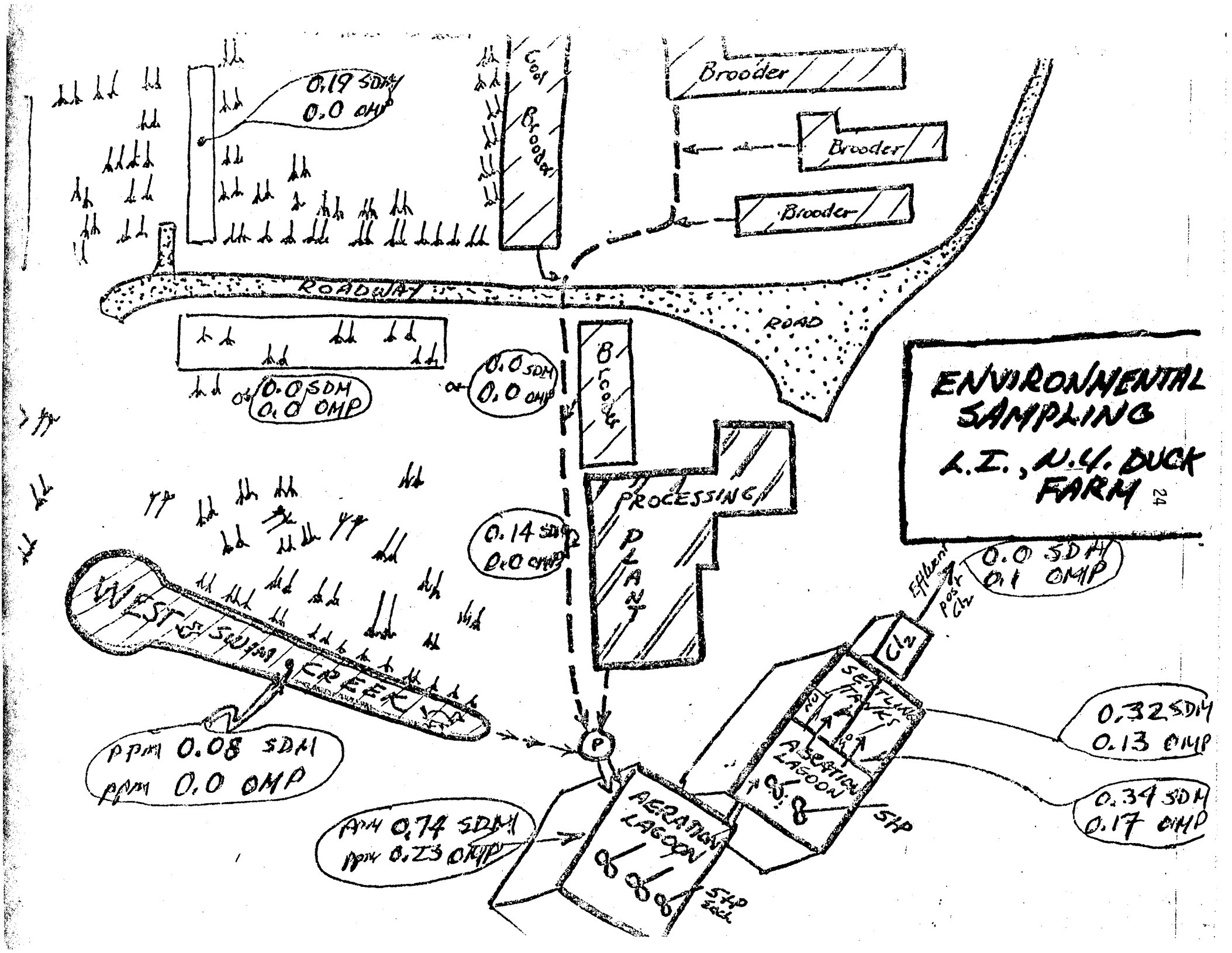
Sulfadimethoxine	0.0 ppm
Ormetoprim	0.0 "

10) Straw Sample From Under 5 Week-Old Ducks Maintained Under Shed.

Sulfadimethoxine	0.19 ppm
Ormetoprim	0.0 "

A site map of the farm is included to put the various sample locations in geographical perspective.

In summary, samples were assayed that were taken from points in the starting house, the straw and sand base in which the birds were being raised, the water in which they were swimming, the various lagoons as part of the waste treatment, and finally, the effluent going to the outside environment. In these samples, it is obvious material is present at a relatively low concentration which is in line with rapid degradation of the material as shown in the laboratory, along with the obvious dilution factor in terms of area being sampled. Most importantly, these data show that the waste treatment process does effectively remove drug remaining so the effluent from the final waste treatment contains no sulfadimethoxine and only 0.1 ppm of ormetoprim.


0.19 SDM
0.0 OMP
Cool
Brooder
Brooder
Brooder
Brooder
ROADWAY
ROAD
0.0 SDM
0.0 OMP
0.0 SDM
0.0 OMP
Brooder
ENVIRONMENTAL SAMPLING
L.I., N.Y. DUCK FARM
24
PROCESSING
PLANT
0.14 SDM
0.0 OMP
0.0 SDM
0.1 OMP
Effluent
Pos Clz
Clz
SEDIMENTATION TANKS
ACTIVATION LAGOON
5HP
0.325DM
0.13 OMP
0.34 SDM
0.17 OMP
WEST SUFFOLK CREEK
PPM 0.08 SDM
PPM 0.0 OMP
AMM 0.74 SDM
NPM 0.23 OMP
AERATION LAGOON
5HP Each

The possible effect of sulfadimethoxine and ormetoprim from the manure of ducks fed ROFENAID®-40 was evaluated using the two compounds alone and in combination with soils versus six plant types directly. This is the worst case model, since only the compounds are included with no manure present.

The concentrations used in the test systems were calculated to approximate those estimated in soil from duck manure spread at a rate of 5 tons per acre (the maximum use level) on a dry manure basis. A second concentration series was also included at 4 times the maximum concentration, or 20 tons per acre on a dry weight basis.

The concentration of sulfadimethoxine and ormetoprim in duck feces from ducks receiving 0.08% ROFENAID®-40 in their feed on an "as-is fresh" basis was reported as 34 ppm and 30 ppm, respectively, earlier in this submission. A very conservative estimate of a dry manure concentration of both sulfadimethoxine and ormetoprim is based on a fresh manure water content of 75-80% for an estimate of 150 ppm for both on a dry basis for uniformity.

Using a 6-inch depth, the weight of an acre is 2 MM lbs; therefore, at 5 tons per acre, a ratio of 1 part manure to 200 parts of soil is obtained. At 150 ppm of each in the dry manure, an application rate of 5 tons per acre yields 150 x 1/200 = 0.75 ppm in the soil. At 4 times the maximum manure rate of 20 tons per acre, a 4 x 0.75 = 3.0 ppm of each in the soil would be obtained.

The test concentrations in soil were thereby set based on the above calculation for 1 and 5 ppm of each of the compounds in soil as individual systems.

Samples of each compound were mixed with potting soil to investigate the effects of sulfadimethoxine and ormetoprim on plant growth. A positive control was prepared using sodium azide while a negative control had no medication added. The seven treatments used were:

Treatment 1 - Sulfadimethoxine 1 ppm
" 2 - " 5 "
" 3 - Ormetoprim 1 ppm
" 4 - " 5 "
" 5 - Sulfadimethoxine 5 ppm + Ormetoprim 5 ppm
" 6 - Control
" 7 - Positive control (sodium azide 50 ppm)

For each of the seven test soils, 10 flats of 20 seeds each were planted for each of the following six species: corn, soybean, cucumber, barley, tomato and ryegrass. Flats were maintained under normal growth conditions with watering done from the bottom up so as not to flush out the medications. The flats were kept under conditions of controlled temperature and humidity and received 12 hours of illumination per 24 hours.

The number of seeds germinating per flat was recorded on day 7, and the approximate average seedling height[2] (cm) per flat was determined by measuring 25% of the existing shoots after planting. On day 14 after planting, the germination count on a short height measurement was repeated.

The plants in each flat were then clipped at the soil line and weighed immediately. An average (wet) shoot weight (g) per flat was then calculated. Due to seed variability, some seedlings died before completion of the test. The number of dead seeds was subtracted from the number germinating at day 14 for use as a divisor in calculating average shoot weight.

The raw data and statistical treatment are included in the basic report submitted to NADA 40-209V on July 12, 1982. The statistical analysis is presented in bar graph form in the next six figures by species for the five variables analyzed.

Comparison of the 7- and 14-day observations of germination and shoot height provide a time course evaluation of the variable measured. Comparison of the three variables at 14 days, i.e., germination, shoot height and shoot weight, can be used as an index of toxicity with a toxic effect defined as a negative effect on all three variables. The figures can be described in terms of their 14-day data as follows:

Figure I (corn seeds). The SDM 5 ppm and OMP (1 and 5 ppm) treatment groups had significantly higher average shoot weight than the untreated controls. There were no significant differences between treatments with respect to germination rate and average shoot height.

Figure II (cucumber seeds). There were no significant differences between treatments with respect to germination rate; however, both the OMP 5 ppm and SDM + OMP treatment groups had significantly lower average shoot height and weight than the control group.

Figure III (soybean seeds). The SDM 5 ppm, OMP 5 ppm, and SDM + OMP had a significantly higher germination rate than the control. However, all these groups and the OMP 1 ppm group had significantly lower average shoot weight than the controls. Both OMP levels had significantly lower average shoot height than the control group.

Figure IV (tomato seeds). There were no significant differences between treatments with respect to germination rate. The OMP 1 ppm had significantly higher average shoot height and weight than controls, while SDM + OMP had significantly lower average shoot weight than control.

Figure V (barley seeds). The SDM + OMP had a significantly lower germination rate than control, while each of the SDM levels had significantly higher average shoot weight than the control. There were no significant differences between treatments with respect to average shoot height.

Figure VI (ryegrass). The SDM + OMP treatment group had a significantly lower germination rate and average shoot weight than the control group, while the SDM 1 ppm group also had significantly lower average shoot weight than the control group. There were no significant differences between groups for average shoot height.

Inspection of the six figures shows no consistent toxic effect as defined previously for any of the plant types with any of the treatments tested. There is no difference between levels of sulfadimethoxine and ormetoprim. The data indicate there will be no significant toxic effect to plants related to sulfadimethoxine and ormetoprim where duck droppings from ducks receiving up to 0.08% ROFENAID®-40 in their ration are spread as manure at 5 tons per acre (dry basis) or 20 tons per acre (4X normal rate).

Figure 1. Corn

1. SDM 1 ppm
2. SDM 5 ppm
3. OMP 1 ppm
4. OMP 5 ppm
5. SDM 5ppm + OMP 5ppm
6. Untreated Control
7. Treated Control

Significance of Comparison to Untreated Control
* $P < 0.05$ ** $P < 0.01$

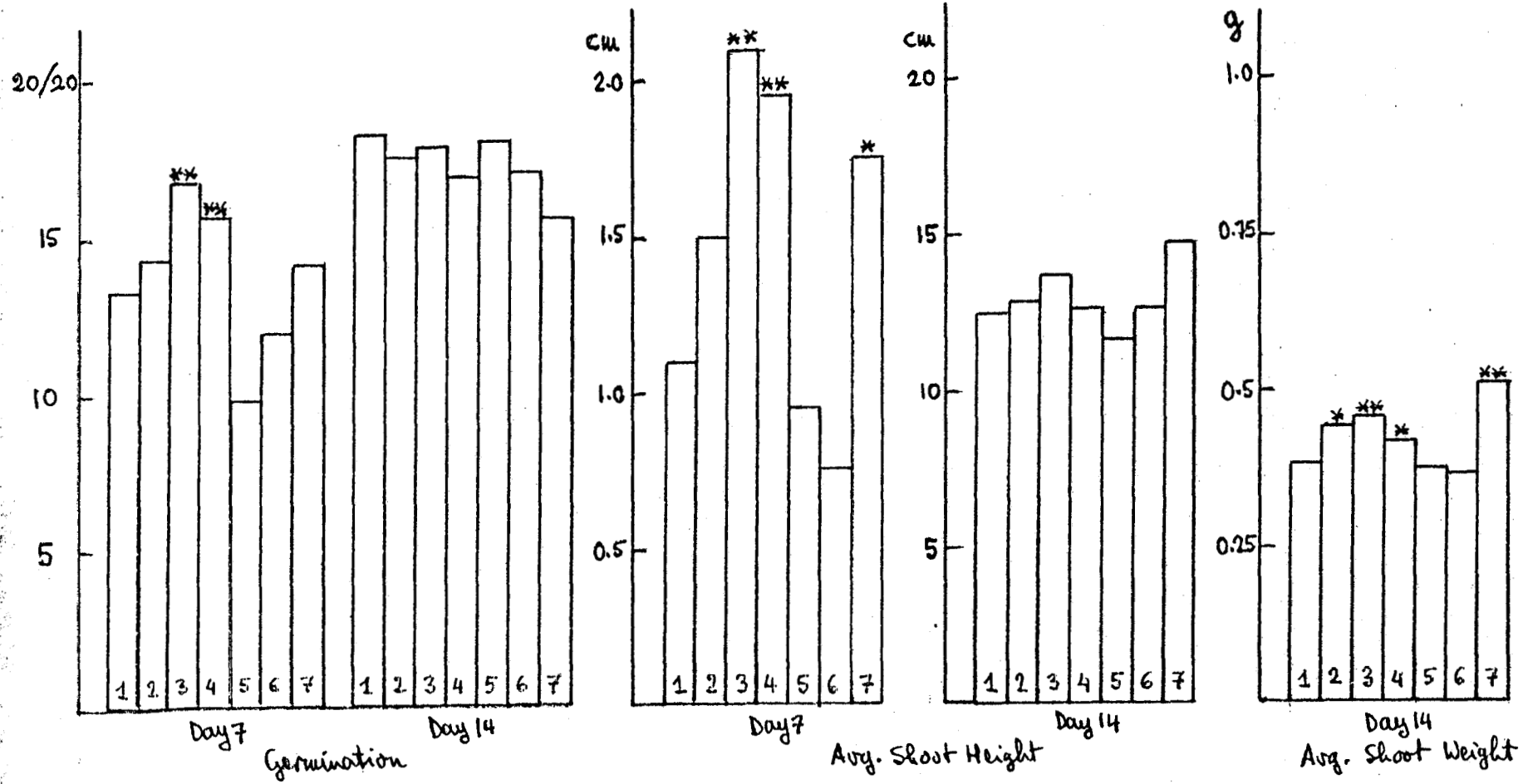
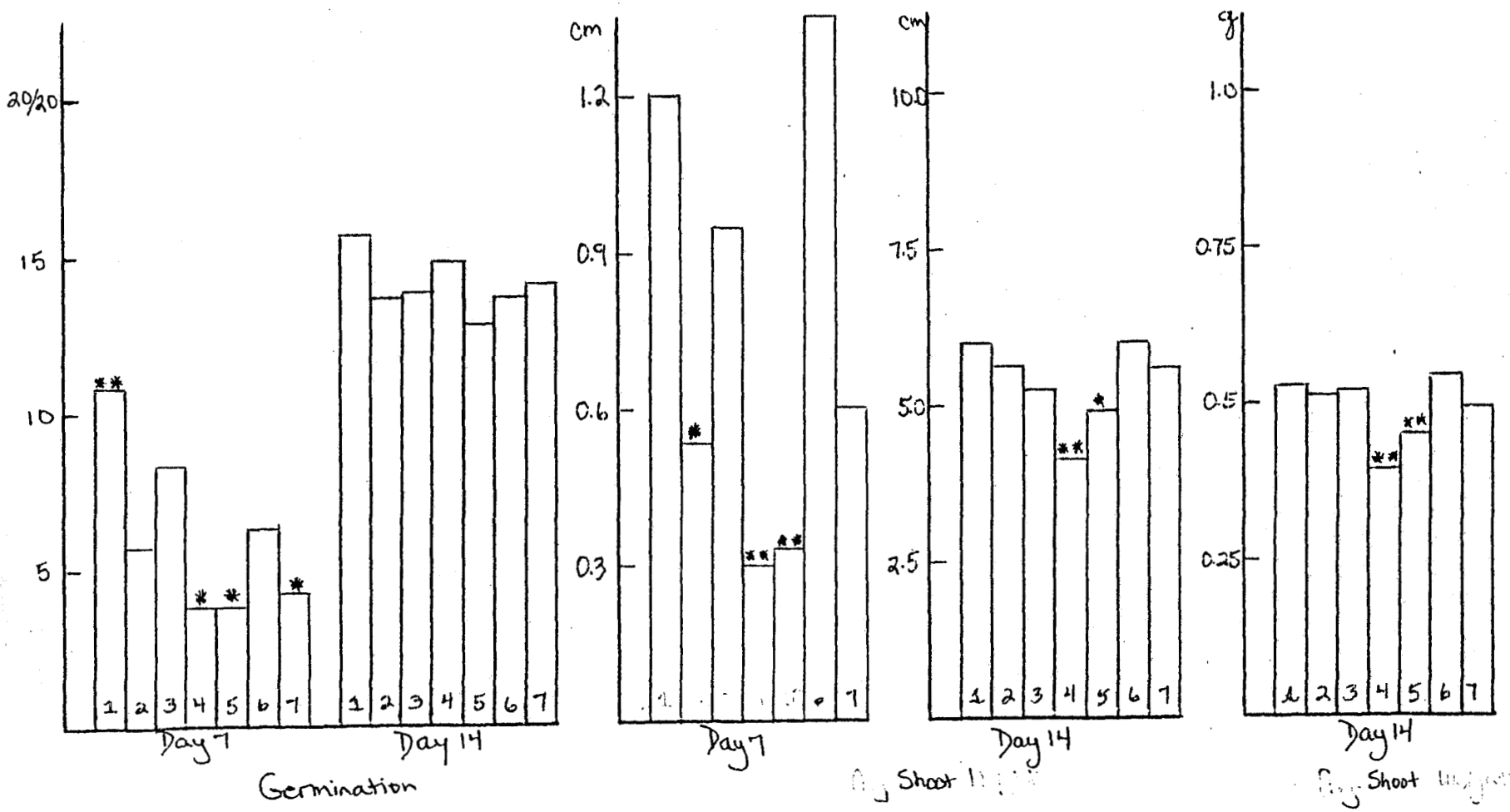


Figure 2 Cucumber

1: SDM 1 ppm
2: SDM 5 ppm
3: OMP 1 ppm
4: OMP 5 ppm
5: SDM 5 ppm + OMP 5ppm
6: Untreated Control
7: Treated Control

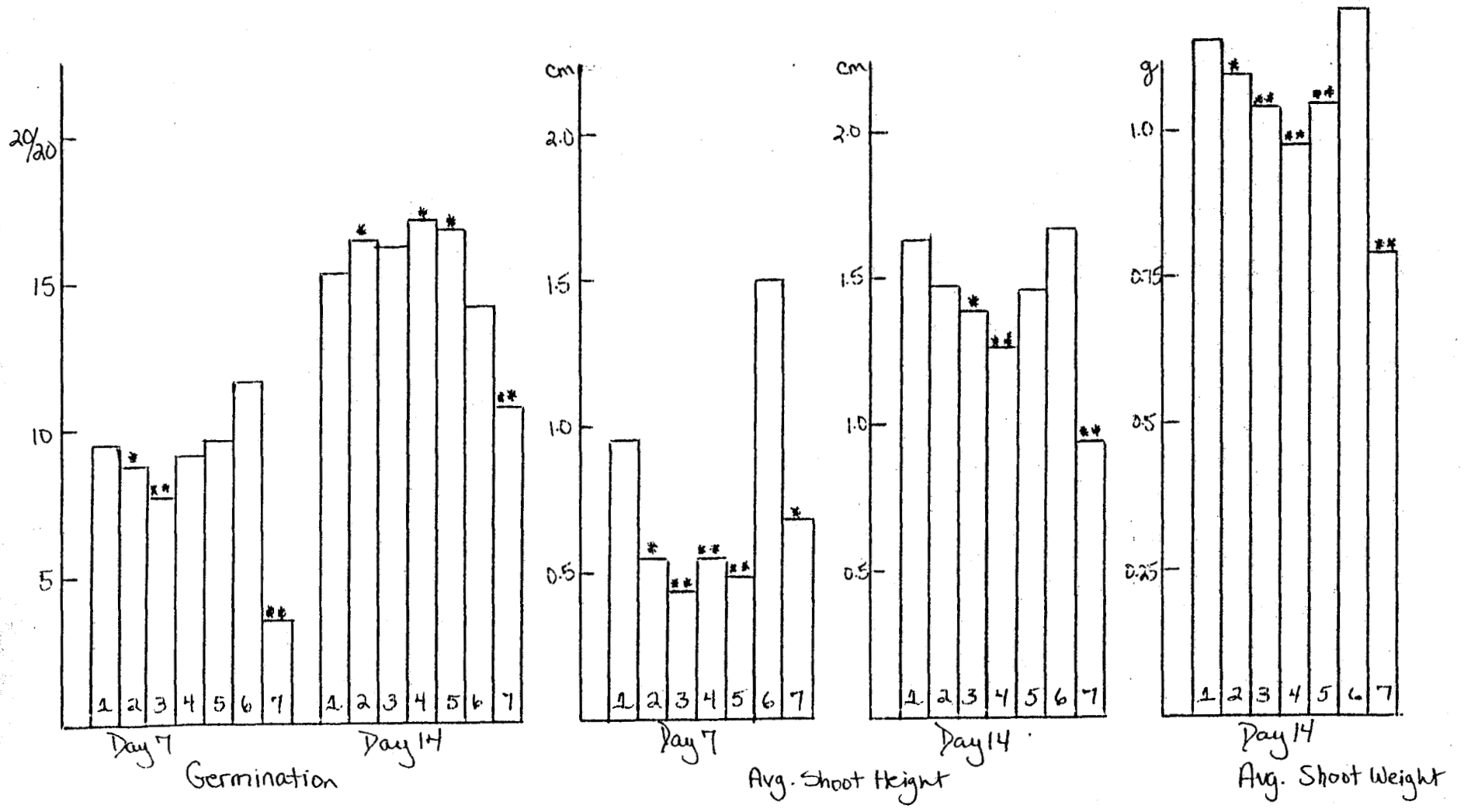
Significance of comparison to untreated control
* $P < 0.05$ ** $P < 0.01$



1 SDM 1ppm
2 SDM 5ppm
3 OMP 1ppm
4 OMP 5ppm
5 SDM 5ppm + OMP 5ppm
6 Untreated Control
7 Treated Control

Figure 3 Soybean

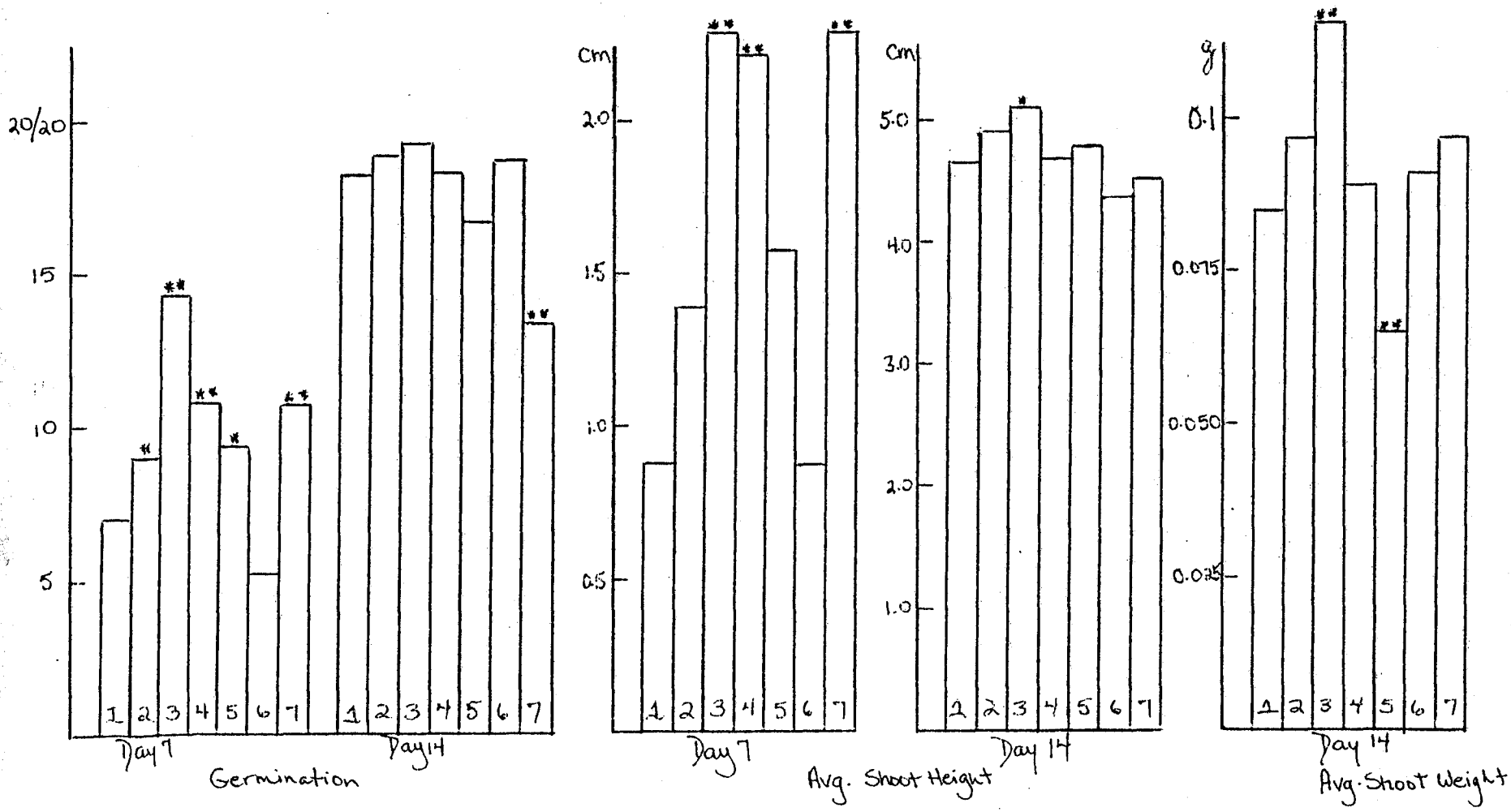
Significance of comparison to untreated control
* $P < 0.05$ ** $P < 0.01$



1 SDM 1ppm
2 SDM 5ppm
3 OMP 1ppm
4 OMP 5ppm
5 SDM 5ppm + OMP 5ppm
6 Untreated Control
7 Treated Control

Figure 4 Tomato

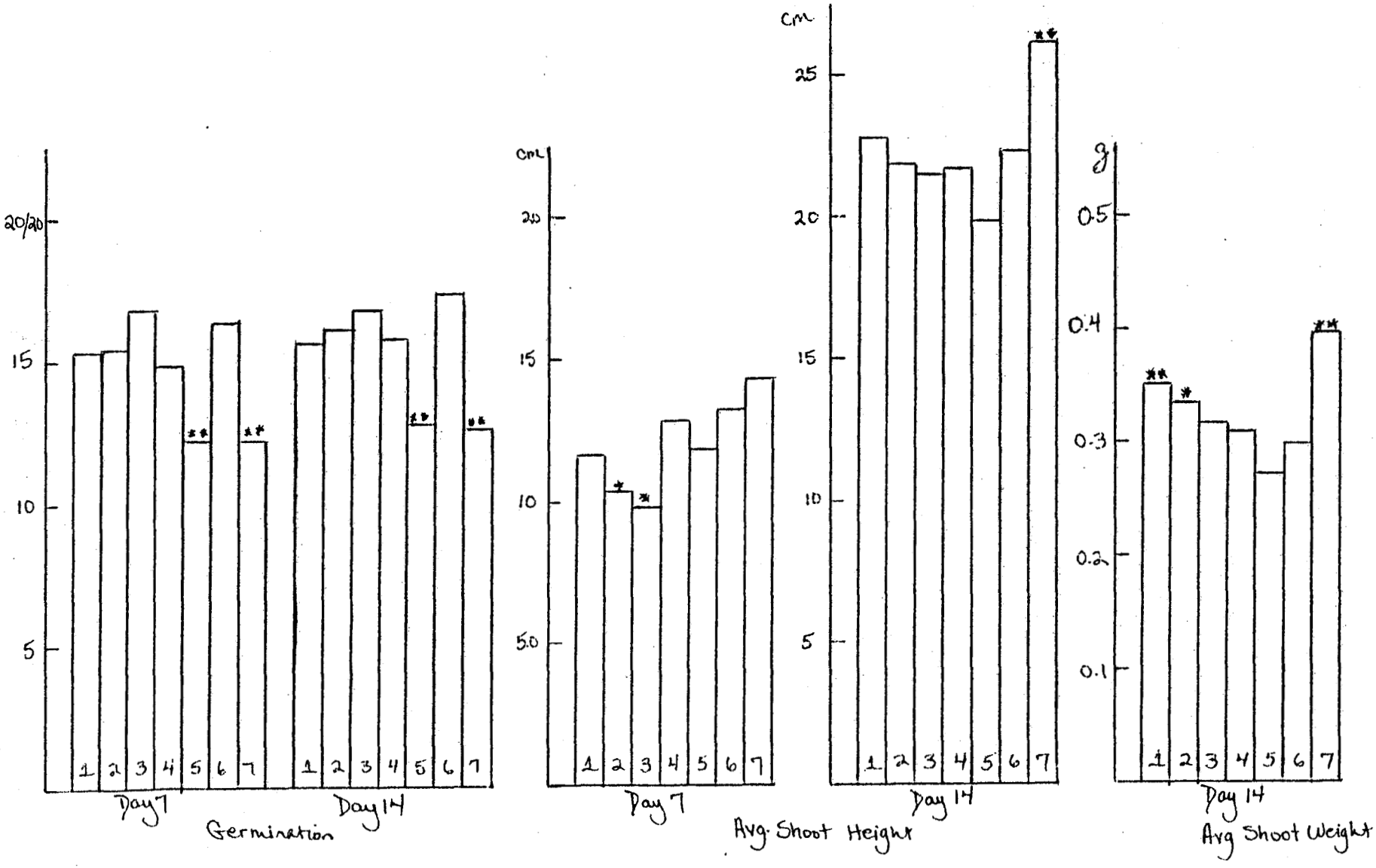
Significance of comparison to untreated control
* $P < 0.05$ ** $P < 0.01$



1 SDM 1ppm
2 SDM 5ppm
3 OMP 1ppm
4 OMP 5ppm
5 SDM 5ppm + OMP 5ppm
6 Untreated Control
7 Treated Control

Figure 5 Barley

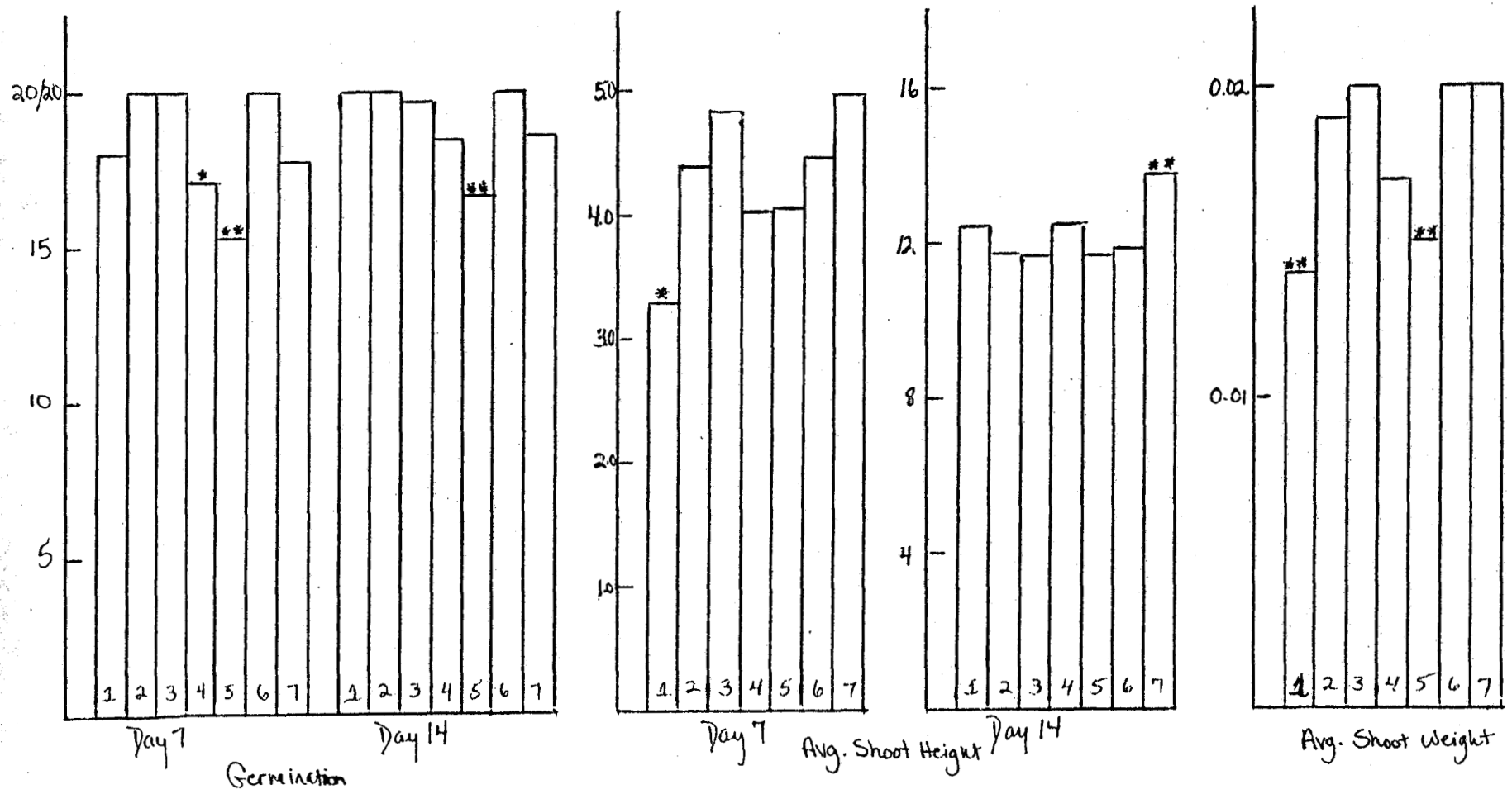
Significance of comparison to untreated control
* $P < 0.05$ ** $P < 0.01$



1 SDM 1ppm
2 SDM 5ppm
3 OMP 1ppm
4 OMP 5ppm
5 SDM 5ppm + OMP 5ppm
6 Untreated Control
7 Treated Control

Figure 6 Ryegrass

Significance of comparison to untreated control
* $P < 0.05$ ** $P < 0.01$



Rofenaid and its individual components have been evaluated versus three aquatic species using static systems.

The acute toxicity of sulfadimethoxine, ormetoprim and ROFENAID®-40 to bluegill (Lepomis macrochirus), water flea (Daphnia magna) and fresh water alga (Selenastrum capricornutum) was determined by E.G.&G. Bionomics. The bluegill and water flea work was done at the E.G.&G. Aquatic Toxicology Laboratory in Wareham, MA and the fresh water alga work at the E.G.&G. Marine Research Lab in Pensacola, FL. The original reports were submitted to NADA 40-209V on March 16, 1979.

Procedures used in the 96-hour acute toxicity test for bluegill followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians by the Committee on Methods for Toxicity Tests with Aquatic Organisms," U. S. EPA, April, 1975 (EPA-660/3-75-009 Ecological Research Series).

Procedures used in the 48-hour acute toxicity test for water flea (Daphnia magna) followed those described in "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians by the Committee o n Methods for Toxicity Tests with Aquatic Organisms," U.S. EPA, April, 1975 (EPA-660/3-75-009 Ecological Research Series).

The 96-hour toxicity test with fresh water alga (Selenastrum capricornutum) was based on "The Algal Assay Procedure: Bottle Test," National Eutrophication Research Program, Pacific Northwest Water Laboratory, Corvallis, OR (U.S. EPA, 1971) and R.H. Hall, "An Algal Toxicity Test Used in the Safety Assessment of Detergent Components," presented before the 36th Annual Meeting of the American Society of Limnology and Oceanography, Inc., Salt Lake City, Utah (1973).

The maximum exposure times were used for the acute toxicity values listed below for the three species.

Compound	Acute Toxicity to Bluegill (96-hr) (LC_{50} mg/liter)
Sulfadimethoxine	No mortality noted in a saturated solution
Ormetoprim	No mortality noted in a saturated solution
ROFENAID®-40	No mortality noted in a saturated solution

The ormetoprim and Rofenaid test fish were stressed by low dissolved oxygen concentrations (less than 40%) in all but one Rofenaid test concentration at 96 hours. This low dissolved oxygen concentration obviously resulted in more effects than would be expected for the test chemical concentrations used.

Compound	Acute Toxicity to Water Flea (48-hr) LC_{50} mg/liter (± 95% confidence interval)
Sulfadimethoxine	53 (26-105)
Ormetoprim	33 (18- 60)
ROFENAID®-40	38 (23- 61)

Compound	Acute Toxicity to Fresh Water Alga (96-hr) LC_{50} mg/liter (± 95% confidence interval)
Sulfadimethoxine	170 (42-688)
Ormetoprim	90 (21-378)
ROFENAID®-40	38 (6-238)

The wide variabilities in the 95% confidence intervals indicate these determinations are probably affected by the low water solubility of the drugs relative to the concentrations used.

The following additional data were gathered on trout and catfish by the U.S. Fish & Wildlife Service at Leetown, W. VA and LaCross, WI during their evaluation of Ro 5-0037 which has a ratio of 5:1 (sulfadimethoxine: ormetoprim) as compared to ROFENAID®-40 at 5:3 (sulfadimethoxine:ormetoprim).

The following additional data were gather on a variety of fish by the U.S. Fish & Wildlife Service at Leetown, W. VA and LaCrosse, WI during their evaluation of Ro 5-0037 in a ratio of 5:1 as compared to ROFENAID®-40 at 5:3.

The National Fish Health Research Laboratory in Leetown, W. VA evaluated Ro 5-0037 by medicating the feed to trout to provide a dose up to 400 mg/kg/day for 14 days at 13°C water temperature with no signs of toxicity.

The National Fishery Research Laboratory at LaCross, WI initially evaluated the dry powder 30% Ro 5-0013 dry premix vs solutions of each drug and the formulation of 5% solution Ro 5-0037 as a source of drug for fish toxicity testing.

The stock solutions of sulfadimethoxine and ormetoprim were prepared in base and acid, respectively with a Ro 5-0037 formulated to yield a 5% solution were used in the test. It must be noted that use of the above solutions do not insure solubility of the drug in control pH aqueous systems.

Methods for conducting the toxicity tests are standardized according to the Committee on Methods for Toxicity Tests with Aquatic Organisms, EPA-60/3-75-009. Most of the materials were so non-toxic that LC_{50}'s could not be determined, and those results are reported as the highest concentration exposure that produced no mortality as shown in the following table I (page 36). (Appendix B contains the reference data.)

The liquid in the initial aeration lagoon of the working duck farm represents the worst case situation with sulfadimethoxine and ormetoprim concentration of 0.74 ppm and 0.23 ppm, respectively.

These data can be used to calculate the factors before any toxicity would be evident based on the most sensitive species for each component and the combination. The water flea is the most sensitive species for sulfadimethoxine and ormetoprim individually with 26 mg/liter and 18 mg/liter, respectively at the -95% confidence interval yielding 35-fold and 78-fold factors for sulfadimethoxine and ormetoprim. A factor of 6-fold is calculated using the 6 mg/liter (-95% confidence interval) values for ROFENAID®-40 in fresh water alga and the sum of the sulfadimethodine and ormetoprim concentration in the lagoon water.

SUMMARY

The impact of sulfadimethoxine and ormetoprim in duck fecal material for birds that have received ROFENAID®-40 at concentrations up to 0.08% in their feed has been evaluated. Laboratory studies using fecal matter from ducks receiving ROFENAID®-40 have evaluated the stability of sulfadimethoxine and ormetoprim in fecal matter itself and in soil and water mixtures. Aerobic oxidation and soil percolation studies were also utilized with this fecal sample. The laboratory studies indicate that both sulfadimethoxine and ormetoprim are decreased rapidly and also are adsorbed on soil surfaces. Data from a working duck farm using ROFENAID®-40 confirms the very small amounts of sulfadimethoxine and ormetoprim present in actual practice.

The tissue residue data show that no bioaccumulation takes place in ducks while on use concentrations and, therefore, eliminates this concern for wild flying birds. The toxicity data in five species show that the compounds are basically non-toxic. The bluegill and water flea data also indicate that the compounds are shown to be basically non-toxic to these environmental monitors. The acute toxicity to fresh-water algae reinforces this pattern of non-toxicity. There is no consistent toxic effect for six species of crop and non-crop mono- and dicot plants at four times the maximum that would be obtained via manure application.

The laboratory, working farm and toxicity data show that ROFENAID®-40 use in ducks will not present an environmental concern to the area.

Clearly beneficial effects will result from the implementation of the proposed action, including the more efficient production of ducks with the concomitant savings in feed and energy, as well as other benefits. This will be discussed more fully in Section 5.

A secondary environmental consequence results from the discharge of pollutants into the ecosphere during manufacturing. This aspect is considered quantitatively and from a regulatory point-of-view in Section 3.

Table 1. Toxicity of R05-0037 and its individual components to rainbow trout and channel catfish in soft, reconstituted water.

Species	Temp. (°C)	Chemical	Toxic unit	96-h LC_{50} and 95% confidence interval or highest exposure concentration producing no mortality
Rainbow trout	12	R05-0037	mg/l	400
		Sulfadimethoxine	mg/l	400
		Ormetoprim	mg/l	400
		Placebo for R05-0037	µl/l	20,000
Channel catfish	17	R05-0037	mg/l	600 378-952
		Sulfadimethoxine	mg/l	400
		Ormetoprim	mg/l	200 163-245
		Placebo for R05-0037	µl/l	20,000

[a]All concentrations are based on the active ingredient.

D. (cont'd.)

3. Describe the probable adverse environmental effects that cannot be avoided

We know of no adverse environmental effects that cannot be avoided other than the minimal contribution of by-products, organic and inorganic, to the environment. Since all manufacturing operations must meet requirements of all Federal, State and Local authorities, such contributions must be considered minimal.

The following constitutes an analysis of the environmental effects of the manufacturing process of sulfadimethoxine and ormetoprim.

Material balance of process per kilogram of sulfadimethoxine

Total input chemicals		6.132 kg
Output from process		
Product (sulfadimethoxine)	1.000 kg	
Solids disposal	0.361 kg	
Air discharge	0.210 kg	
Water (sewer) discharge	4.561 kg	
Total output		6.132 kg

The water (sewer) discharge consists principally of inorganic salts (sodium chloride and sodium carbonate). The air discharge consists of minor amounts of organic solvents lost during solvent recovery. The solids disposal consists principally of carbon used as a decolorizing agent.

Material balance of process per kilogram of ormetoprim

Total input chemicals		8.479 kg
Output from process		
Product (ormetoprim)	1.000 kg	
Liquids disposal	6.468 kg	
Solids disposal	1.011 kg	
Total output		8.479 kg

The liquids disposal consists mainly of dimethylformamide and methanol. The solids disposal consists principally of sodium chloride.

Control of any possible pollutants resulting from manufacturing operations is in accord with all Federal, State and Local emission requirements.

Air Emissions

1. Sulfadimethoxine Production, Nutley, New Jersey

The sulfadimethoxine process was installed at the Nutley plant in 1956. Equipment installed in New Jersey prior to 1968 is grandfathered under New Jersey Bureau of Air Pollution Control regulations and does not require an air pollution permit. However, in 1980 an air emissions survey was conducted to assure that the volatile organic emissions from this process conform with 7:27-16 (Subchapter 16) of the New Jersey Administrative Code. Since the sulfadimethoxine vents conform with these most recent regulations (Subchapter 16), no permits are required for ROFENAID®-40 production.

Sulfadimethoxine air emissions for ROFENAID®-40 premix are summarized below:

Component	Emission (tons/year)
Toluene	0.03
Pyridine	0.08

A two percent increase in sulfadimethoxine production will be required to meet the anticipated demand for ROFENAID®-40 premix.

2. Ormetoprim Production, Nutley, New Jersey

Ormetoprim process equipment such as reactors, centrifuges, receivers and dryers operate under the following New Jersey Department of Environmental Protection Air Permits and Certificates:

Certificate No.	Issue Date	Certificate No.	Issue Date
43816	8/18/80	43830	8/18/80
43817	8/18/80	43831	8/18/80
43818	8/18/80	43832	8/18/80
43819	8/18/80	43833	8/18/80
43820	8/18/80	43834	8/18/80
43822	8/18/80	43835	8/18/80
43823	8/18/80	43836	8/18/80
43824	8/18/80	43837	8/18/80
43825	8/18/80	43838	8/18/80
43826	8/18/80	43839	8/18/80
43827	8/18/80	43840	8/18/80
43829	8/18/80	43841	8/18/80

Ormetoprim process air emissions for ROFENAID®-40 premix are as follows:

Component	Emission (tons/year)
Dimethylformamide	0.18
Methanol	0.92

A ten percent increase in current ormetoprim production will be required to meet the anticipated demand for ROFENAID®-40 premix. These permits would allow for the increased production of ormetoprim for ROFENAID®-40.

3. Dry Blending Operation, Fresno, California

ROFENAID®-40 will be prepared by dry blending sulfadimethoxine and ormetoprim with an inert carrier at the Fresno Premix Plant. Particulate emissions generated in the mixing operation are controlled by bag filters as regulated by California Air Resources Board Permit Number 104 0070 104, issued in 1978.

Waste Disposal

1. Sulfadimethoxine Production, Nutley, New Jersey

A summary of wastes generated during sulfadimethoxine production follows:

Component	Solid (tons/year) Increase Due to ROFENAID®-40	Discharge to Passaic Valley Sewage Commission Treatment Works (tons/year) Increase Due to ROFENAID®-40
Organics		0.60
Inorganics		5.42
Charcoal and Dicalite	0.53	

Solid Wastes - Recovered solid wastes are disposed of in an industrial landfill licensed by the New Jersey Department of Environmental Protection to accept these types of wastes.

2. Ormetoprim Production, Nutley, New Jersey

A summary of wastes generated during ormetoprim production follows:

Component	Liquids Disposal (tons/year) Increase Due to ROFENAID®-40	Discharge to Passaic Valley Sewage Commission Treatment Works (tons/year) Increase Due to ROFENAID®-40
Organics		5.88
Inorganics		3.74
Waste Solvents	7.71	

Liquid Wastes are bulked and used as a fuel blend by Northeast Solite, Saugerties, New York or other licensed hazardous waste disposal operations.

3. Dry Blending Operation, Fresno, California

Solid wastes generated in the blending process consist primarily of particulate matter filtered in baghouse operations. These wastes are sent to sanitary landfills licensed to accept industrial wastes. Any waste waters generated in equipment washups are directed to the local wastewater treatment plant.

4. Evaluate alternatives to the proposed action:

We know of no acceptable alternatives that will accomplish control of the animal diseases as described above. Attempts to utilize other preparations such as other antibacterials or immunizing agents that do not afford the same degree of efficacy can only result in greater environmental risks and greater losses in food production and lesser degrees of efficiency in such food production.

There are no feasible alternatives to the raw materials used in the manufacture of sulfadimethoxine, ormetoprim and ROFENAID®-40 premix, which would result in a lesser contribution to the environmental burden.

5. Describe the relationship between local short-term use of the environment with respect to the proposed action and the maintenance and enhancement of long-term productivity:

Short-term effects upon the environment are negligible as discussed in Sections 2 and 3. There is no cumulative adverse effect upon the environment since potential pollutants are added and dispersed at a low controlled rate as described in Section 2. Because of these factors, there will be no long-term detrimental effect upon the productivity of the environment.

Considerable overall benefits will accrue from the proposed use of ROFENAID®-40 in exchange for possible minimal local effects due to the manufacture and use of the product.

The use of ROFENAID®-40 for the prevention and treatment of disease will result in higher survival rates and lowered morbidity with the corresponding efficient use of the provided feedstuffs.

Increasing the efficiency of duck production means that more pounds of meat for human consumption will be produced per ton of feed and kilowatt-hour of energy. In the long run, this means feeding a larger number of people without increasing the environmental burden resulting from the production of feed, fertilizer and energy, and from the disposal of animal wastes.

6. Describe any irreversible and irretrievable commitment of resources that would be involved if the proposed action should be implemented:

A portion of the raw materials used in the manufacture of sulfadimethoxine and ormetoprim will be discharged ultimately into the ecosphere, as indicated in Sections 2 and 3. The organic portion of the waste products will be biodegraded and ultimately returned to the natural pool of carbon dioxide and ammonia. Due to the economics and thermodynamics of the processes involved, such chemical entities are irretrievable and, therefore, the original commitment of resources may be regarded as irreversible.

7. Discuss the objections raised by other agencies, organizations or individuals that are known to the applicant:

ROFENAID®-40 has been an approved and used product for poultry use for over 11 years in the United States without any apparent adverse effects upon the environment. No apparent adverse environmental effects have been noted during the treatment to date of over five million ducks.

8. If the proposed action should be taken prior to 90 days from the circulation of a draft environmental impact statement or 30 days from the filing of a final environmental impact statement, explain why:

The information presented herein obviates the requirements for an environmental impact statement, since the proposed action will result in no significant or cumulative adverse effects upon the environment.

9. Risk-benefit analysis:

Implementation of the proposed action with regard to the subject drug will be of significant value to the techniques of duck husbandry with the forseeable benefits outlined in Sections 2 and 5: A further foreseeable benefit will be an increase in the supply of duck meat and an increase in the wholesomeness of this product. An additional benefit is provided by the more efficient utilization of natural resources such as feed and energy in the production of duck meat for human consumption.

Further, the approval of this use of ROFENAID®-40 for disease control in ducks (diseases for which no known effective, alternative drug exists) will assist in maintaining the duck growing industry as a viable industry-permitting it to continue as an employer and to make contributions to our Gross National Product (GNP).

9. (cont'd.)

There is only minimal potential risk due to the introduction of ROFENAID®-40 into the environment through the duck droppings or from the emission of by-products during manufacture. Irretrievable depletion of natural resources due to the manufacture of ROFENAID®-40 is so small as to be meaningless in practical terms.

The benefit to the public of the use of the subject drug greatly outweighs any potential present or future risk to the environment.

E. Certification

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

31 June 83 (Date)

(Signature of responsible official)

Dept. Director (Title)

Animal Science Research

INTEROFFICE CORRESPONDENCE

ROCHE

TO Dr. A. MacDonald

DATE December 16, 1976

FROM J. Westheimer

SUBJECT Assay of Free and Total Sulfadimethoxine in Environmental Samples Derived from Rofenaid-Treated Ducks

Summary

A tissue residue method to determine sulfadimethoxine in swine and cattle feces and urine described earlier (IOM, Kaykaty and Gonzales to Fellig, Determination of Sulfadimethoxine in Swine and Cattle Urine and Feces) has been modified. Its use is thus extended to the analysis of environmental samples generated in the treatment of ducks with Rofenaid-medicated feed.

The modification was necessitated because assay of the materials under study in this work by the method cited above yielded high blanks and low recoveries with soil samples.

Experimental

The following materials are of primary interest in this work:

1) Feces from treated or untreated ducks
2) Soil-feces, 20:1 mixtures
3) Water-feces, 20:1 mixtures
4) Tap water
5) Loamy soil

Free SDM

The sample is extracted twice with acetone; the solvent is evaporated to a small volume. After addition of water, the drug is extracted with chloroform and subsequently partitioned into dilute aqueous ammonia. After acidification, the unconjugated sulfonamide is quantitated via the Bratton-Marshall Reaction as described earlier.

Total SDM (Includes Free, Acylate and Conjugated Forms)

After acetone extraction of the sample, hydrochloric acid is added to the solvent and the mixture heated for one hour. The residue is neutralized with dilute base and adjusted to pH 7 with phosphate buffer. The resulting solution is extracted with chloroform which in turn is back extracted with dilute aqueous ammonia. The sulfadimethoxine is then determined colorimetrically via the Bratton-Marshall Reaction.

Procedure

Free SDM

1) Place 5 ml or 5 g of well-mixed sample into a 50-ml glass-stoppered centrifuge tube, add 15 ml acetone and homogenize for one minute at slow speed with a Polytron homogenizer.
2) Centrifuge the contents and decant into another 50 ml centrifuge tube.
3) Repeat steps (1) and (2).
4) Evaporate the combined acetone extract to near dryness and quantitatively transfer the residual solvent to a 125 ml separatory funnel with several portions of water.
5) Extract the aqueous phase with two 50 ml portions of chloroform; then re-extract the combined solvent with 30 ml 1% (v/v) aqueous ammonia, containing 2.5% sodium chloride.
6) Quantitate the drug via the Bratton-Marshall Reaction as described earlier.(1)

Total SDM

1) Carry out steps (1) to (3) as described previously for the assay of free SDM.
2) To the combined solvent portions add 10 ml 0.5 N hydrochloric acid and heat for one hour in a heating block set to maintain the contents of the tube at 80 . .
3) Neutralize the remaining solution by first adding 10 ml of water, followed by 1 ml 1 N sodium hydroxide. Quantitatively transfer the solution to a 125 ml separatory funnel.
4) Adjust the pH to 7± with phosphate buffer prepared as described below, extract the solution with two 50-ml portions of chloroform, and continue as in step (5) under Free SDM, "then re-extract the combined solvent . . ."

The buffer was prepared by mixing 39.0 ml monobasic sodium phosphate and 61.0 ml dibasic sodium phosphate.

Results and Discussion

The efficacy of the modifications described in this report was established by first treating a series of 5-gm control feces samples via the Total SDM procedure. This resulted in values corresponding to 0.06 ppm SDM equivalent.

Blank values obtained with all other substrates were approximately 0.02-0.05 ppm SDM equivalent using the modifications outlined for both free and total SDM.

Recovery data from all substrates were obtained by following the method for free SDM described herein. This was deemed sufficient because once it was established that the modification for total SDM was equivalent to the earlier method cited above, the efficiency of acetone as an extracting solvent could adequately be demonstrated with the shorter procedure. In any case, conjugated sulfadimethoxine with which to fortify the samples was not available.

The recoveries obtained are listed in the following table.

Table I

Recovery of Sulfadimethoxine from Experimental Samples

Sample	Percent Recovery
Feces	83.1 ± 0.7%
Water:Feces, 20:1	90.6 ± 1.8%
Soil:Feces, 20:1	73.9 ± 1.1%
Water	93.9 ± 1.4%
Soil	79.8 ± 0.7%

J. Westheimer

JW:cb

Reference:
(1) Fellig, J., and Westheimer, J., J. Agricultural & Food Chemistry, 16, 738 (1968).

INTEROFFICE CORRESPONDENCE

ROCHE

TO Dr. A. MacDonald

DATE December 8, 1976

FROM J. Westheimer

SUBJECT Assay of Ormetoprim in Environmental Samples from Ducks Receiving Feed Medicated with Rofenaid Using Regulatory Assay

Introduction

The work described in this report was undertaken to determine the applicability of the AHRD method for ormetoprim (01.0-Determination of Ormetoprim in Animal Tissues) to environmental samples obtained from ducks. This was found to be the case except with aqueous samples where a minor modification was required.

Experimental:

Feces from untreated ducks and from animals maintained on 0.08% Rofenaid were obtained from Moriches Duck Farm, Moriches, L.I. The "control" fecal matter was used to prepare soil-feces and water-feces mixtures in a 20:1 ratio. The soil used in these studies was of the loamy type. The method was validated by analyzing the following substrates:

1. Feces
2. Water:feces suspension (20:1)
3. Soil: feces mixture (20:1)
4. Tap water
5. Loamy soil

Appropriate samples of each of the above materials were fortified at the 10 ppm level. Replicate assays were performed using the method cited above.

The modification on the introduction was made necessary by the substrates containing water. The modified procedure follows:

1. Place 5.0 ml of well-stirred aqueous fecal suspension into a 250 ml centrifuge bottle.

2. Add 2 ml of 20% (v/v) aqueous ammonium hydroxide solution, 10 ml methylene chloride and 90 ml ethyl acetate.

3. Homogenize for one minute with a Polytron homogenizer at medium speed.

4. Decant the entire contents of the bottle through Whatman #111V filter paper containing about 25 g anhydrous sodium sulfate into a 250 ml separatory funnel.

5. Rinse the centrifuge bottle with 50 ml ethyl acetate and repeat step 4.

6. Continue as described in the original procedure, starting at step 8: "Extract the combined organic phase twice" . . . .

Results and Discussion:

To check the performance and precision of the method, whenever possible six replicate samples of the substrates listed above, both dosed and undosed were assayed. Recovery data are listed in the following table.

Material	Recovery of Ormetoprim from Environmental Sample		
	Ormet. Conc.	Recovery %	Std. Deviation
Feces	1 ppm	72.5	± 3.0
Feces	10 ppm	82.1	± 3.6
Soil-Feces, 20:1	5 ppm	87.0	± 2.1
Water-Feces, 20:1	10 ppm	94.3	± 2.9
Tap Water	10 ppm	97.6	± 0.7
Loamy Soil	10 ppm	91.4	± 3.0

In the assay procedure, the recommended Whatman #2V filter paper was found to be inconveniently slow; thus, the switch to the faster #111 paper and sodium sulfate.

Blank values were found to be 0.03 ppm ormetoprim equivalent for soil-feces mixture and water, 0.01 ppm for water-feces, 0.08 ppm for soil and 0.09 ppm equivalent for feces.

J. Westheimer

JW:cb

Appendix B

United States Department of the Interior

FISH AND WILDLIFE SERVICE

IN REPLY REFER TO:

National Fishery Research Laboratory
P.O. Box 818
La Crosse, Wisconsin 54601

R 2/14

February 11, 1980

G. Maestrone, D.V.M.
Asst. Res. Group Chief
Animal Health Research Dept.
Hoffmann-LaRoche Inc.
Nutley, NJ 07110

Dear Dr. Maestrone:

Please find attached the data we have generated on R05 and its components. Methods for conducting the toxicity tests are standardized according to the Committee on Methods for Toxicity Tests with Aquatic Organisms, EPA-60/3-75-009. Most of the materials were so nontoxic that LC_{50}'s could not be determined, and those results are reported as the highest concentration exposure that produced no mortality (Table 1).

The large quantities of chemicals that were required in these tests altered the pH in test solutions (Table 2). Chemical buffers were added to the test water before fish were introduced to readjust the pH to around 7.6, the pH found in the control test.

I'm also attaching a copy of the test methodology for your use.

Sincerely yours,

Leif L. Marking
Chemist

LLM:ajh

Table 1. Toxicity of R05-0037 and its individual components to rainbow trout and channel catfish in soft, reconstituted water.

Species	Temp. (°C)	Chemical	Toxic unit	96-h LC_{50} and 95% confidence interval or highest exposure concentration producing no mortality
Rainbow trout	12	R05-0037	mg/l	400
		Sulfadimethoxine	mg/l	400
		Ormetoprim	mg/l	400
		Placebo for R05-0037	µl/l	20,000
Channel catfish	17	R05-0037	mg/l	600 378-952
		Sulfadimethoxine	mg/l	400
		Ormetoprim	mg/l	200 163-245
		Placebo for R05-0037	µl/l	20,000

[a]All concentrations are based on the active ingredient.

Table 2. Effect of R05-0037 on the pH of test solutions.

Concentration (mg/l)	pH of test solution
Control	7.63
25	8.40
50	8.54
100	8.81
200	9.00
400	9.08
600	9.11