ENVIRONMENTAL IMPACT ANALYSIS REPORT

Romet(R)-30 (Sulfadimethoxine + Ormetoprim) Medicated Premix for Control of Certain Salmonid Diseases

A. Date - February 22, 1984

B. Name of Applicant - Hoffmann-La Roche Inc.

C. Address - Nutley, New Jersey 07110

D. Environmental Information

SUMMARY

The applicant has filed an original submission to New Animal Drug Application 125-933 providing for the use of Romet(R)-30 premix for the manufacture of medicated feeds for the prevention and therapy of certain infectious salmonid diseases. The continuous use of the feed medicated with Romet(R)-30 for salmonids at a level of 50 mg/kg is restricted to a maximum of five days time. The salmonids species are considered by FDA to be a minor meat producing species.

1. Describe the proposed action

This application provides for the use of Romet(R)-30 premix for the manufacture of medicated fish feeds for the therapy of bacterial infectious diseases of salmonids caused by Aeromonas salmonicida (furunculosis) and by Yersinia ruckeria (enteric red mouth). The continuous use of the medicated feed is restricted to a maximum of 5 days. The medication dose is 50 mg/kg of body weight per day for the maximum 5 day treatment period. The salmonids species are considered by FDA to be minor meat producing species.

The environment can potentially be affected by this action in the following ways.

(a) through the excretion of Romet(R)-30 components (sulfadimethoxine and ormetoprim) by the treated fish

(b) through the unavoidable but controlled discharge of some pollutants into the ecosphere during the manufacture of Romet(R)-30

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

The present application provides for the use of Romet(R)-30 as an additive for salmonids feeds which is medicated so as to supply 50 mg of drug activity per kilo of body weight of the fish. The active medication in Romet(R)-30 is a 5:1 mixture of sulfadimethoxine (5 parts) and the potentiator ormetoprim (1 part). The two same active ingredients, sulfadimethoxine and its potentiator ormetoprim, are present in a related medicated premix product, Rofenaid(R)-40.

Rofenaid(R)-40, a 40% premix comprised of 25% sulfadimethoxine and 15% oremtoprim, is presently used in poultry feeds as an approved drug at a concentration of 0.02% 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 (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.

A supplemental application to NADA 40-209 provides for use of Rofenaid(R)-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.

The animal efficacy to include in vitro activity, in vivo aquariam and raceway trials as well as field studies under commerical conditions is summarized in the F.O.I. statement.

Romet(R)-30 is a broad spectrum antibacterial premix containing sulfadimethoxine and ormetoprim used in the preparation of medicated feeds. Each of these drugs exhibits antibacterial efficacy alone. However, when they are combined in a pound of premix at a ratio of 113.5 g (25%) of sulfadimethoxine and 22.7 g (5%) of ormetoprim, a greater and broader degree of efficacy at a lower dosage is observed.

D. 2. (cont'd.)

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.

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 use of sulfadimethoxine and ormetoprim at the selected ratio of 5:1 in Romet(R)-30 yields 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 -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 synthesis 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 aicd, 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.

D. 2. (cont'd.)

The salmonids raising operation is a highly sophisticated and limited industry, restricted to those areas where the geographic and environmental conditions (water availability and temperature) facilitate rearing and management for commercial purposes. Additionally, salmonids are raised in suitable geographic areas by federal and state agencies for restocking purposes.

The geographic distribution of the use of Romet(R)-30 for salmonids would be mainly limited to the North Western part of the U.S., the location of commercial salmonid raising. The raising of salmonids for restocking purposes could result in a small amount of drug use in the hatcheries in the various states.

Since only a limited amount of trout are produced each year, approximately 20,000 tons per year, the maximum drug use would be limited. On a worst case assumption, 50% of the trout would require treatment when all the trout are full grown, (this would involve the annual total use of 2250 kg of Romet(R)-30. As noted earlier Romet(R)-30 is a 5:1 combination of sulfadimethoxine:ormetoprim which are the active ingredients in a 5:3 ratio for Rofenaid(R)-40 which is approved for use in poultry.

In this connection, it may be of interest to compare the salmonids use value to the relative size of the three segments of the poultry industry and their consumption of Rofenaid(R)-40. 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(R)-40 in 1982 was 72,730 kg of the 40% premix representing 29,092 kg of drug substance. The turkey industry used 14,387 kg of Rofenaid(R)-40 (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(R)-40 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(R)-40 drug substance (or 2% of this total) to treat 450,000 of the 12 million ducks raised in 1982.

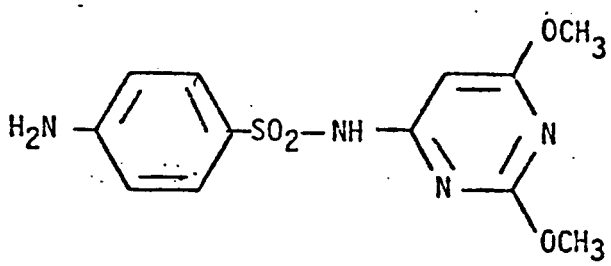
Thus, it is clear that the salmonids industry, while important by itself as a source of meat consumed by humans, is relatively insignificant as related to the rest of the poultry industry which represents the current major use of Rofenaid(R)-40 medicated feed.

D. 2. (cont'd.)

Romet(R)-30 contains a combination of five parts sulfadimethoxine and one part ormetoprim (5:1 ratio). The chemical data for sulfadimethoxine and its potentiator is as follows:

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:



Its solubility in various solvents and systems is:

(g/100 ml at 25°C)

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	g/ml
pH 8.6	0.1	g/ml
pH 8.1	0.05	g/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 = 707.

Sulfadimethoxine is stable in water.

D. 2. (cont'd.)

Sulfadimethoxine is known to undergo three principal color reactions:

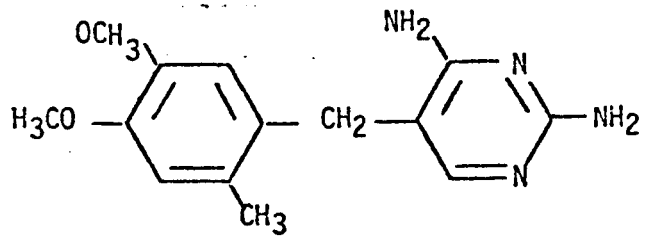
1. Bratton-Marshall reaction
2. With ferricyanide in aqueous potassium hydroxide, a reddish-brown color is produced
3. With cupric sulfate in aqueous sodium hydroxide, a yellow precipitate is produced

No degradation of sulfadimethoxine could be detected when a 1 mg percent solution in 0.01N NaOH of sulfadimethoxine 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 other medicated premixes, such as Rofenaid(R)-40 and its outstanding stability on extended storage in animal feeds, as well as during commercial pelleting operations (see NADA 40-209V for specific details.

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:



D. 2. (cont'd.)

Its solubility in various solvents is:

(gms/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 $E^{1\%}$ of 274.
Ormetoprim is stable in water.

Ormetoprim 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 of ormetoprim could be detected when a one 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 very stable in medicated premixes such as Rofenaid-40 as well as medicated animal feeds, even on extended storage and in commercial pelleting of medicated feed. (see NADA 40-209V for specific details)

D. 2. (cont'd.)

The toxicity of ROFENAID(R)-40 as a combination and each of its components has been evaluated using the array of animal models listed below and is the basis for the 5:1 combination in Romet(R)-30:

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

3. Acute oral toxicity in mice (single oral dose via suspension in 5% gum acacia w/a 72-hour 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

D. 2. (cont'd.)

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 groups 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.

Mutagenicity Testing

8. The mutagenicity of Romet(R)-30 and its components, sulfadimethoxine and ormetoprim, were evaluated under the sponsorship of the U.S. Fish and Wildlife Service, National Fishery Research Laboratory, P.O. Box 818, LaCrosse, WI 54601, by the EG&G Mason Research Institute, 1530 East Jefferson St., Rockville, MD 20852.

The evaluation of Romet(R)-30 and its two components, sulfadimethoxine and ormetoprim, in the Salmonella/mammalian-microsome plate incorporation mutagenicity assays indicate that all three compounds do not cause a significant increase in the number of revertants per plate of any of the tester strains neither with nor without metabolic activation by rat liver microsomes.

Safety for Ducks

9. The safety of SDM + OMP (5+3) to ducks has been evaluated and reported to FDA in NADA 40-209V. Sulfadimethoxine plus ormetoprim (5+3) has been fed at 0.04% or 0.08% to over 1.8 million ducks under commercial growing conditions for five days, the period 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.

D. 2. (cont'd.)

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 or 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.

The regulatory procedure for sulfadimethoxine has been compared using both gas and liquid chromatography to validate the colorimetric readout using five tissues (liver, muscle, skin, kidney and intestinal fat) from five species (duck, chicken, beef, swine and turkey) with all results found to be equivalent. This study was reported to NADA 40-209V on February 9, 1977.

A tissue depletion study of Romet(R)-30 in rainbow trout was conducted by Dr. G. Bullock at the National Fish Health Research Laboratory at Leetown, W. VA and submitted to INAD 2208 on August 23, 1978. A concrete raceway study was used with an average fish weight of 131 g and a water temperature of 10°C (50°F). The fish were dosed in their feed pellets at a rate of 50 mg Romet(R)-30/kg of fish/day for a total of five consecutive days. Fish were sampled after 3- and 5-days of treatment and at 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18 and 20 weeks after last dose.

A fillet of muscle with adhering skin/scales was taken from five fish at each sampling period. The fillets were assayed as a total sample (muscle, skin, scales) and as discreet muscle, skin and scales aliquots. A total calculated value for the whole fillet was calculated using the muscle, skin, scales assay values, and the proportion of each by weight: muscle - 88.1%, skin - 8.7% and scales - 3.2%.

The regulatory methods described were used for both sulfadimethoxine and ormetoprim for all four sample types with the method validation recovery values determined as part of the study. Sensitivity of the methods was 0.05 ppm.

The data is summarized in table I, page 12, and supports a six week withdrawal time following a five day treatment of Romet(R)-30 at 50 mg/kg of fish.

Table I

Roret(R)-30 Tissue Clearance in Trout

Ormetoprim Concentration (ppm)

	Feeding Period (Days)		Post-Treatment [Week(s)]								
	3	5	1	2	3	4	5	6	8	10	13
Fish - Total Assay Value	1.61	2.41	0.50	0.32	0.26	0.19	0.16	ND	ND	ND	ND
Skin	5.99	15.61	2.89	2.02	1.91	1.15	0.72	0.61	0.54	0.31	0.20
Scales	2.23	1.41	1.37	0.94	0.92	0.96	0.88	0.56	0.56	0.34	0.11
Muscle	0.53	1.70	0.10	ND	ND	ND	ND	ND	ND	ND	ND
Fish - Total Calc. Value	1.06	2.90	0.38	0.21	0.20	0.13	0.09	0.07	0.06	0.04	0.02

Sulfadimethoxine Concentration (ppm)

Fish - Total Assay Value	5.77	11.70	ND				
Skin/Scales	7.50	17.25	ND	ND	ND	ND	ND
Muscle	5.50	10.67	ND	ND	ND	ND	ND
Fish - Total Calc. Value	5.74	11.45	ND				

ND = 0.05 ppm

Muscle - 88.1%
Skin - 8.7%
Scales - 3.2%

By Weight

The following 27 pages are reproduced in tact from the EIAR submitted to NADA 40-209 for Rofenaid(R)-40 in ducks dated February 3, 1983. The pages from the Rofenaid(R)-40 in ducks EIAR are 9 through 36. The areas covered in these pages are:

- the environmental stability of SDM and OMP under a variety of conditions,
- the effect of SDM and OMP on plant growth and the aquatic toxicity of SDM, OMP, Rofenaid and Romet-30 (Ro 5-0037).

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.

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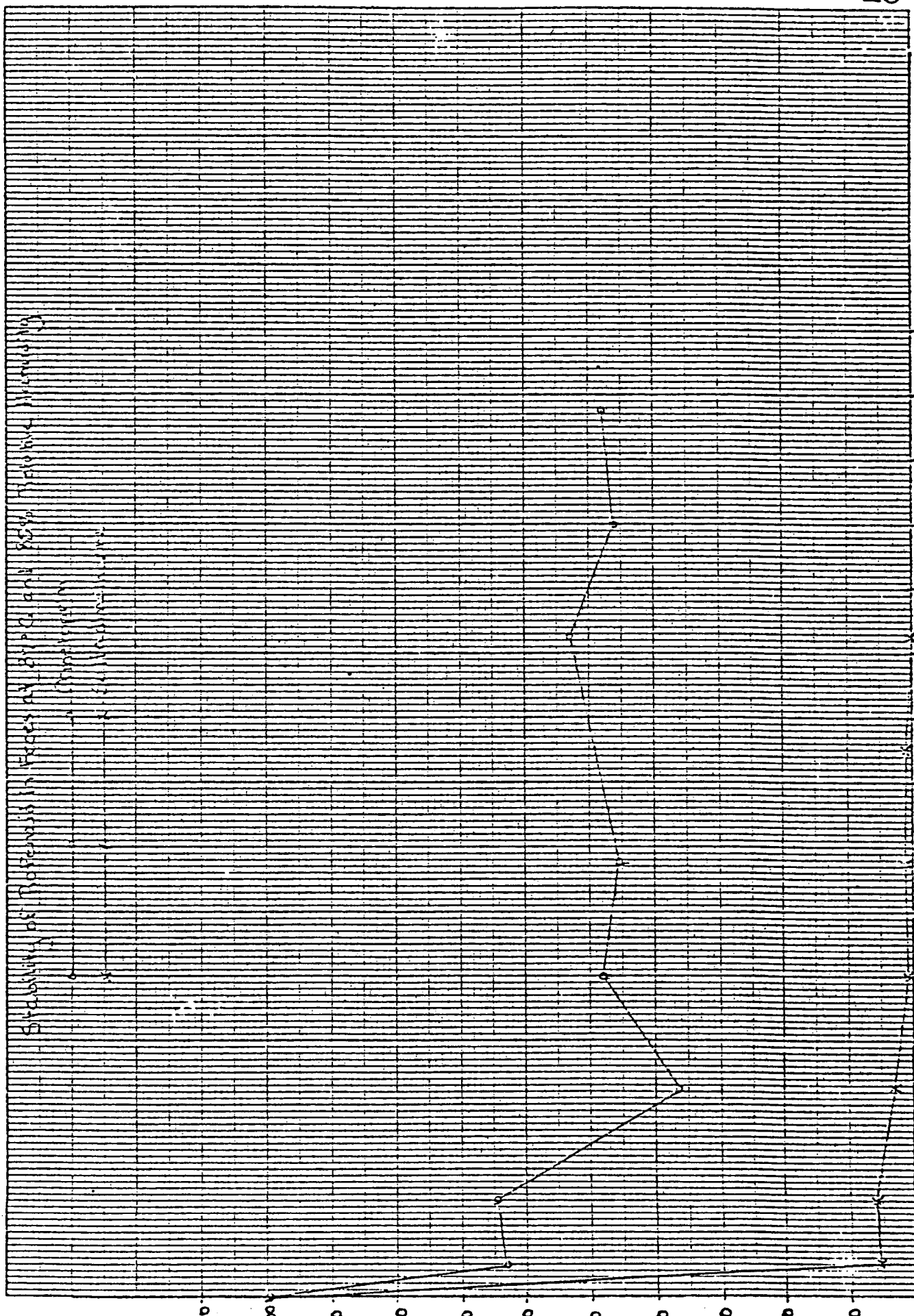
DIETZGEN CORPORATION
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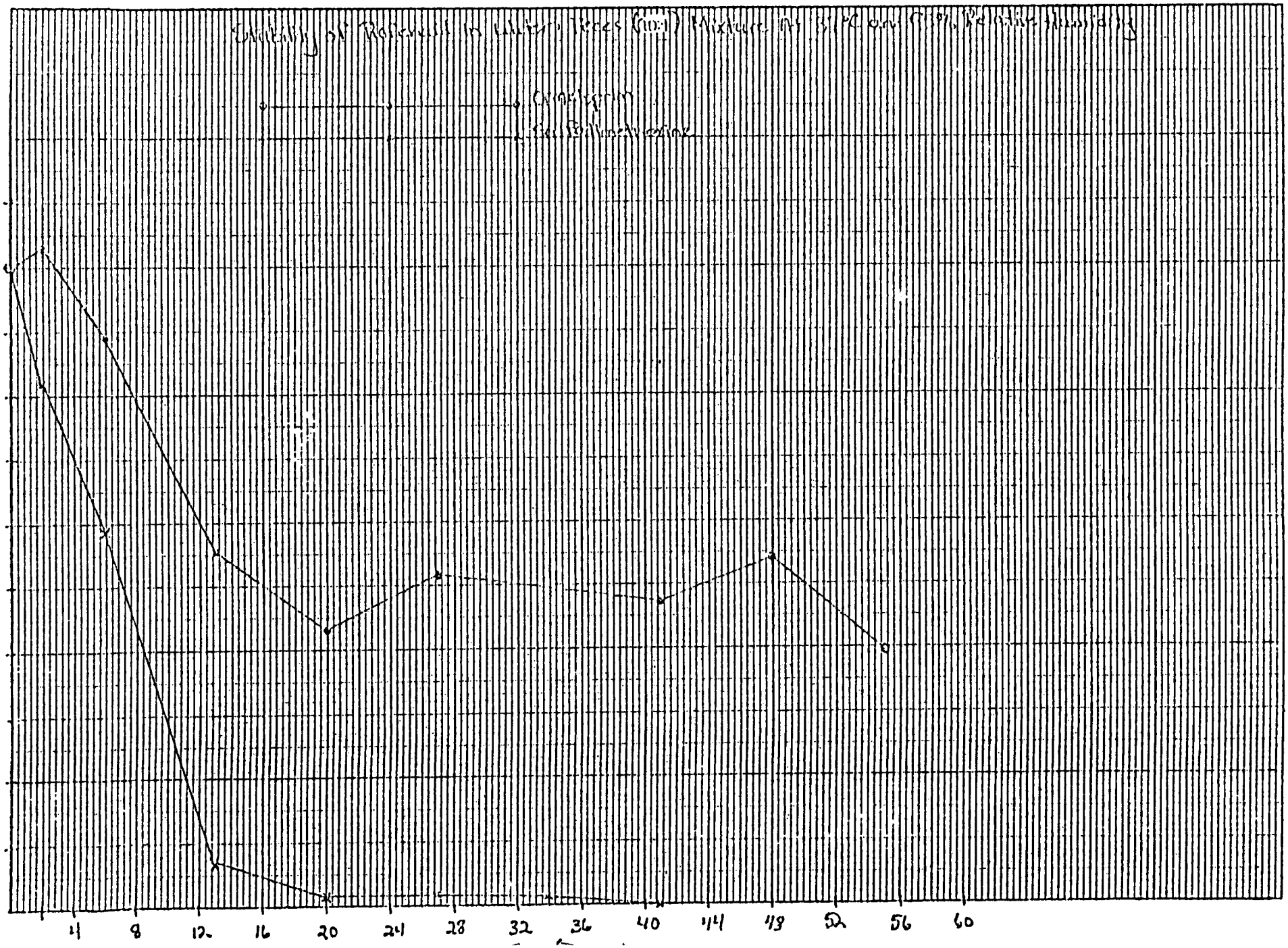
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This 5-inch quantity of soil was adequate in the case of sulfadimethoxine to adsorb anywhere from 125-475 mg, depending upon the type of soil. The material is definitely adsorbed and not degraded and can be extracted from the soil's surface by a pH 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 0.03% ROFENAID®-40 in the feed for at least two weeks.

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August 16, 1973

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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 I (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-Sanith 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	45%	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.

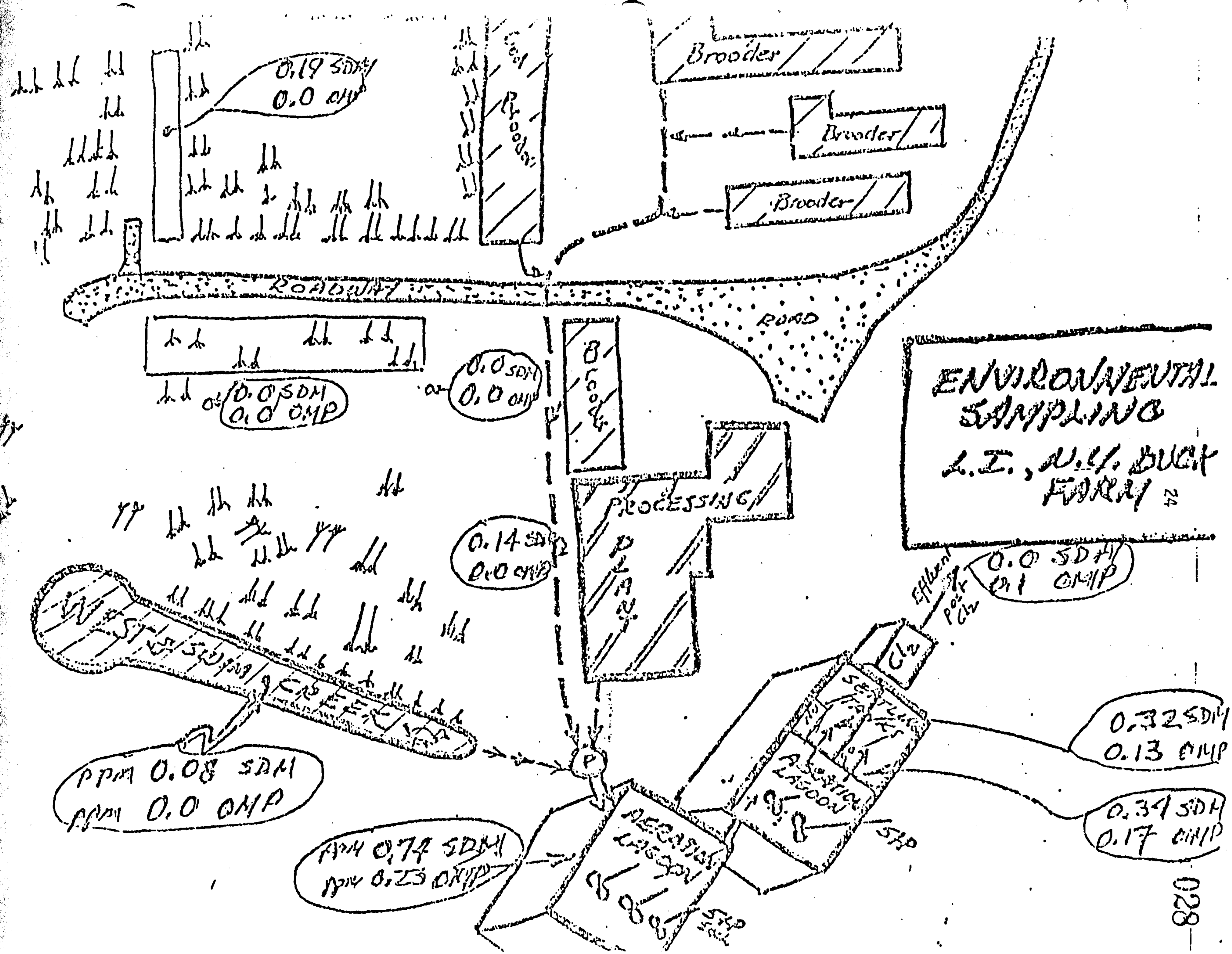
Sulfadimethoxine	0.0 ppm
Ormetoprim	0.0 "

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.


ENVIRONMENTAL SAMPLING
L.I., N.Y. DUCK FARM
24
Brooder
Brooder
Brooder
Brooder
ROADWAY
ROAD
PROCESSING PLANT
0.19 SDM
0.0 OMP
0.0 SDM
0.0 OMP
0.0 SDM
0.0 OMP
0.14 SDM
0.0 OMP
0.0 SDM
0.1 OMP
Effluent
Cl2
SETTLING TANKS
AERATION LAGOON
RECEIVING LAGOON
0.32 SDM
0.13 OMP
0.34 SDM
0.17 OMP
PPM 0.74 SDM
PPM 0.25 OMP
PPM 0.08 SDM
PPM 0.0 OMP
P
SAP

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.
* $P < 0.05$ ** $P < 0.01$

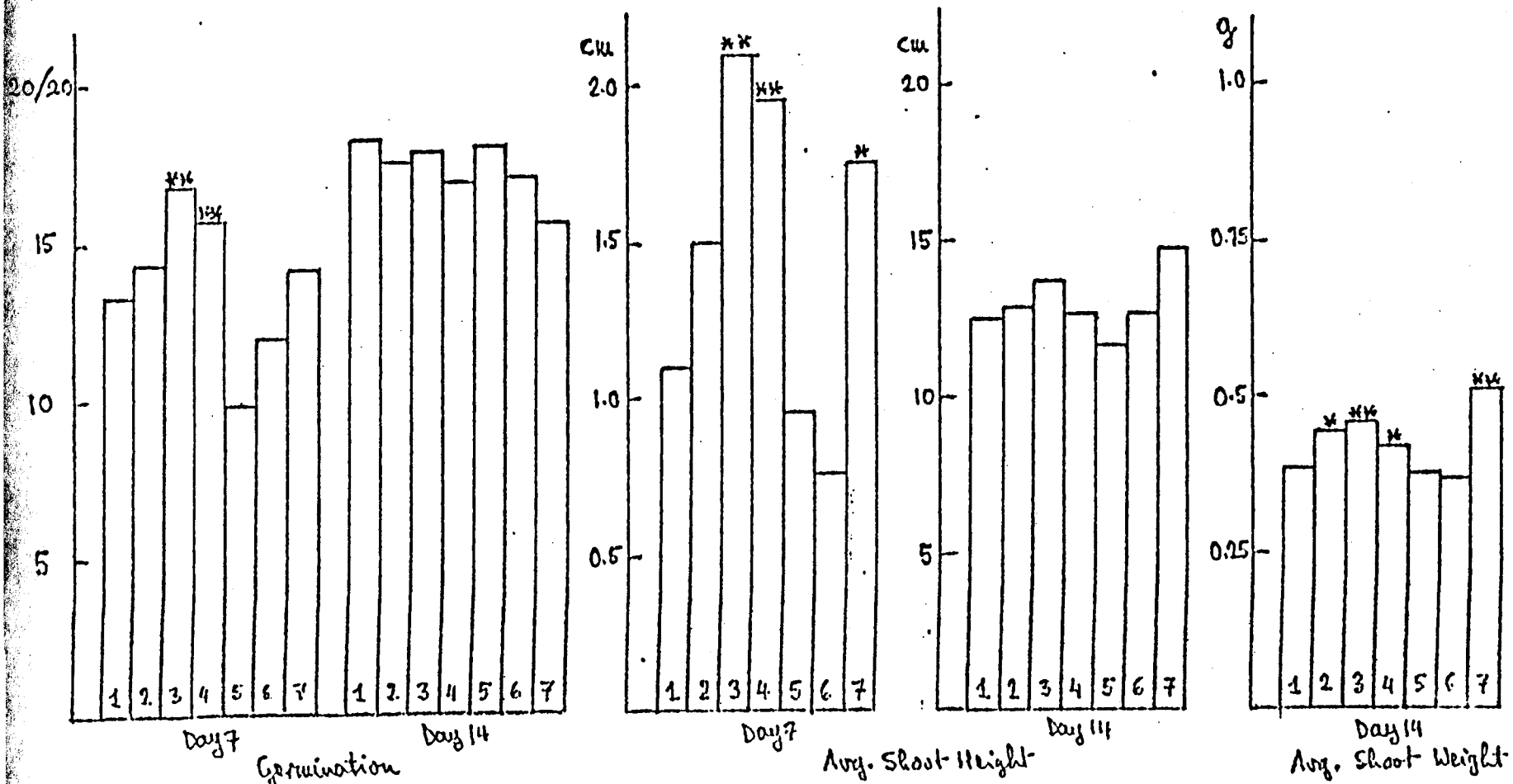


Figure 2 Cucumber

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

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

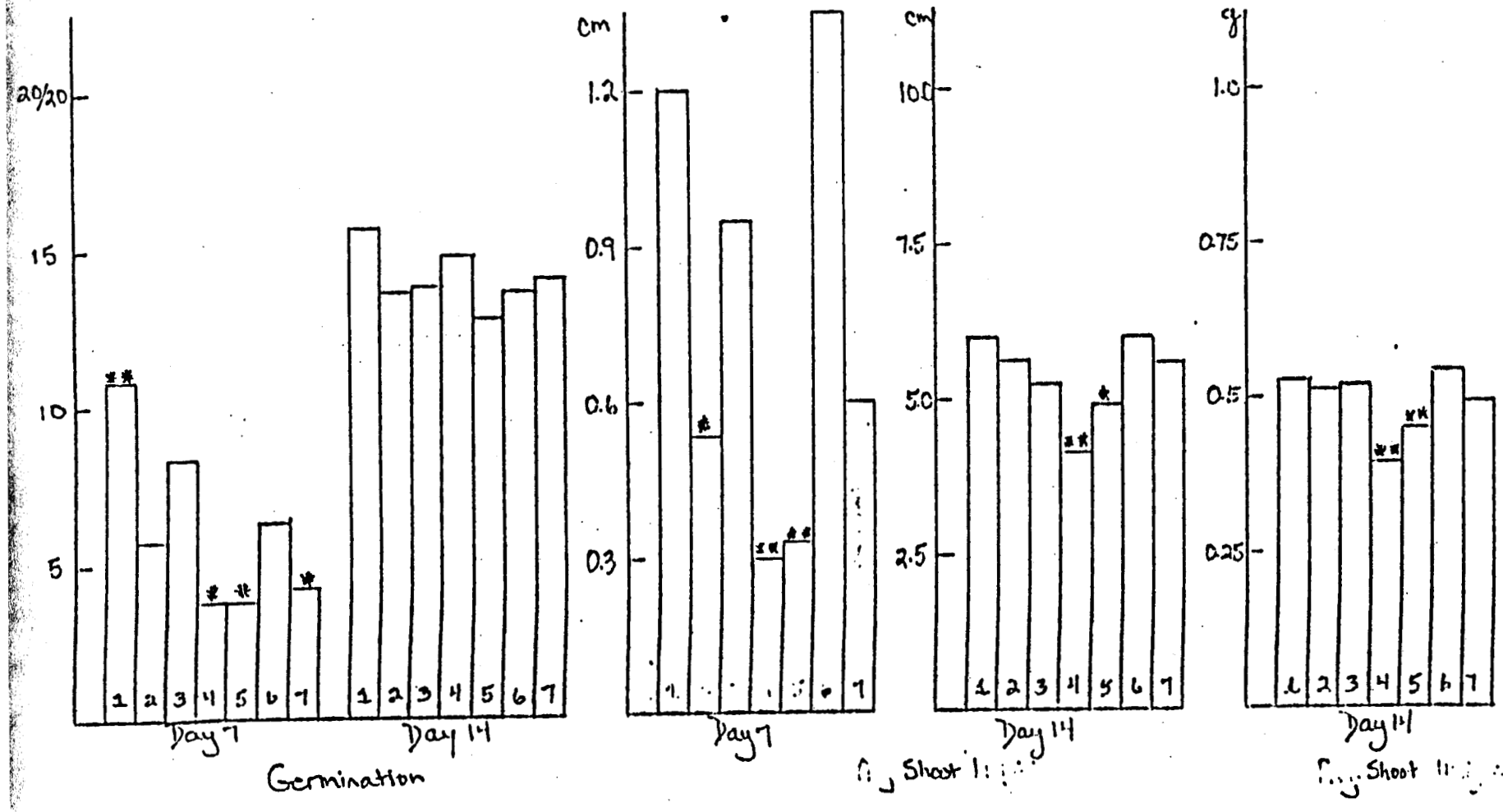
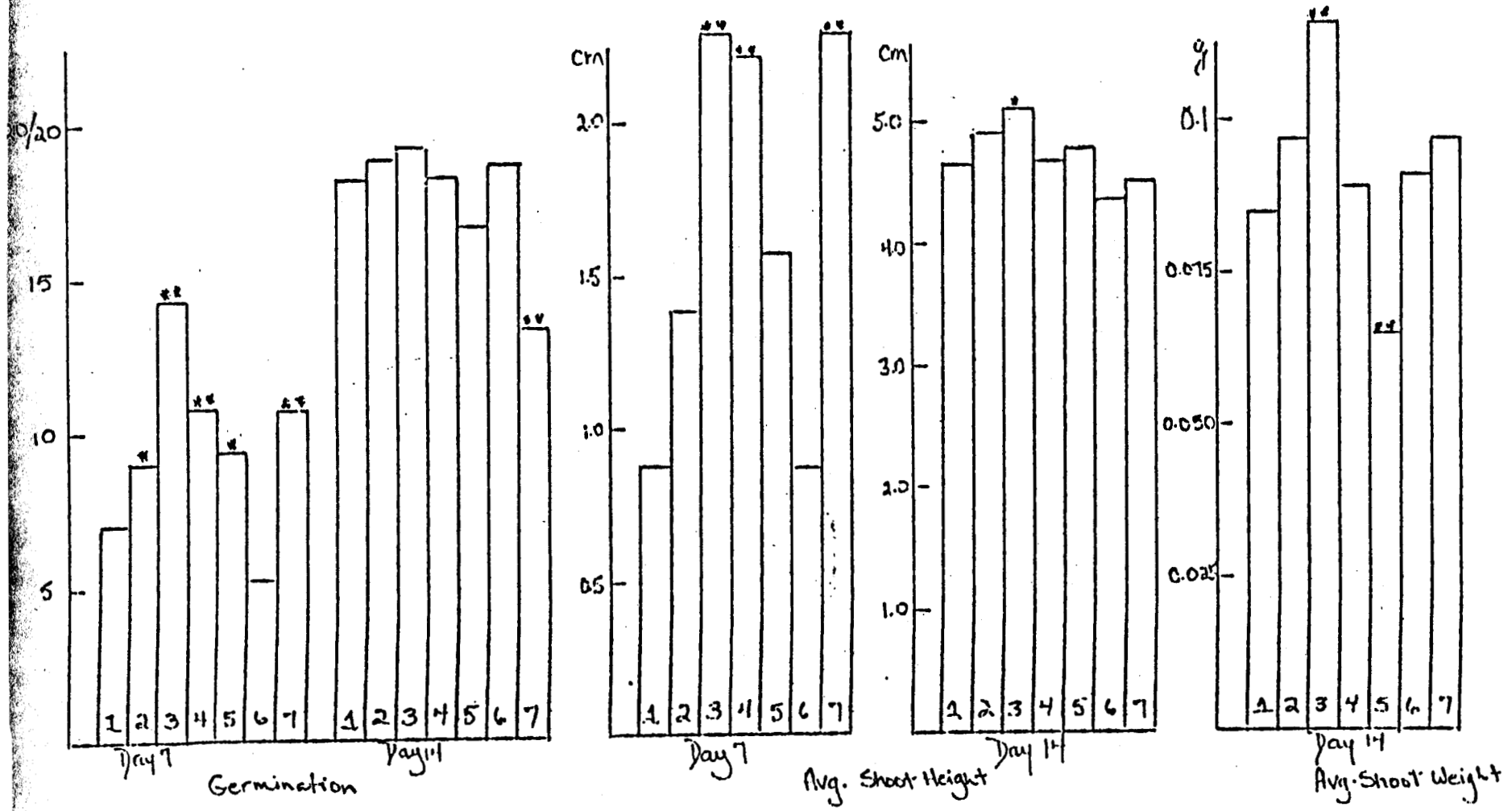


Figure 3 Soybean

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

Figure 4 Tomato

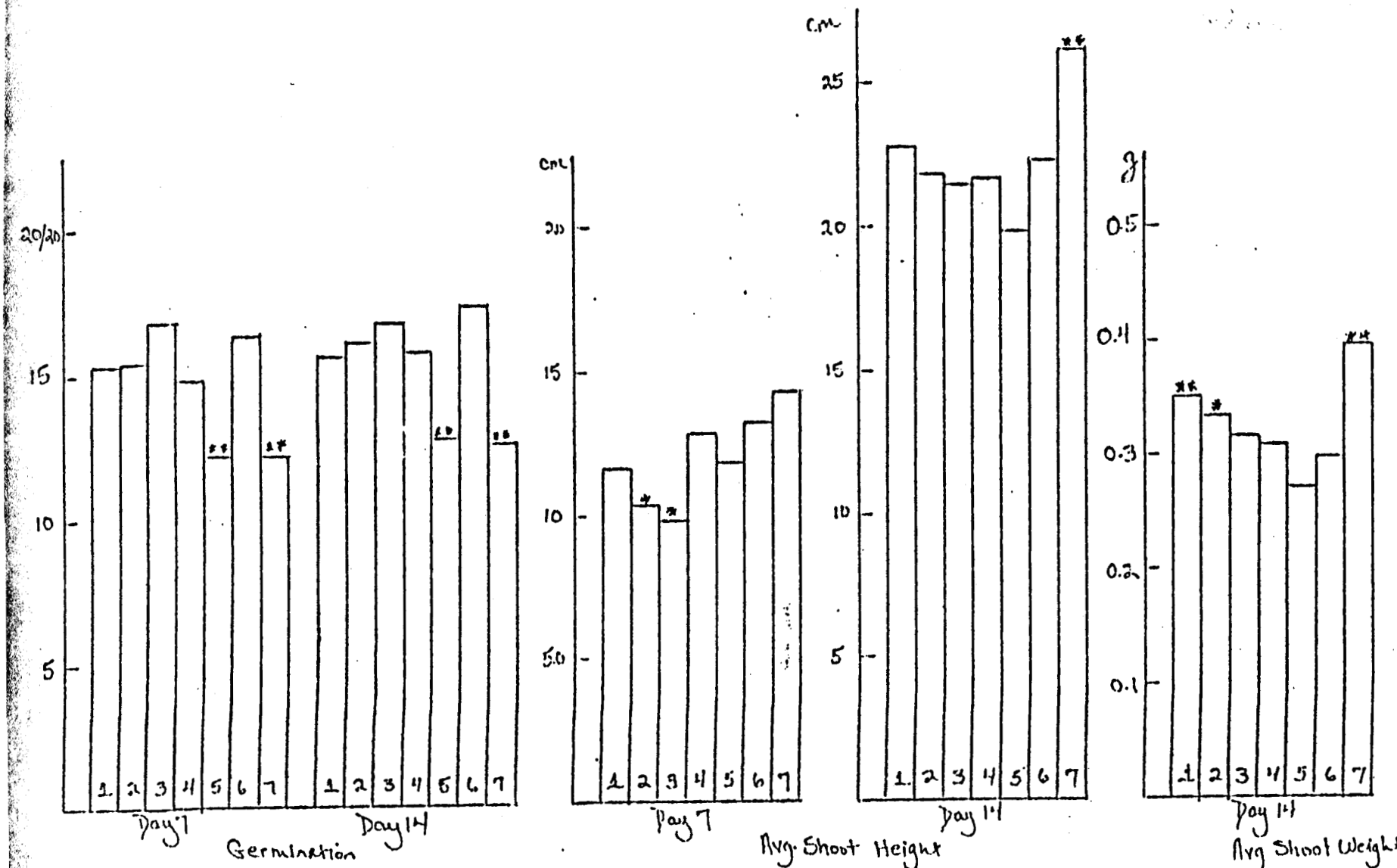
Significance of comparison to untreated control
* $P < 0.05$ ** $P < 0.01$



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

Figure 5 Barley

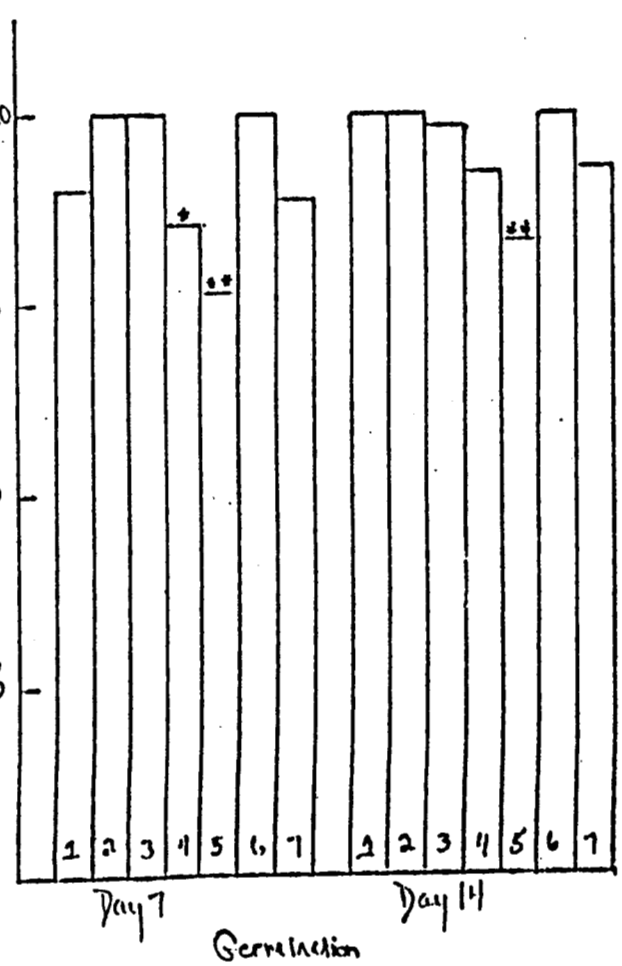
Significance of comparison to untreated control
* $P < 0.05$ ** $P < 0.01$

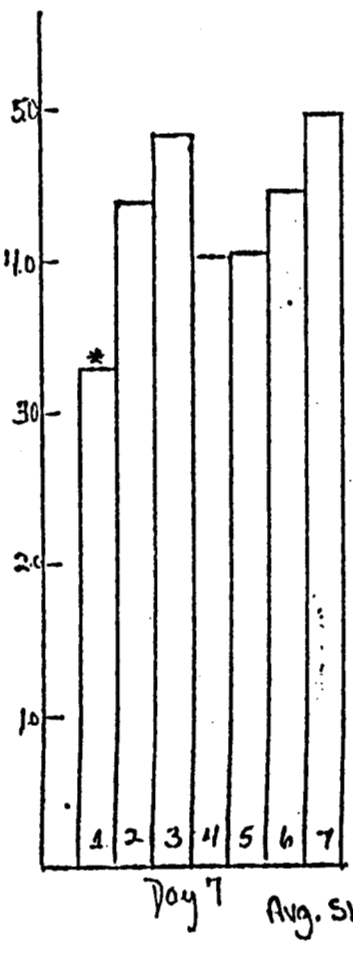


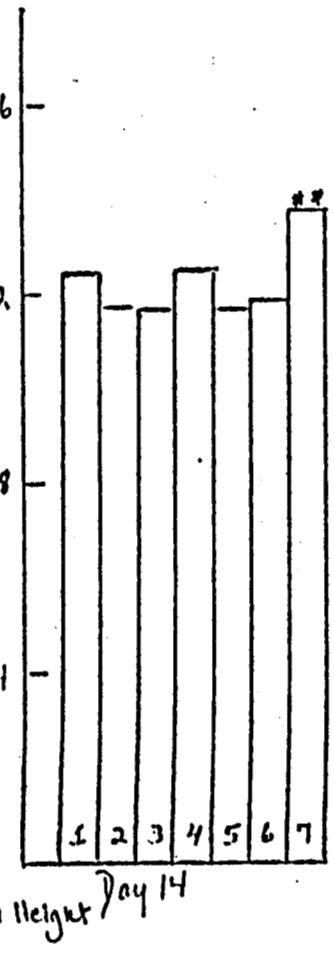
1. SDM 1ppm
2. SDM 5ppm
3. OMP 1ppm
4. OMP 5ppm
5. SDM 5ppm + OMP 5ppm
6. Untreated Control
7. Treated Control

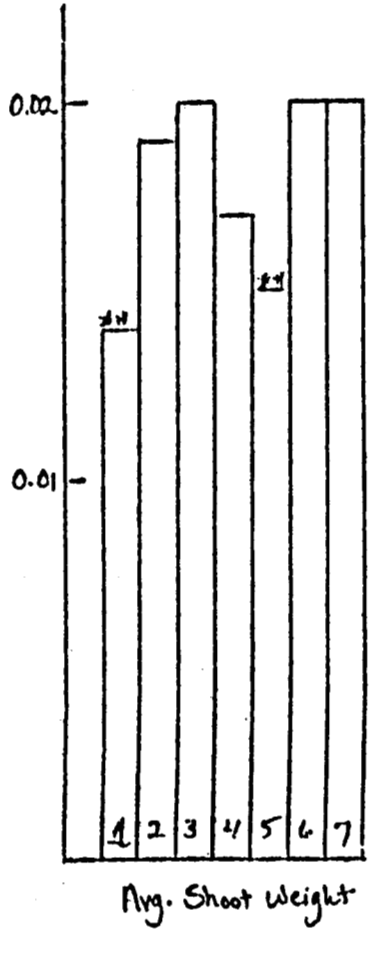
Figure 6 Ryegrass

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 on 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.

TOXICITY STUDIES - Ro 5-0037 (Romet(R)-30)

Reproduction, fertility, conditions of the offspring for salmon, percentage of egg hatch, survival of all stages were not different in the Ro 5-0037 injected fish than in the non-medicated salmon as reported to NADA 125-933 on 4/8/83.

These data are obtained from a preliminary evaluation of the influence of Ro 5-0037 administration to Atlantic salmon brood stock, by the parenteral route. It was initiated in 1979 at the Craig Brook, National Fish Hatchery, East Orland, Maine under the supervision of Dr. G. Bullock and Mr. M. Hendrix, Hatchery Manager.

The following studies were conducted to evaluate the safety of Ro 5-0037.

1. Effect of the direct contact of Ro 5-0037 5% solution on salmon egg incubation
2. Effect of prespawning injections of Ro 5-0037 on salmon egg incubation
3. Incidence of mean percent eggs obtained from brood stock in 1979 through 1981.
4. Mean eggs per pound of spawning weight.
5. Survival rate from hatching to one year of age at the Craig Brook National Fish Hatchery.

The hatchery manager conducting these studies reported:

"Did not see any significant difference in egg survivability (eye-up) in eggs from injected and non-injected parents and feel comfortable with that information, but we did not keep test and control groups separate among the resulting fry, parr, and smolts and cannot make definite statements about those life stages. What we have said is that there was no apparent harm from Ro 5-0037 during those life stages because our survivability was as good or better than what we have seen in the past when no Ro 5-0037 was used. We keep daily records on all lots of fish from the time they are feeding fry until they are released as smolts, and it is from these records that we make comparison." . . .

"We did keep elaborate records on egg survivability for our own information and have provided that data but, since we were engaged in no formal experimentation at other life stages, no other raw data are available."

TOXICITY STUDIES - Ro 5-0037 (cont'd.)

The results from these studies are as follows establishing the safety of Ro 5-0037 to the target species.

1. Effect of Direct Contact of Ro 5-0037 on Atlantic Salmon Egg Incubation Conducted in Fall 1979 at Craig Brook National Fish Hatchery

The purpose of this experiment was to see if Ro 5-0037 at the 5% concentration, as used for injecting the Atlantic salmon brood stock, applied directly to eggs was lethal. If it has been, a decision had to be made as to whether or not to inject late arriving fish. The egg sacs are quite large in late run fish (protruding well below the injection site) and there was concern about injecting Ro 5-0037 directly onto the ovareis.

Eggs from one female Atlantic salmon were divided into three lots. One lot was bathed in 5% Ro 5-0037 solution immediately before fertilization, one lot was bathed in 5% Ro 5-0037 solution immediately after fertilization, and one lot was bathed in water-the normal procedure-to serve as a control.

Five milliliters of both Ro 5-0037 5% solution and water were used for the bath, since 5 ml is the amound usually required for injecting each brood fish. The eggs were then disinfected in Wescodyne, enumerated by displacement, and mortality was picked weekly. According to the hatchery manager, the "direct contact" experiment is more valid than other tests, because the same female could be used as the source of eggs.

Results

	Green Eggs	Eyed Eggs	% Eyed
Ro 5-0037 before Fertilization	2393	2359	98.6
Ro 5-0037 after Fertilization	2175	2152	98.9
Control (Water)	2683	2664	99.3

Since the test showed no deleterious effect when Ro 5-0037 was added directly to the eggs, it was concluded that injections onto the ovaries of late run fish should not be deterimental.

TOXICITY STUDIES - Ro 5-0037 (cont'd.)

2. Effect of Prespawning Injection of Ro 5-0037 on Atlantic Salmon Egg Incubation Conducted in Fall 1979 at Craig Brook National Fish Hatchery

All fish had received 50 mg/kg Ro 5-0037 by injection upon mid-summer arrival at the hatchery. Approximately two weeks before spawning (on October 15), test fish received a second injection of Ro 5-0037. Fish were then spawned in the normal manner and the eggs were held separately. The eggs were disinfected in Wescodyne, enumerated by displacement, and mortality was picked weekly. Egg mortality records were maintained through shocking.

Results

	Fish	Green Eggs	Eyed Eggs	% Eyed
Late Injection	1	4034	1298	32.2*
	2	6006	5733	95.5
No Late Injection	3	4278	3720	87.0
	4	6120	5250	85.8
	5	7041	6801	96.6

*Probably a bad female rather than any problem with Ro 5-0037.

It is evident from this table that the incidence of eyed eggs obtained from brood stock treated with one or two doses of Ro 5-0037 was very similar, except for those obtained from fish #1. As the hatchery manager commented, the lower incidence of eyed eggs was probably due to a bad female, rather than to Ro 5-0037.

According to the hatchery manager, "The problem with arriving at anything conclusive when comparing prespawning injections of incoming adults with non-injected controls is that there is so much variation in egg survivability between individual females regardless of whether you do anything to them or not. Therefore, a large number of fish is required to neutralize the effects of such things as a bad female (the one shown in the experiment that had only a 32.2% eye-up is an example)."

TOXICITY STUDIES - RO 5-0037 (cont'd.)

3. Incidence of Eyed Eggs Obtained from Brock Stock Injected or Not Injected with Ro 5-0037

The data obtained in a three year study involving 239 fish are reported in the following table:

		Mean % Eyed	
Year	N	X	Arcsin % Transformation 95% Confidence Interval
Ro 5-0037 Injected			
1979	11	79.6%	69.3-88.2%
1980	142	91.0%	89.6-92.4%
1981	69	87.4%	83.4-90.8%
Ro 5-0037 Not Injected			
1979	4	88.0%	85.2-90.5%
1980	13	92.7%	89.8-95.3%

It is evidenced from the above data that the mean % eyed eggs incidence was very similar independently from the prespawning injection of Ro 5-0037.

Mean Eggs/Lb Spawning Weight

Data collected in 1980 on the number of eggs produced by 149 fish (136 injected with Ro 5-0037 and 13 not injected) are presented in the following table:

	N	X	SE	95% Confidence Interval
Ro 5-0037 Injected	136	825.5	11.1	803.7-847.4
Ro 5-0037 Not Injected	13	852.8	32.1	782.9-922.7

It is evidenced from the above data that the number of eggs/lb spawning weight was very similar in both experimental groups.

TOXICITY STUDIES - RO 5-0037 (cont'd.)

4. Atlantic Salmon Survival Rate from Hatching Up to One Year of Age

Data obtained on an initial population of 653,147 Atlantic salmon hatched in 1979 through 1981 at the Craig Brook National Fish Hatchery and followed up to at least one year of age, are reported in the following table:

Craig Brook NFH Production

Brood Year	Fry Alive at Initial Feeding	Stocked to Raceways No. (%)	Survival at One Year No. (% Stocking)	Survival at 2/01/83 No. (% Stocking)
1979[1]	189,341	134,700 (71.1)	111,000 (82.4)[3]	-
1980[2]	212,659	177,465 (83.5)	164,822 (92.9)	158,075 (89.1)
1981[2]	241,147	201,092 (83.3)		186,000 (92.5)[4]

[1]Fry derived from Ro 5-0037 and non-Ro 5-0037 injected parents (mixed).

[2]Fry derived from Ro 5-0037 injected parents.

[3]All fish stocked at end of first year, contracted furunculosis.

[4]Fish less than one year old at this reporting date (2/1983).

Romet(R)-30 will be used to control septicemic diseases of fishes by coating pelleted food with this drug or by including the drug into the mash before pelletization. Even though fish will consume the medicated food, and the drug is not readily water soluble, a small percentage of Romet(R)-30 will be released into the water mainly from fecal material of treated fish. Because fish are not raised under uniform conditions, certain standards must be applied to reach a realistic figure of the amount of drug which may be found in water. In determining this figure, the following assumptions will be used:

Standard Raceway

Although larger or smaller ponds or raceways may be used for fish culture, a raceway which is 60' x 6' with water 1.5' deep, is most often used. With these dimensions, the standard raceway contains 540 cu. feet of water.

Density of Fish

This represents the amount of fish which are held in a standard raceway. Most fish culturists do not exceed a density of 0.5. This figure means that fish are cultured at a rate of one-half their length as expressed in lbs/cu feet. Thus, if 6-inch fish are to be placed in a raceway there would be 3 lbs/cu feet of water or 3x540 cu ft = 1620 lbs fish/raceway.

Flow Index

This reflects the relationship of pounds of fish per gallon per minute (gpm) water flow to fish size. This value varies with temperature and altitude (see table in attached material).

Water Flow (I)

This is determined using flow index, weight of fish, and length of fish:

$$I = \frac{\text{Weight of Fish (lb)}}{\text{Flow Index (gpm) x Length of Fish (in)}}$$

With the above information, estimates of Romet(R)-30 concentration in water containing fish can be made.

Example 1

A raceway of 3-inch fish are to be treated for 5 days with Romet(R)-30 at the rate of 50 mg/kg/day. Weight of fish in raceway = 1.5 lbs fish/cu. ft. water x 540 cu. ft. water or 810 lbs. Drug will be used at 50 mg/kg/day or 22.7 mg/lb x 810 lbs or 18.4 g pure Romet(R)-30/day.

$$\text{Water Flow into Raceway} = \frac{810}{1.61^* \times 3} = 168 \text{ gpm}$$

*Flow index at 52°C and 1000' elevation

168 x 3.8 = 637 liters/min.
637 x 60 x 24 = 917,280 liters/day

If it is assumed that none of the drug were utilized by the fish and all 18.4 g were found in the water, the concentration of Romet(R)-30 in a 24 h volume of water would be: 18,400 mg/917,280 liters or 20 ppb.

Example 2

Treat a raceway of 9-inch fish with Romet(R)-30 for 5 days at 50 mg/kg/day.

Weight of fish = 4.5 x 540 = 2340 lbs

22.7 mg Romet(R)-30/lb x 2430 lbs = 55.2 g Romet(R)-30/day

$$\text{Flow Rate} = \frac{2430}{1.61 \times 9''} = 168 \text{ gpm}$$

168 x 3.8 = 637 liters/min.

637 x 60 x 24 = 917,280 liters/24-hr

Again, assuming that none of the drug were absorbed by the fish, this would be 55.200 mg/917,280 liters or 60 ppb.

From these two examples, which represent the size extremes that would receive food medicated with Romet(R)-30, it is apparent that even if none of the drug were absorbed, there would be only 20-60 ppb in the water per day on each day of treatment. However, since most of the drug administered would be absorbed and slowly released by the fish, the actual concentration of drug in the water would be considerably lower.

A model raceway study was conducted at the National Fish Health Research Laboratory to mimic the worst case situation noted above. This study is included as Appendix I to this report.

Examination of this data shows that very low concentrations of SDM and OMP are encountered in the effluent water both during and post-dosing. A single value of 0.025 ppm SDM in the water was noted; however, it must be balanced with the 30 (out of 43) samples which had less than 5 ppb of SDM. The OMP assays showed one water value at 6 ppb and the rest of the 43 samples at less than 5 ppb OMP in the water.

The total collected sediment from this study showed 7.2% of the dosed SDM still present and less than 0.3% of the OMP.

SUMMARY

The impact of SDM and OMP has been reviewed previously for the use of Rofenaid in Ducks (EIAR dated 2 February, 1983 to NADA 40-209) and is included with this report.

The SDM and OMP concentrations measured in the raceway water were very low, with thirty (of 43) water samples having less than 5 ppb and only one at 25 ppb. The OMP assays showed 42 of 43 samples at 5 ppb or less. The accumulated sediment for the whole study accounted for 7.2% of the dosed SDM drug and less than 0.3% of the dosed OMP drug.

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 100 and 300 fold factors for sulfadimethoxine and ormetoprim. A factor of 24 fold is calculated using the 6 mg/liter (-95% confidence interval) values for ROFENAID^{R}-40 in fresh water alga and the sum of the highest sulfadimethoxine and ormetoprim concentrations in any water sample.

The raceway and toxicity data show that Romet^{R}-30 use in fish 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 salmon 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.

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 ROMET-30 premix are summarized below:

Component	Emission (kg/yr)
Toluene	2.45
Pyridine	2.18

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	5/18/80	43838	5/18/80
43817	"	43839	"
43818	"	43840	"
43819	"	43841	"
43820	"	44981	4/15/80
43821	"	44982	"
43822	"	44983	"
43823	"	44984	"
43824	"	44985	"
43825	"	44986	"
43826	"	46066	12/12/80
43827	"	46067	"
43828	"	46068	"
43829	"	46069	"
43830	"	46070	"
43831	"	46071	"
43832	"	46072	"
43833	"	46073	"
43834	"	46074	"
43835	"	46075	"
43836	"	48014	"
43837	"	61188	8/23/82

Ormetoprim process air emissions for ROMET-30 premix are as follows:

Component	Emission (kg/yr)
Dimethylformamide	4.90
Methanol	25.08

3. Dry Blending Operation, Fresno, California

ROMET-30 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 (kg/year) Increase Due to ROFENAID®-40	Discharge to Passaic Valley Sewage Commission Treatment Works (kg/year) Increase Due to ROFENAID®-40
Organics		36.76
Inorganics		332.09
Charcoal and Dicalite	32.46	

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 (kg/year) Increase Due to ROFENAID®-40	Discharge to Passaic Valley Sewage Commission Treatment Works (kg/year) Increase Due to ROFENAID®-40
Organics		360.88
Inorganics		229.16
Waste Solvents	472.41	

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 prooosed 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 ROMET-30 exchange for possible minimal local effects due to the manufacture and use of the product.

The use of ROMET-30 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 salmonids' production means that more lbs. 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, a closely related product with both containing the same active ingredients, 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.

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 salmonid husbandry with the foreseeable benefits outlined in Sections 2 and 5. A further foreseeable benefit will be an increase in the supply of salmonid 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.

9. (cont'd.)

There is only minimal potential risk due to the introduction of ROMET-30 into the environment through the salmonid's treatment or from the emission of by-products during manufacture. Irretrievable depletion of natural resources due to the manufacture of ROMET-30 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.

March 8 1984
(Date)

M. R. Wouters
(Signature of Responsible Official)

Manager Regulatory Affairs
(Title)

573-2* 01154

INTEROFFICE CORRESPONDENCE

AMD4-73
ROCHE 82AMD4

Appendix I

TO Ro 5-0037 Environmental File DATE March 6, 1984

FROM A. MacDonald

SUBJECT Ro 5-0037 Raceway Water Study -- Concentration in Water During Dosing and Post-Dosing

The model raceway study of Ro 5-0037 in trout is described in the study protocol (attachment I) and the report by Herman (attachment II). The raceway parameters were:

Water Temperature	11.5°C.
pH	7.2-7.3
Raceway Dimensions	L - 53", W - 14.5", H - 9.5" (water ht)
Raceway Volume	4.225 cu. feet
Water Flow Rate	2.93 gal/min (11.1 liter/min)
Feeding Rate	1% of body weight/dry (83.4 g/dry)
Total Weight of Fish	18.4 lb (8340 g)
Individual Weight	126.4 g
Length	8.9"
Number of Fish	66
Oxygen Conc. (in)	9.8-10 ppm
(out)	4.5-6 ppm

$$\text{Flow Index} = \frac{18.4 \text{ lb}}{8.9" \times 2.93 \text{gpm}} = 0.71$$

Treatment Time	5 days
Dose Per Day	50 mg/kg/day Ro 5-0037
Total Dose Per Day	417 mg/day
Total Dose 5 Days	2,085 mg Ro 5-0037 or 1737.5 mg SDM + 347.5 mg OMP

The fish were fed three times a day and the water was sampled as per the protocol schedule with nine water samples on days 1 and 2, five water samples on days 3, 4 and 5, three water samples on days 6, 7 and 8. The sediment from eight days was collected for assay.

Very low concentrations of SDM and OMP were found in the effluent water. The listing below shows the frequency of assays in the given ranges:

SDM Conc.(ppm)	No. of Samples
N.D.	14
N.D. to 0.005	16
0.006 to 0.01	9
0.01 to 0.02	3
0.02 to 0.03	1

OMP Conc.(ppm)	No. of Samples
N.D.	35
N.D. to 0.005	7
0/006 to 0.01	1

The sediment was filtered with the wet sediment and the filtrate assayed separately.

Total Volume of Filtrate	=	2310 ml
Total Weight of Sediment	=	402.9 g
Ave SDM Filtrate	=	9.02 ug/ml
Ave SDM Sediment	=	255.3 g/g
Total SDM Filtrate (9.02) x 2310 ml	=	23.14 mg
Total SDM Sediment (402.9) x 255.3 g	=	102.87 mg
Total SDM	=	126.01 mg
Total SDM Dosed	=	1737.50 mg
% of Dosed SDM in Sediment/Filtrate	=	7.2
Ave OMP Filtrate	=	0.73 ug/ml
Ave OMP Sediment	=	7.36 ug/g
Total OMP Filtrate (0.73) x 2310 ml	=	1.68 mg
Total OMP Sediment (7.36) x 402.9 g	=	2.97 mg
Total OMP	=	4.65 mg
Total OMP Dosed	=	347.50 mg
% of Dosed OMP in Sediment/Filtrate	=	0.28

Attachment I - Study Protocol
Attachment II - Dr. Herman's Data
Attachment III - Assay for SDM in Raceway Water and Sediment w/Methods
Attachment IV - Assay for OMP in Raceway Water and Sediment w/Methods

A. MacDonald

AMD:kg

Attachment I

060

ENVIRONMENTAL STUDY PROTOCOL

The environmental study protocol provides the basic outline of the study and delegates responsibility for each section. Each section will prepare a working protocol for that segment of the study. These working protocols along with a copy of the raw data will be attached to the final section report.

TITLE: Ro 5-0037 Raceway Water Study: Concentration in Water During Dosing and Post-Dosing

PURPOSE: To determine the concentration of Ro 5-0037 in raceway water during the 5-day dosing period and for 3 days post-final dose

TEST ARTICLE: Ro 5-0037

NATURE OF STUDY: Environmental impact of feeding Ro 5-0037 to trout as a therapeutic

TEST SYSTEM: Model raceway with trout

PERSONNEL:

A. MacDonald, General Study Director, Hoffmann-La Roche Inc., Nutley, NJ

A. Raceway model with trout feeding and water collection at National Fish Health Research Lab.

Dr. Roger Herman
Fish and Wildlife Service
Leetown, W. Virginia

B. Water and sediment analysis for sulfadimethoxine and ormetoprim by

Dr. Gloria Chen
Animal Science Research
Hoffmann-La Roche, Inc.
Nutley, New Jersey

STUDY LOCATION: Model Raceway Section:

Fish & Wildlife Lab
Leetown, W. Virginia

Assay Section:

Animal Science Research
Hoffmann-La Roche Inc.
Nutley, New Jersey

RECORDS MAINTENANCE: Records will be kept by each section for their portion during the study itself. Upon completion, a copy of both sections' records to include all raw data will be filed along with the final report in the Environmental File, Animal Science Research, Hoffmann-La Roche Inc., Nutley, New Jersey

PROCEDURE: (Model Raceway Section)

Model Raceway Set-Up: To be equivalent to standard raceway in terms of fish density, water temperature, pH and flow to model worst case raceway treatment in terms of most drug used. Model raceway to set-up to collect all sediment for collection at end of study.

Source & Fish Size: Acclimatization of fish before treatment

Study Length: 8 days with 5 days of treatment followed by 3 days post last treatment.

Dose: 50 mg/kg/day in the feed

Feeding: Twice a day, 8:00 AM and 4:00 PM

Water Sampling: Fifty ml size in plastic bottle collected at mid race at one-half light

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Water Sampling Schedule: One liter before dosing (control water)

Study Day	Dose Time	Water Sample Time (time from feeding)
1 Treatment	8:00 AM	8:15 AM (15 mins)
		9:00 " (1 hr)
		10:00 " (2 ")
		11:00 " (3 ")
		12:00 Noon (4 hr)
		1:00 PM (5 hr)
		2:00 " (6 ")
		3:00 " (7 ")
	4:00 PM	4:00 " (8 ") (collect just before feeding)
2 Treatment	8:00 AM	8:00 AM (16 hr) (collect just before dosing)
		Rest of day-- same as Day 1
	4:00 PM	
3 Treatment	8:00 AM	8:00 AM (16 hr) (collect just before dosing)
		9:00 AM (1 hr)
		10:00 AM (2 ")
		12:00 Noon (4 hr)
	4:00 PM	4:00 PM (8 hr) (collect just before feeding)
4 Treatment	Day 4 Dose and Sampling Same as Day 3	
5 Treatment	Day 5 Dose and Sampling Same as Day 3	
6 No Dose		8:00 AM
		12:00 Noon
		4:00 PM
7 No Dose	Same as Day 6	
8 No Dose	Same as Day 6	

Study ends at 4:00 PM on Day 8.

Model raceway is drained so that all collected sediment from 8 days is saved and bottled.

PROCEDURE ASSAY METHODS: Water and sediment will be assayed for sulfadimethoxine and ormetoprim via hplc methods specific for each compound.

[signature] 8 Feb 83

General Study Director
Hoffmann-La Roche Inc.
Nutley, New Jersey

Date

Fish & Wildlife Service
Leetown, W. Virginia

Date

[signature] 8 Feb. 1983

Animal Science Research
Hoffmann-La Roche Inc.
Nutley, New Jersey

Date

ROCHE CHEMICAL DIVISION

February 7, 1983

Dr. Roger Herman
U. S. Dept. of the Interior
Fish & Wildlife Service
National Fish Health Research Lab
Leetown, Route 3, Box 50
Kearneysville, West Virginia 25430

Dear Roger:

I enjoyed meeting you on February 2 and discussing the environmental raceway study with Ro 5-0037. As per our discussion, I have enclosed the following:

1) A copy of the revised general environmental protocol

2) Self-addressed mailing labels for shipment of the boxes

3) A copy of the BVM Minor Use of Drugs in Animals Guidelines (for information purposes only--not study related)

4) Copy of the Good Laboratory Practice Regulations (for information purposes only--not study related)

5) Two feed sample bags. Please take two samples of the finished Ro 5-0037 feed and mail to me for assay. Indicate the expected concentration of Ro 5-0037 in the space provided.

Under separate cover via UPS, I have forwarded four insulated shipping containers with forty-eight 500 ml bottles and four 1-liter bottles for the samples of control water, treated water and sediment.

As we discussed, the study will start on February 14 and terminate on February 21 at 4:00 PM. I would appreciate your shipment of the water samples as they are collected, so we can assay them with a minimum of delay.

Again, thank you for your assistance.

Sincerely,

HOFFMANN-LA ROCHE INC.

Alexander MacDonald, Ph.D.
Assistant Director
Animal Science Research

AMD:kg
Enclosures

Attachment II

United States Department of the Interior

066

FISH AND WILDLIFE SERVICE
NATIONAL FISH HEALTH RESEARCH LABORATORY
LEETOWN, BOX 700
KEARNEYSVILLE, WEST VIRGINIA 25430

March 2, 1983

Dr. Alex McDonald
Hoffman-LaRoche, Inc.
Nutley, New Jersey 07110

Dear Alex:

The enclosed page copies give the essentials of the environmental partition study.

Your protocol was followed with these exceptions:

1. Fish fed three times per day after first day because fish did not seem to like taste of feed.

2. One water sample missed on 2/18.

3. Sediment collected morning of 2/22 instead of evening of 2/21.

Twice as much feed as needed was prepared - samples sent to you - fish fed their alloted amount and the remainder is in the freezer.

Gelatin coating of feed was as per Fish Hatchery Management, Piper, ed.

Sincerely,

Roger Lee Herman

Roger L. Herman
Histopathologist

Enclosure

Fish from APDS mixed strains

adjusted weight of fish (number) water flow to meet limited fish 5-6 ppm O_2

Total wt fish 8340 gm (18.4#) ave wt 126.4 gm
" no. " 66 (3.6/#)
flow 11.1 lpm (2.93 gpm)
Temp 11.5 °C
TH ave 280 on spring supply } historical
pH ave 7.2-3 " "

Tank 53" x 14.5" x 9.5" water = 4.22 ft³ water

F.I = 18.4# / (8.9" x 2.93 gpm) = 0.71

O_2 in 9.8-10 ppm
out 4.5-6 ppm during acclimation

feed rate 1.0% bw/day = 83.4 gm/day
x5
417 total feed

R05-0037 @ 50 mg/kg fish/day 0.417 mg/day
x5
2.085

Premix (30%) 3.33 gm
.417 mg
1.38861
x5
6.94305 total premix

Two feed samples mailed to A. MacDonald

on Partition R050037 Project Book No. — COPY

068

[illegible] Flush end of tank to remove sediment
start feed 41.7 temp 11.5°C. O_2 out at 8am 6.3 ppm
4pm 41.7 gm feed temp 10.5 O_2 out 6.6

2/15 O_2 9.5 in 6 out Temp 10°C 8Am
fish not enthusiastic about feed
Offer some feed at noon (after sample)

2/16 8Am Temp 11°C
feed 3x (total 83.4 gm)
not feeding well

2/18 8Am Temp 10.5°C O_2 out 5.8
missed 9am water sample

2/19 noon Temp 11°C O_2 out 6.0

" sediment collected 7am - siphoned into bucket
let settle - siphoned 3 liters

Remaining water samples + sediment shipped VBS

2/15 - 18 feed three times day 8am - 12n - 4pm
due slow feeding response

no mortality during test

Oxygen measured with YSI model 54ARC meter
calibrated against Winkler

Rice Hull premix lot no A 11661-01

Coated by gelatin on 3/16" commercial trout pellets

ed & Understood by me, | Date | Invented by | Date

069

Appendix H

Drug Coatings for Feed Pellets

Either gelatin or soy oil may be used as drug carriers for coating feed pellets. A representative sample of pellets should be checked for adequate coatings before the operation is terminated.

3.15 l 45.46 kg

Gelatin: 125 grams gelatin in 3.0 quarts water per 100 pounds of pellets.

(1) Slowly dissolve the gelatin into hot tap water.
(2) Stir the drug into the gelatin solution until all lumps are gone.
(3) Slowly add the drug-gelatin mixture to pellets as they are stirred by hand or in a small cement mixer. To avoid pellet breakage, stir gently and only long enough to assure an even drug coating.

Soy oil: 2–3 pounds per 100 pounds of pellets.

(1) Mix drug evenly in warm (100–120° F) oil.
(2) Pour or spray mixture over pellets.

CERTIFICATE OF ANALYSIS
F.A.P. ANALYTICAL LABORATORY
HOFFMANN-LA ROCHE INC. NUTLEY, NEW JERSEY 07110

ROCHE

NAME	[illegible] Research / Dr. [illegible]
STREET	[illegible]
CITY / STATE / ZIP	[illegible]

–DO NOT KEYPUNCH ABOVE THIS LINE–

CARD 1

CERTIFICATE NUMBER (1–5)	CUSTOMER CODE PLANT NO. (7–11)	CUSTOMER CODE MILL NO. (12–14)	DATE RECEIVED MO. DAY YR. (16–21)	DATE MIXED MO. DAY YR. (23–28)
[illegible]			[illegible]	[illegible]

FEED NAME AND FEED LOT NUMBER (30–71)	ROCHE PREMIX LOT NUMBER (73–78)	80
[illegible]		1

CARD 2 DUPLICATE COLUMNS 1 THRU 11 FROM CARD 1

SULFADIMETHOXINE

PF (13)	LABEL CLAIM (15–18)	ASSAY VALUE (20–22)	SAMPLE % OF CLAIM	ANALYST (24–26)	BOOK NO.	PAGE NO.
				[illegible]	[illegible]	[illegible]

ORMETOPRIM

PF (28)	LABEL CLAIM (30–33)	ASSAY VALUE (35–37)	SAMPLE % OF CLAIM	ANALYST (39–41)	BOOK NO.	PAGE NO.
				[illegible]	[illegible]	[illegible]

IPRONIDAZOLE

PF (43)	LABEL CLAIM (45–48)	ASSAY VALUE (50–52)	SAMPLE % OF CLAIM	ANALYST (54–56)	BOOK NO.	PAGE NO.

LASALOCID NA

PF (58)	LABEL CLAIM (60–63)	ASSAY VALUE (65–67)	SAMPLE % OF CLAIM	ANALYST (69–71)	BOOK NO.	PAGE NO.

REMARKS:

DATE OF ASSAY

MO. DAY YR. (73–78)	80
[illegible]	2

SUPERVISOR

THE ABOVE ASSAY APPLIES ONLY TO THE SAMPLE SUPPLIED. THE APPLICABILITY OF THIS ASSAY TO THE FEED BATCH AS A WHOLE IS DEPENDENT UPON THE SAMPLE BEING REPRESENTATIVE OF THE BATCH AND IS THE RESPONSIBILITY OF THE SUPPLIER OF THE SAMPLE. ALL VALUES ARE REPRESENTED AS PARTS PER MILLION.

CERTIFICATE OF ANALYSIS
F.A.P. ANALYTICAL LABORATORY
HOFFMANN-LA ROCHE INC. NUTLEY, NEW JERSEY 07110

ROCHE

071

NAME
[illegible] Research / Dr. R. [illegible]
STREET
[illegible], Rt. 3 Box 50
CITY STATE ZIP
[illegible] [illegible]

–DO NOT KEYPUNCH ABOVE THIS LINE–

CARD 1

CERTIFICATE NUMBER	CUSTOMER CODE PLANT NO.	CUSTOMER CODE MILL NO.	DATE RECEIVED MO. DAY YR.	DATE MIXED MO. DAY YR.
1 2 3 4 5	7 8 9 10 11	12 13 14	16 17 18 19 20 21	23 24 25 26 27 28
[illegible]			[illegible]	[illegible]

FEED NAME AND FEED LOT NUMBER	ROCHE PREMIX LOT NUMBER	
30 31 32 … 71	73 74 75 76 77 78	80
[illegible]		1

CARD 2 DUPLICATE COLUMNS 1 THRU 11 FROM CARD 1

SULFADIMETHOXINE

PF	LABEL CLAIM	ASSAY VALUE	SAMPLE % OF CLAIM	ANALYST	BOOK NO.	PAGE NO.
13	15 16 17 18	20 21 22		24 25 26		

ORMETOPRIM

PF	LABEL CLAIM	ASSAY VALUE	SAMPLE % OF CLAIM	ANALYST	BOOK NO.	PAGE NO.
28	30 31 32 33	35 36 37		39 40 41		

IPRONIDAZOLE

PF	LABEL CLAIM	ASSAY VALUE	SAMPLE % OF CLAIM	ANALYST	BOOK NO.	PAGE NO.
43	45 46 47 48	50 51 52		54 55 56		

LASALOCID NA

PF	LABEL CLAIM	ASSAY VALUE	SAMPLE % OF CLAIM	ANALYST	BOOK NO.	PAGE NO.
58	60 61 62 63	65 66 67		69 70 71		

REMARKS:

DATE OF ASSAY MO. DAY YR.	
73 74 75 76 77 78	80
	2

SUPERVISOR

THE ABOVE ASSAY APPLIES ONLY TO THE SAMPLE SUPPLIED. THE APPLICABILITY OF THIS ASSAY TO THE FEED BATCH AS A WHOLE IS DEPENDENT UPON THE SAMPLE BEING REPRESENTATIVE OF THE BATCH AND IS THE RESPONSIBILITY OF THE SUPPLIER OF THE SAMPLE. ALL VALUES ARE REPRESENTED AS PARTS PER MILLION.

Attachment III

F 575-2* 01154

INTEROFFICE CORRESPONDENCE

ROCHE

GC3-19

072

TO Dr. A. MacDonald

DATE March 25, 1983

FROM G. Chen & T. Egan

cc: Dr. H. Eoff

SUBJECT Assay for Sulfadimethoxine (SDM) in Raceway Water and Sediment (Book #10459, pp 170-71, 173-75, -77)

Forty-three raceway water, three filtrate of sediment, and three precipitate of sediment samples were assayed for SDM concentration. Raceway water and filtrate of sediment samples were assayed singly according to the procedure in IOM, GC3-13 (March 16, 1983), wet precipitate of sediments were assayed in duplicate based on the procedure in IOM, GC3-14 (March 16, 1983). The water content of wet precipitate of sediments were also studied.

Average recovery value from fortified control raceway water was used to correct the SDM content in water samples. No recovery data is available for both filtrate and precipitate of sediments.

Results are listed in tables I-III.

G. Chen

GC:kg

Attachments

Table I

The Concentration of Sulfadimethoxine in Raceway Water

ASR #	Description	Lab Code No.	Conc. (ug/ml) Corrected
89-590	2/14/83 NFHRL S #1 8:15AM	10459-171-01	0
89-591	2/14/83 NFHRL S #2 8:30AM	10459-170-02	0.009
89-592	2/14/83 NFHRL S #3 9:15AM	-03	< 0.001
89-593	2/14/83 NFHRL S #4 10:15AM	-04	N.D.
89-594	2/14/83 NFHRL S #5 11:15AM	-05	N.D.
89-595	2/14/83 NFHRL S #6 12:15PM	-06	N.D.
89-596	2/14/83 NFHRL S #7 1:15PM	-07	N.D.
89-597	2/14/83 NFHRL S #8 2:15PM	-08	N.D.
89-598	2/14/83 NFHRL S #9 3:15PM	-09	N.D.
89-599	2/14/83 NFHRL S #10 4:00PM	-10	N.D.
89-600	2/15/83 NFHRL S #11 8:00AM	10459-175-07	0.003
89-601	2/15/83 NFHRL S #12 8:15AM	10459-170-12	0.025
89-602	2/15/83 NFHRL S #13 9:00AM	-13	0.012
89-603	2/15/83 NFHRL S #14 10:00AM	-14	0.003
89-604	2/15/83 NFHRL S #16 12:00Noon	-15	0.003

Corrected for recovery = 83.9%

N.D. < 0.0005 ug/ml

074

Table I (cont'd.)

ASR #	Description		Lab Code No.	Conc. (ug/ml) Corrected
89-629	2/15/83 S #15	NFHRL 11:00AM	01459-171-02	0.003
89-630	2/15/83 S #17	NFHRL 1:00PM	-03	0.008
89-631	2/15/83 S #18	NFHRL 2:00PM	-04	0.007
89-632	2/15/83 S #19	NFHRL 3:00PM	-05	0.005
89-633	2/15/83 S #20	NFHRL 4:00PM	-06	0.007
89-634	2/15/83 S #21	NFHRL 8:00AM	-07	0.004
89-635	2/16/83 S #22	NFHRL 9:00AM	-08	0.004
89-636	2/16/83 S #23	NFHRL 10:00AM	-09	N.D.
89-637	2/16/83 S #24	NFHRL 12:00Noon	-10	0.003
89-638	2/16/83 S #25	NFHRL 4:00PM	-11	0.003
89-639	2/17/83 S #26	NFHRL 8:00AM	-12	0.003
89-640	2/17/83 S #27	NFHRL 9:00AM	-13	0.001
89-641	2/17/83 S #28	NFHRL 10:00AM	-14	0.004
89-642	2/17/83 S #29	NFHRL 12:00Noon	-15	N.D.
89-643	2/17/83 S #30	NFHRL 4:00PM	-16	0.001

Table I (cont'd.)

ASR #	Description		Lab Code No.	Conc. (ug/ml) Corrected
89-644	2/18/83 S #31	NFHRL 8:00AM	10459-171-17	0.002
89-645	2/18/83 S #33	NFHRL 10:00AM	10459-173-02	0.011
89-646	2/18/83 S #34	NFHRL 12:00Noon	-03	0.005
89-647	2/18/83 S #35	NFHRL 4:00PM	-04	0.008
89-648	2/19/83 S #36	NFHRL 8:00AM	-05	0.014
89-649	2/19/83 S #37	NFHRL 12:00Noon	-06	0.007
89-650	2/19/83 S #38	NFHRL 4:00PM	-07	0.007
89-651	2/20/83 S #39	NFHRL 8:00AM	-08	0.007
89-652	2/20/83 S #40	NFHRL 12:00Noon	-09	N.D.
89-653	2/20/83 S #41	NFHRL 4:00PM	-10	N.D.
89-654	2/21/83 S #42	NFHRL 8:00AM	-11	N.D.
89-655	2/21/83 S #43	NFHRL 12:00Noon	-12	N.D.
89-656	2/21/83 S #44	NFHRL 4:00PM	-13	0.006

Table II

The Concentration of Sulfadimethoxine in Sediment

ASR #	Description	Lab Code No.	Concentration (ug/ml or ug/g)
89-657	2/22/83 NFHRL S #45 FOS*	10459-175-09	8.69 ug/ml
-658	2/22/83 NFHRL S #46 FOS	-10	9.27 "
-659	2/22/83 NFHRL S #47 FOS	-11	9.11 "
89-657	2/22/83 NFHRL S #45-ppt of Sediment	10459-177-01	214.56 ug/g
-657	" "	-02	305.64 "
89-658	2/22/83 NFHRL S #46-ppt of Sediment	-03	240.92 "
-658	" "	-04	251.74 "
89-659	2/22/83 NFHRL S #47-ppt of Sediment	-05	260.62 "
-659	" "	-06	258.56 "

*Filtrate of Sediment

No recovery data is available

Table III

Summary of Sediment

ASR #	S #	Total Volume of Filtrate (ml)	Total Weight of Wet Precipitate (g)	Water Content* of Wt ppt (%)
89-657	45	695	171.5	73.65
-658	46	795	118.2	74.85
-659	47	820	113.2	73.30

*Average of two

INTEROFFICE CORRESPONDENCE

TO Dr. A. MacDonald

DATE March 16, 1983

FROM G. Chen

cc: Dr. H. Eoff

SUBJECT High Performance Liquid Chromatographic Pocedure for Assay of Sulfadimethoxine (SDM) in Raceway Water at 0.01 ug/ml Level (Book #10459, pp. 170-175)

A simple hplc method for assay of sulfadimethoxine (Ro 4-0517, lot #742079) in water at 0.01 ug/ml/10 ml sample size has been developed and validated in the range of 0.01-0.05 ug/ml with an average recovery of 83.9% (ref. standard deviation = 7.9%).

Sulfadimethoxine is extracted from water into methylene chloride at pH 6.0 ± 0.1. An aliquot of methylene chloride extract is evaporated just to dryness under a stream of nitrogen in a water bath at approx. 40°C. The residue is reconstituted in a suitable volume of mobile phase (In this case, one ml of the mobile phase is used.), and a 50 ul aliquot is injected onto the hplc partisil column. The effluent is monitored by a UV detector with a wavelength at 280 nm, and the SDM peak area is registered and measured by HP integrator. Three points of reference standards are applied in this study (0.05-0.20 ug/ml).

EXPERIMENTAL

A. Apparatus

1. Hplc consists of:

   a. Pump - Spectra-Physics 3500B
   b. Injector - Waters WISP 710B
   c. Column - Whatman Partisil PXS 10/25 (10 micro microparticulate silica, 25 cm x 4.6 mm I.D.)
   d. Detector - LDC Spectro-Monitor III, spectrophotometer with a sensitivity of 0.005 AUFS
   e. Recording integrator - Hewlett-Packard 3380A

A. (cont'd.)

2. Balances - Sartorius, d ± 0.1 mg;
   Mettler P1200, d = 10 mg
3. Volumetric pipettes
4. Syringe - Hamilton, 100 ul
5. Centrifuge tubes - Sterile, polypropylene, 50 ml with screw cap (Corning)
6. pH Meter - Corning Digital 110
7. Nitrogen - Prepurified
8. Evaporator - N-EVAP model 106, Organomation
9. Water bath - Thelco model 83
10. Shaker - Reciprocal, model R-7, New Brunswick Scientific Co.
11. Volumetric flasks
12. Graduated cylinders
13. Vortex tube mixer - Lab-line super mixer
14. Aspirator
15. Centrifuge - CRU-5000, I.E.C.

B. Reagents

1. Solvents - distilled in glass (B & J): methylene chloride, methanol, chloroform and acetonitrile
2. Ammonium hydroxide (Baker): reagent grade
3. Hydrochloric acid (Baker): reagent grade
4. Phosphate buffer (I.M.): Mix 1M potassium hydrogen phosphate with 1M potassium dihydrogen phosphate such that pH = 5.25
5. 0.85M HCl/(1M phosphate buffer, pH 5.25), (1:1, v/v)

C. Mobile Phase

Chloroform/(methanol:distilled water:ammonium hydroxide) = 500/10 (150:9:1), v/v

Shake mixture for few seconds, filter through millipore FH filter (Fluoropore 47 mm), then degas for five minutes. Mobile phase should be tightly capped and stored. It is stable for a minimum of one week.

Chromatography

A partisil PXS 10/25 (Whatman) silica column was conditioned with the mobile phase prior to use in order to achieve a stable response at a flow rate of 1.2 ml/minute. Three levels of reference standard solutions (SDM, lot #742079) were injected daily and the SDM peak areas were measured.

C. Chromatography (cont'd.)

A set of replicated spiked samples was prepared to study the applicability of the extraction technique. The assay was performed by comparison of absorbances between the reference standards and the spiked samples and/or samples of interest. Approximate retention time = 4.6 minutes.

D. Preparation of SDM standard solutions

1. External standards (hplc reference standards)

   a. 100 ug/ml - Weigh exactly 10.0 mg SDM into a 100 ml volumetric flask, add chloroform to dissolve, and dilute to volume with chloroform

   b. 2.0 ug/ml - Dilute 1 ml of (1-a) to 50 ml with hplc mobile phase

   c. 0.20 ug/ml - Dilute 5 ml of (1-b) to 50 ml with hplc mobile phase

   d. 0.10 ug/ml - Dilute 5 ml of (1-b) to 100 ml with hplc mobile phase

   e. 0.05 ug/ml - Dilute 5 ml of (1-d) to 10 ml with hplc mobile phase

2. Sample fortification standards

   a. 2.0 ug/ml - Dilute 1 ml of (1-a) to 50 ml with acetonitrile

   b. 4.0 ug/ml - Dilute 2 ml of (1-a) to 50 ml with acetonitrile

   c. 10.0 ug/ml - Dilute 5 ml of (1-a) to 50 ml with acetonitrile

Store stock solutions in stoppered volumetric flasks, sealed with parafilm in refrigerator. Solutions may be kept for a minimum of one week.

E. Preparation of fortified samples

1. Pipette 10 ml aliquots of control raceway water into a 50 ml polypropylene centrifuge tube.

E. (cont'd.)

2. Add 50 ul sample fortification standards into respective centrifuge tubes which contain 10 ml control raceway water each, then mix.

(i.e., 0.01 ug/ml - 50 ul of 2.0 ug/ml SDM
0.02 " - " 4.0 " "
0.05 " - " 10.0 " ")

3. Adjust pH to 6.0 (± 0.10) by adding 1:1 0.85M HCl/1M phosphate buffer (pH 5.25).

4. Add 30 ml methylene chloride, screw capped, shake for 10 minutes at a reciprocating shaker at maximum speed.

5. Let two phases separate completely, aspirate off all of the top layer, and discard.

6. Measure 25 ml methylene chloride extract (lower layer) back to original tube, and evaporate just to dryness under a stream of nitrogen at 40°C.

7. Reconstitute the residue with suitable volume of hplc mobile phase within the calibration curve range (i.e., 1 ml for 0.01 and 0.02 ug/ml level, and 2 or 3 ml for 0.05 ug/ml level).

8. Inject 50 ul onto hplc column through Waters WISP auto injector.

F. Sample preparation

Pipette 10 ml aliquots of raceway water sample into a 50 ml polypropylene centrifuge tube, then process according to steps E-3 to E-8.

All raceway water samples are stored in refrigerator at 0-4°C prior to assay.

G. Calculations

1. Fortified sample recovery.

A comparison between the known quantity of reference standard and the fraction of the sample introduced onto the column.

$$\% \text{ Recovery} = \frac{\text{Corr. Peak Area SDM in the Fortified Sample}}{\text{Peak Area of Reference Standard}}$$

$$\times \frac{\text{Conc. Ref. Std (ug/ml) x Vol. Corr. Factor x 100\%}}{\text{Spiked Drug Quantity (ug)}}$$

2. ug per ml SDM in water sample

$$\text{ug/ml} = \frac{\text{Corr. Peak Area SDM in Sample}}{\text{Peak Area of Reference Standard}}$$

$$\times \frac{\text{Conc. Ref. Std (ug/ml) x Vol. Corr. Factor}}{\text{Sample Volume (ml) x \% Recovery}}$$

Corr. Peak Area = Peak Area minus Control Background

G. Chen

GC:kg

Table 1

Recovery of Sulfadimethoxine from Fortified Control Raceway Water

	Level(ug/ml)		
	0.01	0.02	0.05
% Recovery	77.4	79.4	94.4
	90.3	80.3	90.4
	88.4	82.8	87.3
	72.0	80.1	79.3
	80.9		94.9
			80.5
x	81.8	80.7	87.8
S.D.	7.62	1.48	6.73
Rel. S.D.	9.31	1.84	7.67

Level Average	83.9
S.D.	6.66
Rel. S.D.	7.94

INTEROFFICE CORRESPONDENCE

ROCHE GC3-1084

TO Dr. A. MacDonald DATE March 16, 1983

FROM G. Chen

SUBJECT Assay of Sulfadimethoxine (SDM) in Sediment by Hplc Procedure (Book #10459, pp. 174-175)

A simple hplc method described in IOM, GC3-13, is applied to assay SDM concentration in sediment samples. The sample handling and extraction procedure are outlined:

1. Sample handling.

   Entire sediment is filtered through a 32 cm 2V filter paper to separate filtrate from precipitate. Measure the filtrate and record the volume in ml. Weigh the precipitate and record in grams.

2. Loss of water content from precipitate.

   Weigh exactly 10 g wet precipitate into petri dish (pre-weigh) and place into oven at 105°C overnight; transfer to desiccator, cool to room temperature, then weigh. The weight's difference is the loss of water content.

3. Extraction procedure for sediment ppt.

   a. Weigh exactly one gram sediment ppt into a 50 ml polypropylene centrifuge tube; add 10 ml deionized distilled water to form suspension.

   b. Adjust pH to 6.00 ± 0.10 by adding 1:1 0.85M HCl/1M phosphate buffer (pH 5.25).

   c. Add 30 ml methylene chloride, screw capped, shake for 10 minutes at a reciprocating shaker at maximum speed.

   d. Centrifuge at 2000 rpm for 10 minutes or until two phases separate completely; aspirate off all top layer including sediment and discard.

3. (cont'd.)

   e. Measure 5 ml methylene chloride extract into a new polypropylene tube, then evaporate to just dryness under a stream of nitrogen at ~ 40°C.

   f. Reconstitute the residue with suitable volume of hplc mobile phase within the calibration curve range. Make dilution, if necessary.

   g. Inject 50 ul final extract onto hplc column through Waters WISP auto injector.

4. Extraction procedure for sediment filtrate.

   Proceed raceway water sample preparation (GC3-13, paragraph F).

Recovery from fortification samples is not available.

G. Chen

GC:kg

Attachment 10

F-375-2* 01154

INTEROFFICE CORRESPONDENCE

ROCHE GC3-20 086

TO Dr. A. MacDonald

DATE March 29, 1983

FROM G. Chen & T. Egan

cc: Dr. H. Eoff

SUBJECT Assay for Ormetoprim (OMP) in Raceway Water and Sediment (Book #10459, pp. 182-188)

Forty-three raceway water, three filtrate, and three precipitate of sediment samples were assayed for OMP concentration. Raceway water samples were assayed singly according to the procedure in IOM, GC3-15 of March 21, 1983, filtrate and wet precipitate of sediments were assayed in duplicate based on the procedure in IOM, GC3-17 (March 24, 1983). Average recovery value (93.5 ± 5.0%) from fortified control raceway water was used to correct the OMP content in water samples. No recovery data was available for both filtrate and precipitate of sediments.

Results are listed in tables I and II.

G. Chen

G. Chen

GC:kg
Attachments

Table I

087

The Concentration of Ormetoprim in Raceway Water

ASR #	Description	Lab Code No.	Conc. (ug/ml) Corrected
89-686	2/14/83 NFHRL S #1 8:15AM	10459-182-01	0
89-591	2/14/83 NFHRL S #2 8:30AM	-02	0.0005
89-592	2/14/83 NFHRL S #3 9:15AM	-03	N.D.
89-593	2/14/83 NFHRL S #4 10:15AM	-04	N.D.
89-594	2/14/83 NFHRL S #5 11:15AM	-05	N.D.
89-595	2/14/83 NFHRL S #6 12:15PM	-06	N.D.
89-596	2/14/83 NFHRL S #7 1:15PM	-07	N.D.
89-597	2/14/83 NFHRL S #8 2:15PM	-08	N.D.
89-598	2/14/83 NFHRL S #9 3:15PM	-09	N.D.
89-599	2/14/83 NFHRL S #10 4:00PM	-10	N.D.
89-600	2/15/83 NFHRL S #11 8:00AM	-11	N.D.
89-601	2/15/83 NFHRL S #12 8:15AM	-12	0.006
89-602	2/15/83 NFHRL S #13 9:00AM	-13	< 0.001
89-603	2/15/83 NFHRL S #14 10:00AM	-14	N.D.
89-604	2/15/83 NFHRL S #16 12:00Noon	-15	N.D.
89-629	2/15/83 NFHRL S #15 11:00AM	-16	N.D.
89-630	2/15/83 NFHRL S #17 1:00PM	-17	0.005

Corrected for recovery = 93.5%

N.D. = $<$ 0.0005 ug/ml

Table I (cont'd.)

ASR #	Description	Lab Code No.	Conc. (ug/ml) Corrected
89-631	2/15/83 NFHRL S #18 2:00PM	10459-183-02	0.003
89-632	2/15/83 NFHRL S #74 3:00PM	-03	0.003
89-633	2/15/83 NFHRL S #20 4:00PM	-04	0.001
89-634	2/16/83 NFHRL S #21 8:00AM	-05	N.D.
89-635	2/16/83 NFHRL S #22 9:00AM	-06	0.001
89-636	2/16/83 NFHRL S #23 10:00AM	-07	0.001
89-637	2/16/83 NFHRL S #24 12:00Noon	-08	N.D.
89-638	2/16/83 NFHRL S #25 4:00PM	-09	N.D.
89-639	2/17/83 NFHRL S #26 8:00AM	-10	N.D.
89-640	2/17/83 NFHRL S #27 9:00AM	-11	N.D.
89-641	2/17/83 NFHRL S #28 10:00AM	10459-184-02	N.D.
89-642	2/17/83 NFHRL S #29 12:00Noon	-03	N.D.
89-643	2/17/83 NFHRL S #30 4:00PM	-04	N.D.
89-644	2/18/83 NFHRL S #31 8:00AM	-05	N.D.
89-645	2/18/83 NFHRL S #33	-06	N.D.

Table 1 (cont'd.)

ASR #	Description	Lab Code No.	Conc. (ug/ml) Corrected
89-646	2/18/83 NFHRL S #34 12:00Noon	10459-184-07	N.D.
89-647	2/18/83 NFHRL S #35 4:00PM	-08	N.D.
89-648	2/19/83 NFHRL S #36 8:00AM	-09	N.D.
89-649	2/19/83 NFHRL S #37 12:00Noon	-10	N.D.
89-650	2/19/83 NFHRL S #38 4:00PM	-11	N.D.
89-651	2/20/83 NFHRL S #39 8:00AM	-12	N.D.
89-652	2/20/83 NFHRL S #40 12:00Noon	-13	N.D.
89-653	2/20/83 NFHRL S #41 4:00PM	-14	N.D.
89-654	2/21/83 NFHRL S #42 8:00AM	-15	N.D.
89-655	2/21/83 NFHRL S #43 12:00Noon	-16	N.D.
89-656	2/21/83 NFHRL S #44 4:00PM	-17	N.D.

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Table II

The Concentration of Ormetoprim in Sediment

ASR #	Description	Lab Code No.	Conc. (ug/ml) Corrected	or ug/ml
89-657	2/22/83 NFHRL S #45 FFS*	10459-186-01	0.85	0.85
-657	" "	-02	0.84	
89-658	2/22/83 NFHRL S #46 FFS	-03	0.67	0.67
-658	" "	-04	0.66	
89-659	2/22/83 NFHRL S #47 FFS	-05	0.69	0.68
-659	" "	-06	0.66	
				ug/g
89-657	2/22/83 NFHRL S #45 Sediment	-07	8.03	7.84
-657	" "	-08	7.64	
89-658	2/22/83 NFHRL S #46 Sediment	-09	6.75	6.28
-658	" "	-10	5.81	
89-659	2/22/83 NFHRL S #47 Sediment	-11	7.75	7.95
-659	" "	-12	8.14	

*Filtrate from Sediment

No recovery data is available

F 375-2* 01154

INTEROFFICE CORRESPONDENCE

ROCHE GC3-05 091

TO Dr. A. MacDonald DATE March 21, 1983

FROM G. Chen

SUBJECT Determination of Ormetoprim (OMP) in Raceway Water by HPLC Method at 0.01 ug/ml Level (Book #10459, pp. 178 & 180)

Hplc method for analysis of ormetoprim (OMP, Ro 5-9754, lot #167035) in raceway water at 0.01 ug/ml per 10 ml sample size has been developed and validated in the range of 0.01-0.05 ug/ml with an average recovery of 93.5% (rel. std. deviation = 5.33).

Ormetoprim is extracted from water into methylene chloride at pH 10.50 ± 0.10. An aliquot of methylene chloride extract is evaporated just to dryness under a stream of nitrogen at approximately 40°C. The residue is reconstituted in a suitable volume of mobile phase (1-2 ml in this case), a 50 ul aliquot is injected onto hplc partisil column. The effluent is monitored by a UV detector with wavelength at 280 nm (R = 0.005 A.U.F.S.) and the OMP peak area is registered and measured by HP integrator.

Five levels of reference standards are used to establish a calibration curve (0.05-0.80 ug/ml).

EXPERIMENTAL

A. Apparatus

1. Hplc consists of:

   a. Pump - Spectra-Physics 3500B
   b. Injector - Waters WISP 710B
   c. Column - Whatman Partisil PXS 10/25 (10 micro microparticulate silica 25 cm x 4.5 mm I.D.)
   d. Detector - LDC Spectro-Monitor III, spectrophotometer with a sensitivity of 0.005 A.U.F.S.
   e. Recording integrator - Hewlett-Packard 3380A

A. (cont'd.)

2. Balances - Sartorius, d ± 0.1 mg;
   Mettler P1200, d = 10 mg
3. Volumetric pipettes
4. Syringe - Hamilton, 100 ul
5. Centrifuge tubes - Pyrex, 50 ml
6. pH Meter - Corning Digital 110
7. Nitrogen - Prepurified
8. Evaporator - N-EVAP model 106, Organomation
9. Water bath - Thelco model 83
10. Shaker - Reciprocal, New Brunswick Scientific Co., model R-7
11. Volumetric flasks
12. Graduated cylinders
13. Vortex tube mixer - Lab-line super mixer
14. Aspirator
15. Centrifuge - CRU-5000 I.E.C.

B. Reagents

1. Solvents - distilled in glass (B & J): methylene chloride, methanol, chloroform and acetonitrile
2. Ammonium hydroxide (Baker): reagent grade
3. Sodium carbonate, 0.2N
4. Deionized distilled water

C. Mobile Phase

Chloroform/(methanol:distilled water:ammonium hydroxide) = 500/20 (15:19:1), v/v

Shake mixture for few seconds, filter through millipore FH filter (Fluoropore), degas for five minutes. Mobile phase should be tightly capped and stored. It is stable for a minimum of one week.

Chromatography

A partisil PXS 10/25 (Whatman) normal phase column is conditioned with mobile phase prior to use in order to achieve a stable response at a flow rate of 1.2 ml/minutes. Five levels of reference standard soltuions (OMP, 0.05-0.80 ug/ml) are injected daily, and the OMP peak areas are measured.

C. Chromatography (cont'd.)

A set of replicated spiked samples is prepared to study the applicability of the extraction technique. The assay is performed by comparison of absorbances between the reference standards and the spiked samples and/or samples of interest. Approximate retention time is 6.1 min which may vary from column to column without affecting baseline separation.

D. Preparation of OMP standard solutions (lot #167035)

1. External standards (hplc reference standards)

   a. 100 ug/ml - Weigh exactly 10.0 mg OMP into a 100 ml volumetric flask, add chloroform to dissolve and dilute to volume with chloroform

   b. 2.0 ug/ml - Dilute 1 ml of (1-a) to 50 ml with hplc mobile phase

   c. 0.8 ug/ml - Dilute 4 ml of (1-b) to 10 ml with hplc mobile phase

   d. 0.4 ug/ml - Dilute 2 ml of (1-b) to 10 ml with hplc mobile phase

   e. 0.2 ug/ml - Dilute 5 ml of (1-b) to 50 ml with hplc mobile phase

   f. 0.1 ug/ml - Dilute 5 ml of (1-b) to 100 ml with hplc mobile phase

   g. 0.05 ug/ml - Dilute 5 ml of (1-f) to 10 ml with hplc mobile phase

2. Sample fortification standards

   a. 2.0 ug/ml - Dilute 1 ml of (1-a) to 50 ml with acetonitrile

   b. 4.0 ug/ml - Dilute 2 ml of (1-a) to 50 ml with acetonitrile

   c. 10.0 ug/ml - Dilute 5 ml of (1-a) to 50 ml with acetonitrile

Store stock solutions in stoppered volumetric flasks, sealed with parafilm in refrigerator. Solutions are stable for a minimum of one week.

E. Preparation of fortified samples

1. Pipette 10 ml aliquots of control raceway water into a 50 ml Pyrex glass centrifuge tube.

2. Add 50 ul sample fortification standards into respective centrifuge tubes which contain 10 ml control raceway water each (i.e., 0.01 ug/ml - 50 ul of 2.0 ug/ml OMP; 0.02 ug/ml - 50 ul of 4.0 ug/ml; and 0.05 ug/ml - 50 ul of 10.0 ug/ml); mix.

3. Adjust pH to 10.50 (± 0.10) by adding 0.2N sodium carbonate (approximately 3-4 ml).

4. Add 30 ml methylene chloride, screw capped, shake for 10 minutes at a reciprocating shaker at maximum speed.

5. Let two phases separate completely, aspirate off all top layer and discard.

6. Measure 25 ml of methylene chloride extract (lower layer) back to original tube, evaporate just to dryness under a stream of nitrogen at 40°C.

7. Reconstitute the residue with suitable volume of hplc mobile phase within the range of calibration curve.

8. Inject 50 ul onto hplc column through Waters WISP auto injector.

F. Sample preparation

Pipette 10 ml aliquots of raceway water sample into a 50 ml Pyrex glass centrifuge tube, then proceed according to steps E-3 to E-8.

All raceway water samples are stored in refrigerator at 0-4°C prior to assay.

Table 1

Recovery of Ormetoprim (OMP) from Fortified Control Raceway Water

	Level (ug/ml)		
	0.01	0.02	0.05
% Recovery	93.7	91.3	93.2
	99.1	94.4	89.5
	86.1	103.5	89.8
	92.2	101.1	90.3
	86.4		91.4
	97.1		
	97.2		
x	93.1	97.6	90.8
S.D.	5.23	5.69	1.50
Rel. S.D.	5.61	5.83	1.66

Level Average	93.5
S.D.	4.98
Rel. S.D.	5.33

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G. Calculations

1. Fortified sample recovery.

A comparison between the known quantity of reference standard and the fraction of the sample introduced onto the column.

$$\% \text{ Recovery} = \frac{\text{Corr. Peak Area OMP in the Fortified Sample}}{\text{Peak Area of Reference Standard}} \times \frac{\text{Conc. Ref. Std (ug/ml) x Vol. Corr. Factor}}{\text{Spiked Drug Quantity (ug)}} \times 100\%$$

2. ug per ml OMP in water sample

$$\text{ug/ml} = \frac{\text{Corr. Peak Area OMP in Sample}}{\text{Peak Area of Reference Standard}} \times \frac{\text{Conc. Ref. Std. (ug/ml) x Vol. Corr. Factor}}{\text{Sample Vol (ml) x \% Recovery}}$$

Corr. Peak Area = Peak Area minus Control Background

G. Chen

GC:kg

INTEROFFICE CORRESPONDENCE

ROCHE

097

TO Dr. A. MacDonald DATE March 24, 1983

FROM G. Chen

SUBJECT Assay of Ormetoprim (OMP) in Sediment by Hplc Procedure (Book #10459, pp. 186-187)

Hplc procedure reported in IOM, GC3-15 (March 21, 1983) is applied to assay OMP concentration in sediment with minor adjustment. The sample handling and extraction sequence are outlined:

1. Sample handling -- Refer to GC3-14 report (March 16, 1983)

2. Extraction procedure for sediment precipitate (ppt)

   a. Weigh exactly one gram sediment ppt into a 50 ml Pyrex glass centrifuge tube; add 10 ml deionized distilled water to form suspension.

   b. Adjust pH to 10.50 (± 0.10) by adding 0.5N sodium carbonate (~5-6 ml).

   c. Add 30 ml methylene chloride; shake for 10 minutes on a reciprocating shaker at maximum speed.

   d. Centrifuge at 2000 rpm for 10 minutes or until two phases separate completely; aspirate off all top layer including sediment and discard.

   e. Measure 5 ml methylene chloride extract into a new Pyrex glass tube; then evaporate to just dryness under a stream of nitrogen at ~40°C.

   f. Reconstitute the residue with suitable volume of hplc mobile phase within the calibration curve range; make dilution, if necessary.

   g. Inject 50 ul final extract onto hplc column through Waters WISP auto injector.

3. Extraction procedure for sediment filtrate.

   a. Pipette 10 ml aliquots into a 50 ml Pyrex glass centrifuge tube.

   b. Adjust pH to 10.5 (± 0.10) by adding 0.5N sodium carbonate (~5-6 ml).

   c. Add 30 ml methylene chloride; shake for 10 minutes at a reciprocating shaker at maximum speed.

   d. Centrifuge at 2000 rpm for 10 minutes or until two phases separate completely; aspirate off all top layer including interface and discard.

   e. Measure 25 ml methylene chloride extract into a clean centrifuge tube; evaporate to just dryness under a stream of nitrogen at ~40°C.

f & g. Proceed 2-f and 2-g steps.

Recovery from fortified samples is not available.

G. Chen

GC:kg