

TABLE OF CONTENTS

Abstract . . . . . 3

List of figures . . . . . 5

Acknowledgements . . . . . 6

Introduction . . . . . 7

    A. Purpose and Objectives . . . . . 10

    B. Related Research and Activities . . . . . 10

Methods and Procedures . . . . . 18

Principal Findings and Significance . . . . . 21

Conclusions . . . . . 22

Literature Cited . . . . . 24

## ABSTRACT

### EFFECT OF DRYING ON CYROMAZINE LOSS FROM SURFACE-APPLIED CAGED-LAYER MANURE

In Arkansas, much of the environmental concern related to water quality has focused on the high volume of poultry fecal waste spread on the surface of local pastures to fertilize forage grass. Cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine), a feed-through larvicide used to control house flies (Musca domestica), is often a component of caged-layer manure. Cyromazine is quite soluble and stable in water, and previous research has shown that it can be readily washed from pasture plots by intense rainfall. Therefore, a study was conducted to evaluate increased drying time as a best management practice technique for reducing cyromazine runoff losses from pasture fertilized with caged-layer manure. The objective of this study was to compare cyromazine runoff losses from plots with different drying intervals between the manure application and first rainfall event. Fescue plots with uniform slope and dimensions were used to simulate pasture. Each plot had a runoff collector and borders to isolate plot runoff. Manure was analyzed for cyromazine content, applied to the plot surface at 3.76 Mg/ha, and allowed to dry for 1 or 7 days. Simulated rain was then applied at 50 mm/h to generate 30 min of runoff from each

plot. Plots with manure that dried for 7 days had significantly less runoff than both the control plots that dried for 7 days and manure plots that dried for only 1 day. Analyses of runoff samples indicated that increased drying time following manure application reduced the amount of surface runoff and reduced the concentration of cyromazine in the runoff. The resulting decrease in cyromazine runoff loss implies that increased drying time may serve as an effective best management practice for reducing cyromazine losses in runoff from pastures fertilized with caged-layer manure.

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Keywords -- Agriculture / Pesticides / Water Quality / Poultry Manure / Surface Runoff / Solute Transport / Cyromazine

LIST OF FIGURES

	<u>page</u>
Figure 1. Effect of drying time on mean runoff from pasture plots treated with caged-layer manure.....	28
Figure 2. Surface runoff during a simulated rainfall event from two pasture plots treated with caged-layer manure.....	28
Figure 3. Effect of drying time on cyromazine concentration in runoff from pasture plots treated with caged-layer manure.....	29
Figure 4. Cyromazine concentration during a simulated rainfall event in runoff from two pasture plots treated with caged-layer manure.....	29
Figure 5. Effect of drying time on percent loss of cyromazine in runoff from pasture plots treated with caged-layer manure.....	30
Figure 6. Cyromazine percent loss during a simulated rainfall event in runoff from two pasture plots treated with caged-layer manure.....	30

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## INTRODUCTION

Environmental regulatory agencies and the general public have become concerned recently about possible contamination of regional water supplies by various local industries, including agricultural industries. In Arkansas, much of the environmental concern related to water quality has focused on the poultry industry because it is a large, concentrated, growing industry that produces a high volume of fecal waste. In fact, the Arkansas poultry industry is the single largest agricultural industry in the state and led the nation in 1990 by producing over 950 million broilers, 3.6 billion eggs, 22 million turkeys, and approximately 1.7 million metric tons (dry weight) of poultry fecal waste (USDA, 1991; Moore et al., 1994). Most of Arkansas' poultry production is concentrated in the northwestern corner of the state. This same area also receives nearly all of the state's poultry fecal waste, thus allowing producers to avoid the expense of transporting it over long distances.

Disposal of the fecal waste relies heavily on land application without incorporation. Most poultry manure is spread on the surface of nearby pastures to fertilize the forage grass, usually at a rate of 2-5 tons (dry weight) of poultry manure/acre. Although the practice of spreading poultry manure sufficiently meets the fertilizer needs of most forages, it may also cause water quality problems. Heavy

manure applications closely followed by intense rainfall can significantly increase the concentrations of manure constituents in pasture runoff (Edwards and Daniel, 1993), thus reducing the quality of nearby surface water. Constituents of poultry manure that may cause water-quality problems include nitrogen, phosphorus, carbon, microorganisms, and sometimes pesticides.

Pesticides are commonly used in the poultry industry to control insect populations that develop due to dense animal confinement. House flies (Musca domestica) reproduce rapidly in wet manure and can become a particularly serious problem in late spring and summer, when warm, humid weather may facilitate the development of extremely large fly populations. The pesticide commonly used to control fly populations in caged-layer operations is cyromazine (N-cyclopropyl-1,3,5-triazine-2,4,6-triamine), marketed under the trade name Larvadex. This compound, a feed-through larvicide, is intermittently added to hen feed at a rate of 5 mg/kg during the late spring and summer months, passed unaltered through the animal's digestive tract, and excreted in the manure at residue levels capable of preventing the maturation of fly larvae (Miller and Corley, 1978).

Cyromazine is a relatively stable compound compared to other pesticides, allowing it to linger in the manure as an effective larvicide. Brake et al. (1991) reported that cyromazine continued to show larvicidal activity in caged-

layer manure 20 weeks after it was removed from the feed supply. The half-life for cyromazine in aerobic soil is approximately 142 days (CIBA-GEIGY Corporation, 1990). In water or under anaerobic soil conditions, cyromazine is considered to be stable. Because of its high stability, cyromazine has the potential to be very persistent in the environment. Lim et al. (1990) reported that melamine (1,3,5-triazine-2,4,6-triamine) is the primary metabolite produced from cyromazine decomposition.

Cyromazine also has a relatively high water solubility when compared to other pesticides. At 22°C, the solubility of cyromazine in distilled water is 13.6 g L<sup>-1</sup> (CIBA-GEIGY Corporation, 1990). Cyromazine has relatively low adsorption coefficients ( $K_d$  values of 1-5) for soils low in organic matter. This high water solubility and low  $K_d$  facilitate distribution in the manure, but also increase the potential for transport in pasture runoff.

A recent study at the University of Arkansas showed that when cyromazine was present in caged-layer manure spread on grass plots and a runoff event occurred soon after the manure application, some cyromazine was dissolved and transported in the runoff (Daniel et al., 1992). The study also showed that heavier manure applications and/or higher rainfall intensity increased runoff and total cyromazine loss in the runoff. Lysimeters were used to sample soil water in the vadose zone at the 60-cm depth. Since neither cyromazine nor melamine was



detected in any of the soil water samples, the study gave no indication that these compounds would be any threat to ground water quality beneath pastures treated with caged-layer manure. However, the cyromazine percentage losses (up to 23.7%) detected in the surface runoff were relatively large when compared with losses of other pesticides.

A. Purpose and Objectives.

The research reported here was initiated in an effort to identify a best management practice (BMP) that would reduce cyromazine runoff losses from pasture fertilized with caged-layer manure. The experiment was conducted in the summer of 1992, and evaluated increased drying time as a technique for reducing cyromazine runoff loss. The objective was to compare cyromazine runoff losses from plots with different drying intervals between the manure application and first rainfall event.

B. Related Research and Activities.

Aside from the University of Arkansas study mentioned in the introduction of this report, the literature contains no research data regarding pesticide loss as a result of land application of poultry manure. However, a few researchers have studied the contamination of surface and ground water by other components of land-applied poultry waste. Since some of these other components are also very soluble in water, they

may be transported in a manner similar to that of cyromazine. Therefore, a brief review of some other research on transport of manure components in surface or ground water is presented here.

Liebhardt et al. (1979) applied poultry manure to field plots planted in corn (Zea mays). Each plot was 0.402 ha and application rates of poultry manure ranged from 0 to 179 Mg/ha (wet weight). The only water applied was natural rainfall. Soil samples were taken from the profile, and wells were dug to obtain ground water samples. These samples had significant increases in nitrate levels, which were directly related to the application rate of the poultry manure on the plot.

Khaleel et al. (1980) presented a review concerning the transport of nutrients and microorganisms in runoff from land surfaces treated with animal wastes. Several land surfaces were considered, including pastures and rangelands, feedlots, and cropland. Using linear regression analysis, highly significant correlations between the application rates of nutrients (N and P) and their concentrations and losses in runoff were found.

A study of soil loss and microbiological quality of runoff water was conducted by Giddens and Barnett (1980) on land treated with broiler litter. Using a rainfall simulator, they applied rainfall at a rate of 6.35 cm/h for 2 h to a pasture surface with a 7% slope. They found that high rates of broiler litter reduced total runoff water and soil loss,

but increased bacterial levels in the runoff.

Westerman et al. (1983) also used a rainfall simulator to conduct a laboratory study of soil and nutrient losses from bins of soil (1 m x 0.64 m surface area on a 9% slope) treated with poultry waste. They investigated the effects of changing the manure type, application rate, soil series, drying time, and rainfall intensity. Their results showed that the quantity of contaminants in the runoff water was directly related to the application rate of the poultry waste.

McLeod and Hegg (1984) compared the quality of runoff water from pastures treated with different nitrogen amendments, both organic (poultry manure) and inorganic (ammonium nitrate). Test plots with slopes of 3-5% were established in a ladino clover (Trifolium repens L.) and tall fescue (Festuca arundinacea) pasture. Rainfall was controlled with an irrigation system. Runoff samples had relatively low nitrogen and phosphorus contamination.

Research on cyromazine has concentrated primarily on the effectiveness of the compound for controlling various insect pests. Miller and Corley (1978) found that it was very effective in controlling the manure-breeding house fly (Musca domestica L.) and little house fly (Fannia canicularis L.). Levels of 1.25 and 5.0 mg/kg in poultry feed gave > 99% total mortality of the house fly and little house fly, respectively. Residues of the compound were detectable in the poultry eggs, liver, and muscle when it was fed at a rate of 12.5 mg/kg of

feed. Mulla and Axelrod (1983) confirmed the effectiveness of this treatment and also showed that cyromazine effectively controlled flies when it was sprayed directly on the manure surface.

Iseki and Georghiou (1986) tested several different strains of the common housefly (Musca domestica L.) to determine their resistance to cyromazine. Some strains were produced in the laboratory and others developed naturally at various field locations. Only one strain, from a Pennsylvania poultry farm, had developed moderate resistance after previous exposure to cyromazine.

Skovmand (1988) found that cyromazine was effective against houseflies in pig manure when mixed at a rate of 5 mg/kg in the pig feed. The cyromazine was excreted mainly in the pig urine. The effects are more temporary in pig manure than in poultry manure, probably because cyromazine is diluted and washed away more quickly.

Lonsdale et al. (1990) tested cyromazine in Australia as a pour-on prevention for cutaneous myiasis in sheep. They showed that it effectively controlled the sheep blowfly (Lucilia cuprina) which causes the disease. Their research indicated that sheep may be effectively protected for 8 weeks following a pour-on treatment. O'Brien and Fahey (1991) confirmed these results by testing cyromazine on sheep in Ireland. They compared the effectiveness of pour-on cyromazine treatments and conventional diazinon dipping. The

pour-on treatment compared favorably with the conventional dipping, and was effective for as long as 13 weeks after the initial treatment. Retreatment at 8-week intervals gave complete control of the disease.

Schmidtman et al. (1989) showed that feeding cyromazine to dairy calves could help control house flies in outdoor calf hutches. The cyromazine is excreted in the calf urine.

Friedel (1986) found that cyromazine strongly inhibits the development of dog fleas (Ctenocephalides canis). Therefore, it may have potential as a flea larvicide.

Although most triazine compounds are used primarily as herbicides, cyromazine has not been effective in this regard. However, cyromazine is effective in controlling some of the insect pests that can cause damage to plants. For instance, it is often used as a foliar spray to control leaf miners (Liriomyza spp.) in vegetables and ornamentals (Royal Soc. of Chem., 1988).

Hughes et al. (1989) fed cyromazine to larvae of the tobacco hornworm (Manduca sexta L.) at the fifth instar stage. Feeding rates of >20 mg/kg were fatal to the larvae. Hayden and Grafius (1990) found that cyromazine was also effective against onion maggot larvae (Delia antiqua) during the molt between the first and second instar stage. The soil was treated with cyromazine at a rate of 17.5 mg/kg.

Very little information has been published concerning the environmental fate of cyromazine and melamine. Under

laboratory conditions, Jutzi et al. (1982) found that one strain of Pseudomonas sp. bacteria was able to completely metabolize melamine, which was supplied as the only nitrogen source. Melamine was apparently degraded hydrolytically with three successive deaminations to cyanuric acid (2,4,6-trihydroxy-s-triazine), which retains the basic triazine ring structure. The melamine was first converted into equimolar amounts of ammeline and  $\text{NH}_4^+$ . Ammeline was then converted into equimolar amounts of  $\text{NH}_4^+$  and ammelide, which in turn was converted into equimolar amounts of  $\text{NH}_4^+$  and cyanuric acid.

Cook et al. (1984) reported that a strain of Pseudomonas spp. was able to use cyromazine as its sole nitrogen source in laboratory experiments. The degradation process was similar to that observed for the bacterial degradation of melamine. Cyromazine was deaminated first to cyclopropylammeline and then to cyclopropylammelide, which in turn was converted to cyanuric acid plus cyclopropylamine. This bacterial activity continued under both aerobic and anaerobic conditions, and produced 2 moles of  $\text{NH}_4^+$ , 1 mole of cyclopropylamine, and 1 mole of cyanuric acid from each mole of cyromazine. The complete cleavage of the triazine ring structure does not appear to be a major degradation mechanism in the soil, since the pesticide dissipation has not been correlated with carbon dioxide evolution (Kaufman and Kearney, 1970).

Lim et al. (1990) investigated the rate of cyromazine loss from three Brassica spp. vegetables in field trials, and

the rate of cyromazine photodegradation on glass dishes exposed to direct sunlight. Cyromazine was applied to the vegetable crops in a foliar spray at the rate of 0.56 kg/ha (0.5 lb per acre). In 7 days, the total amount of cyromazine in the plant and on its surface declined by an average of 50%, while melamine recovery showed a 3-5 fold increase. However, since the melamine content always remained below 11% of the total residue, some of the cyromazine and/or melamine must have been lost or degraded to other products.

In the photodegradation study (Lim et al., 1990), control dishes that were protected from sunlight showed very little cyromazine loss, so cyromazine losses from plates exposed to the sunlight were attributed to photochemical reactions. The percentage of cyromazine lost from the glass dishes depended on the amount originally applied, with heavier applications resulting in lower percentage losses. The top layers of cyromazine molecules apparently shielded the lower layers from the sunlight, resulting in less photodegradation. The average cyromazine half-life for all of the dishes exposed to direct sunlight was about 5 days. The amount of melamine in the dishes increased with exposure time, but recovery levels accounted for a maximum of only 53% of the cyromazine loss.

Melamine was plated on petri dishes and exposed to sunlight to investigate the possibility that it might also photodegrade. Results showed a relatively slow rate of melamine loss, with an average of approximately 80% remaining

after 7 days.

Photodegradation of cyromazine on soil has been reported to occur at a much slower rate than on glass dishes. When applied to a soil with pH of 7.5, bulk density of 1.28 g/cm<sup>3</sup>, water content of 15.83%, organic matter content of 1.9%, and texture of 63.2% sand, 20.0% silt, and 16.8% clay, cyromazine photodegraded with a half-life of 60 days (CIBA-GEIGY Corporation, 1990). The decomposition of the cyromazine appeared to be a first-order reaction and the primary photoproduct was identified as melamine.

Significant losses of cyromazine by volatilization seem unlikely in the field, since the vapor pressure is only  $3.3 \times 10^{-9}$  mm of Hg at 25°C (CIBA-GEIGY Corporation, 1990). However, relatively small volatilization losses might occur in cases of prolonged exposure to direct sunlight.

Field losses of cyromazine due to crop removal might be fairly significant. When applied to the leaves of plants, cyromazine shows a strong translaminar effect (Royal Soc. of Chem., 1988). When applied to the soil, cyromazine is absorbed by plant roots and translocated upward through the plant (acropetally).

The literature contains little information concerning the possible impact of cyromazine and melamine in surface or ground water. Melamine has been reported to be carcinogenic in laboratory rats (Melnick et al., 1984; Zeiger, 1987), but this may be only a secondary effect from melamine-induced



renal bladder stones (Heck and Tyl, 1985).

Laboratory experiments have shown the lethal cyromazine dose for 50% (LD<sub>50</sub>) of rats to be 3387 mg/kg taken orally, 3100 mg/kg when taken through the skin, and 2.72 mg/L of air when inhaled for 4 hours (Royal Soc. of Chem., 1988). The LD<sub>50</sub> for birds was found to be 1785 mg/kg for bobwhite quail, 2338 mg/kg for Japanese quail, and 2510 mg/kg for mallard ducks. Toxicity tests have shown that the lethal concentration for 50% of fish exposed for 96 hours to cyromazine is 90 mg/L for bluegill sunfish, and 100 mg/L for rainbow trout.

#### METHODS AND PROCEDURES

Pasture was simulated with field plots established in tall fescue (Festuca arundinacea Schreb.) at the University of Arkansas Agricultural Experiment Station in Fayetteville on Captina silt loam (fine-silty, siliceous, mesic Typic Fragiudult). The plots were of uniform slope (5%) and dimensions (1.5 m across the slope and 6 m down the slope). Each plot was surrounded by a border to isolate plot runoff water and fitted with a self-cleaning runoff collector as described by Edwards and Daniel (1993).

To obtain better uniformity in the initial soil water content, all plots were saturated with water using low intensity irrigation and allowed to drain for 96 h before

manure application. Caged-layer manure was sampled and analyzed for cyromazine, melamine, nutrient, and water content before being applied to the plots. It contained 83% water by weight and 1970  $\mu\text{g/L}$  of cyromazine, but no melamine. The manure was applied manually to the surface of the plots as uniformly as possible. The manure application rate was 3.76 Mg/ha (dry weight), so the actual cyromazine application was 43.6 g/ha. At the time of manure application, the fescue provided full ground cover and was cut to a height of 10 cm.

The experiment had two drying intervals (1 day or 7 days) between the manure application and rainfall event. During the drying interval, plots were protected by plastic-covered wooden frames only for the duration of each natural rainfall event. Due to a lack of available plots, a control treatment (0 Mg/ha manure rate) was included only for the 7-day drying interval. There were three replications of each treatment, so 9 plots were required for the experiment. Treatments were assigned to plots in a randomized block design and the runoff results were statistically analyzed using analysis of variance (ANOVA) to determine the least significant difference (LSD) required to separate the means. For cyromazine comparisons (runoff concentration, total loss in runoff, and percent loss in runoff) only two means were being compared, so the p-value is the only statistic given.

After the designated drying interval, a simulator described by Edwards et al. (1992) was used to generate 30

minutes of runoff from each plot by applying simulated rainfall at an intensity of 50 mm/h. Collecting all of the runoff from each plot was not feasible, but a representative composite sample of the runoff from each plot was obtained in the following manner. A discrete runoff sample was collected in the middle of each 5-minute interval of the runoff event. Each sample volume was divided by the time required to take the sample to obtain the mean flow rate for the 5-minute interval. The flow rate was then multiplied by 5 minutes to obtain the total volume of runoff leaving the plot during that interval. The runoff values for all of the time intervals were added together to determine the total volume of runoff from the plot during the rainfall event. Using this runoff volume data, a 1-liter composite sample was constructed from the discrete samples in a flow-weighted manner to be as representative as possible of the total runoff from that plot. All samples were stored in amber glass bottles in the dark at 4°C until extracted and analyzed for the presence of cyromazine and melamine using a procedure described by Daniel et al. (1992). One set of discrete samples from each treatment was analyzed with the composite samples.

## PRINCIPAL FINDINGS AND SIGNIFICANCE

The mean total surface runoff during the simulated rainfall event (Fig. 1) was significantly ( $\alpha=0.05$ ) greater from plots with the 1-day drying time (8.2 mm) than from manure plots that dried for 7 days (3.2 mm). This should be expected since the extra 6 days of drying time allows the soil moisture content to decrease significantly, thus increasing the capacity of the soil for infiltration by rainfall. However, the results surprisingly showed that plots with manure that dried for 7 days also had significantly ( $\alpha=0.05$ ) less runoff than the control plots (7.5 mm) that dried for 7 days. One possible explanation is that the manure that dried and degraded for 7 days may have somehow enhanced the infiltration rate of water into the soil surface.

The discrete sample runoff rate (Fig. 2) showed a fairly steady increase during the course of the rainfall event, from 0.92 (first 5 min of runoff) to 2.32 mm (last 5 min of runoff) for the 1-day manure drying time and from 0.09 to 0.42 mm for the 7-day drying time.

The mean cyromazine concentration in the runoff (Fig. 3) was 47.7 and 23.0  $\mu\text{g/L}$  for 1-day and 7-day drying times, respectively, with a p-value of 0.21. If the 7-day drying time did increase the infiltration rate, more cyromazine may have infiltrated with the resulting flush of additional water into the soil, thus reducing the amount of cyromazine

remaining to be transported in runoff. Discrete sample cyromazine concentrations (Fig. 4) declined steadily during the rainfall event, from 64.7 to 20.1 and 19.4 to 10.3  $\mu\text{g/L}$  for the 1-day and 7-day drying times, respectively.

The total cyromazine runoff loss is reported per unit area of plot surface, and is also given as a percentage of the total cyromazine applied to the plot in the manure. Total cyromazine loss in the surface runoff (Fig. 5) was 3.98 g/ha (9.0%) and 0.82 g/ha (1.9%) for the 1-day and 7-day drying times, respectively, with a p-value of 0.12. The apparent decrease in cyromazine runoff loss with increased drying time was caused by decreases in both the amount of runoff and cyromazine concentration in the runoff.

Discrete samples for the 1-day drying time indicated that cyromazine runoff loss (Fig. 6) during the rainfall event peaked at 0.83 g/ha (1.9%) in the second sampling interval; whereas, the 7-day drying treatment peaked at a 0.04 g/ha (0.1%) loss in the fifth sampling interval.

### CONCLUSIONS

Concentrations of cyromazine detected in runoff were far below the lethal doses reported for large vertebrates. For instance, the  $\text{LD}_{50}$  for bluegill sunfish in 96 h is 90,000  $\mu\text{g/L}$  (Royal Soc. of Chem., 1988). The amount of cyromazine

being used at present is relatively small compared to the use of some other pesticides. Since low levels of cyromazine have not been found to be toxic to humans and other large vertebrates, the immediate danger to them does not appear to be serious at this time. The long-term effect, particularly on many aquatic organisms, soil microorganisms, insect larvae, and earthworms is not known. Since relatively low levels of cyromazine are known to be highly effective in killing several species of insect larvae, it seems likely that it may, at least, affect the insect component of ecological systems. Further investigation of the environmental effects of cyromazine may be helpful.

This study showed that increased drying time following manure application reduced the amount of surface runoff from fescue plots, and reduced the concentration of cyromazine in the runoff. The resulting decrease in cyromazine loss in the surface runoff supports the use of increased drying time as a BMP for reducing cyromazine runoff losses from pastures fertilized with caged-layer manure. If caged-layer manure applications to pastures take place only when rainfall events are unlikely to occur for several days, the loss of cyromazine to surface water may be substantially reduced.

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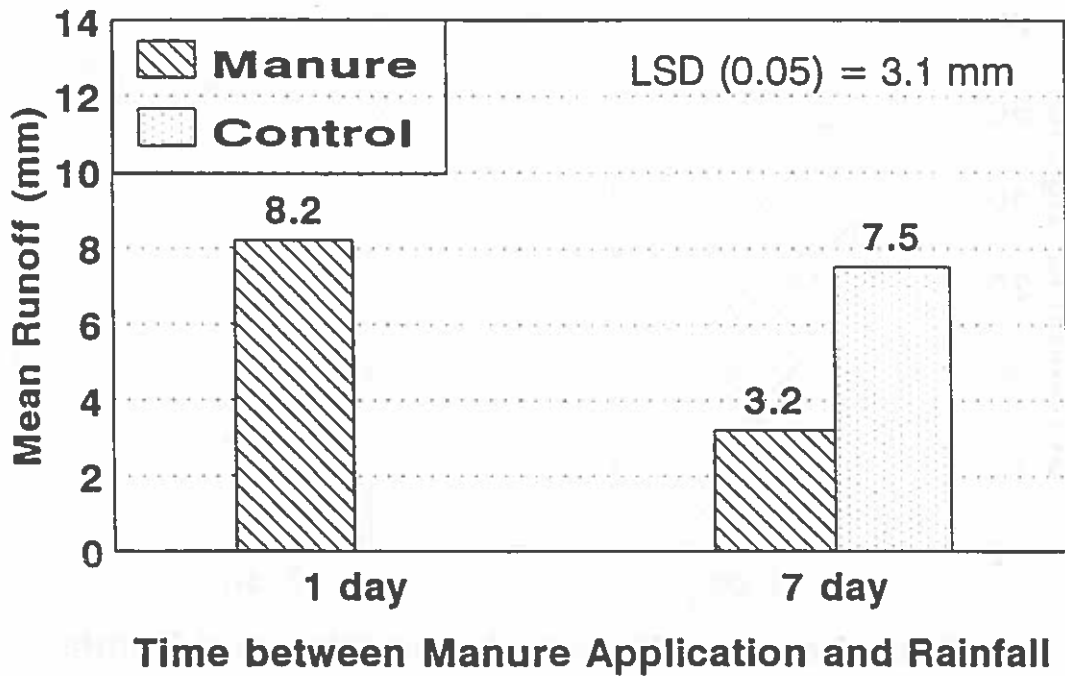


Fig. 1. Effect of drying time on mean runoff from pasture plots treated with caged-layer manure.

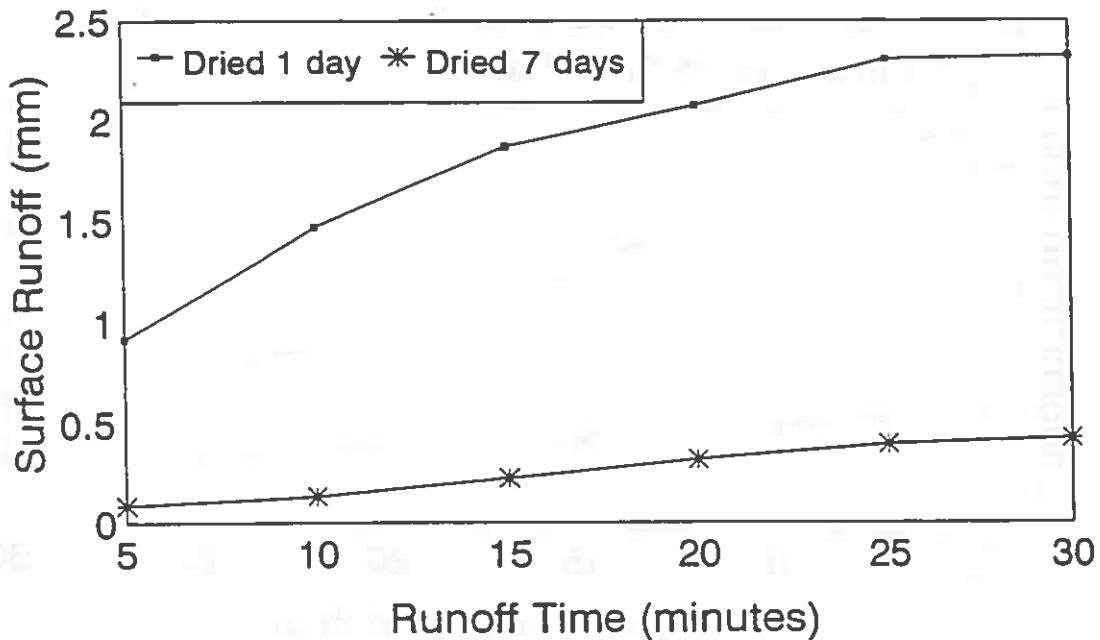
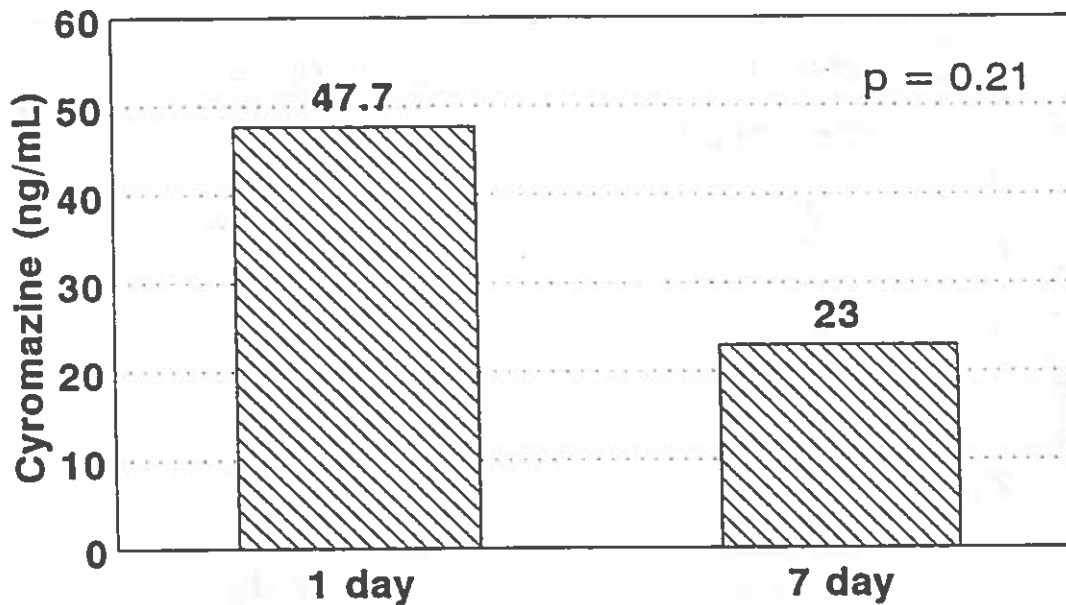
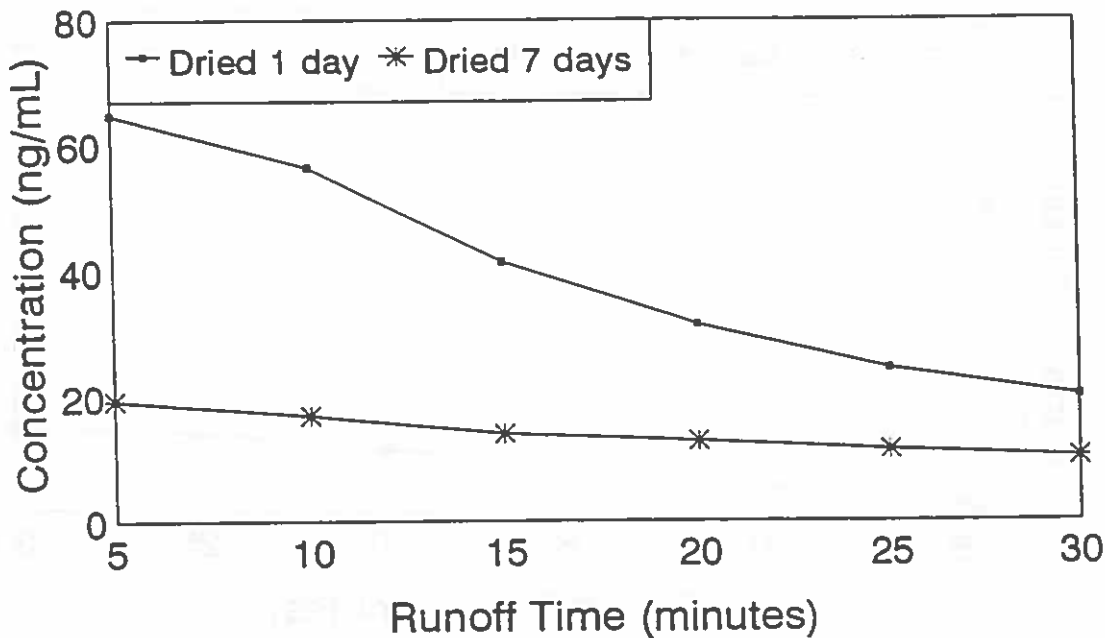


Fig. 2. Surface runoff during a simulated rainfall event from two pasture plots treated with caged-layer manure (developed using discrete samples from only one plot for each treatment).



**Time between Manure Application and Rainfall**

**Fig. 3. Effect of drying time on cyromazine concentration in runoff from pasture plots treated with caged-layer manure.**



**Fig. 4. Cyromazine concentration during a simulated rainfall event in runoff from two pasture plots treated with caged-layer manure (developed using discrete samples from only one plot for each treatment).**

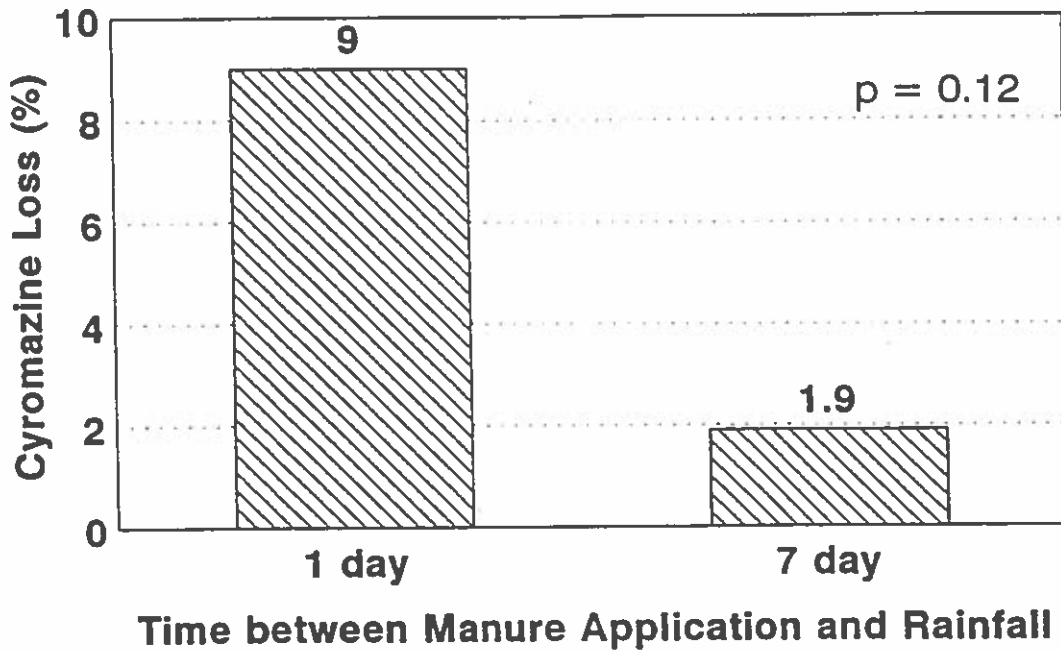


Fig. 5. Effect of drying time on percent loss of cyromazine in runoff from pasture plots treated with caged-layer manure.

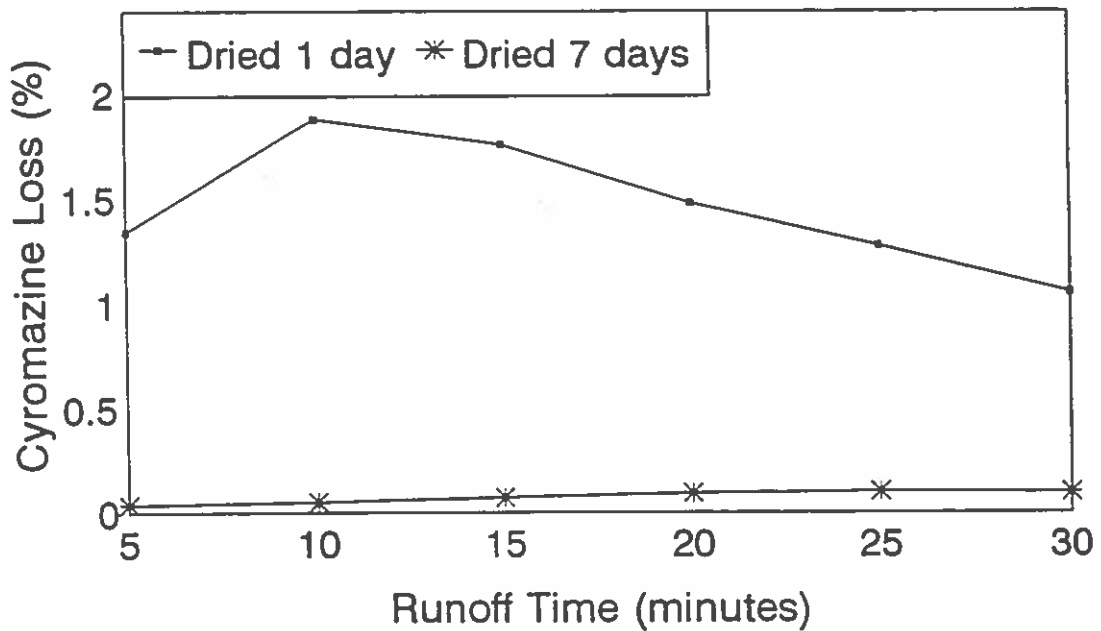


Fig. 6. Cyromazine percent loss during a simulated rainfall event in runoff from two pasture plots treated with caged-layer manure (developed using discrete samples from only one plot for each treatment).

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This ensures transparency and allows for easy verification of the data.

In the second section, the author details the various methods used to collect and analyze the data. This includes both primary and secondary data collection techniques. The analysis focuses on identifying trends and patterns over time, which is crucial for making informed decisions.

The third part of the report addresses the challenges faced during the data collection process. It highlights the need for consistent data entry and the importance of regular audits to catch any errors early on.

Finally, the document concludes with a summary of the findings and recommendations. It suggests that implementing a more robust data management system could significantly improve the accuracy and reliability of the information.

The following table provides a detailed breakdown of the data collected over the past six months. Each row represents a different category, and the columns show the monthly totals and the overall average.

Category	Jan	Feb	Mar	Apr	May	Jun	Average
Category A	120	130	140	150	160	170	143.3
Category B	80	90	100	110	120	130	103.3
Category C	50	60	70	80	90	100	73.3
Category D	30	40	50	60	70	80	53.3
Category E	20	30	40	50	60	70	43.3

Based on the data presented, it is evident that there is a clear upward trend in most categories. This suggests that the current strategies are effective, but it also indicates that there is still room for improvement, particularly in the lower-performing categories.

The recommendations provided at the end of the report are designed to address these issues and ensure that the data remains accurate and up-to-date. Regular training for staff and the use of standardized forms are key to achieving these goals.