Table IV. Recovery of Perthane from 100-Ml. Milk Samples

Type of Sample	Perthane Added		Perthane Recovered	
	μg.	P.p.m.	P.p.m.	%
Milk (control)	0	0	0.060[a]	..
	0	0	0.057[a]	..
	0	0	0.054[a]	..
Milk (fortified)	5	0.050	0.043	85
	10	0.100	0.083	83
	20	0.200	0.160	81
	40	0.400	0.340	86
Milk (fortified after stripping operation)	10	0.100	0.082	82
	20	0.200	0.170	86
	40	0.400	0.320	85
100 ml. H_2O	20	0.200	0.170	86
	20	0.200	0.160	83
	40	0.400	0.340	86

[a] Control values expressed in apparent p.p.m.

tion gave excellent recoveries when a hexane fortifying solution was used, but poor recoveries when Perthane was added in a methanol solvent. It is assumed that the Perthane was not carried first to the butterfat, as is probably the case in a hexane addition. This might indicate, then, since butterfat is always removed in good yield by the hexane extraction, that the Perthane is not carried by the butterfat exclusively but possibly is tied up in milk in other ways. Coinciding with this evidence is the previous observation of the inability of the *n*-hexane extraction to yield a suitable Perthane recovery on stored milk, even though the amount of fat extracted each time remains relatively constant. However, when the acetonitrile-ether method was used, recoveries proved constant over various time periods and the same values were obtained for both the methanol and hexane type fortification.

As in the rat fat procedure, it was necessary to incorporate an acetonitrile extraction prior to dehydrohalogenation to reduce the quantity of fatty residue. Four grams of butterfat when put through the acetonitrile-hexane extraction are reduced to about 0.2 gram. Most of the loss can be traced to the acetonitrile-hexane partition, where Perthane has a partition ratio of 3 to 1 for acetonitrile in this system. Only one extraction with the acetonitrile–diethyl ether is made on the raw milk, since re-extraction does not increase the recovery. When water is substituted for milk, the same per cent recoveries result.

In the rat fat procedure, when adsorption was carried out after the dehydrohalogenation step, high control values resulted; however, in the case with milk, it was necessary to adsorb after dehydrohalogenation in a nonpolar solvent, hexane. The adsorbent adopted was of the same type and formulation as that utilized in the rat fat work.

Results. Results of the chemical assay of 100-ml. samples of raw milk fortified with purified Perthane are shown in Table IV. Samples of untreated milk were fortified before and after the acetonitrile–ether stripping for an indication of losses due to this initial extraction. Both types of fortification exhibited approximately the same per cent recovery. This same recovery was realized when water was substituted as substrate in the procedure. Recoveries were based on determinations using pure Perthane through the basic procedure without substrate, thus reflecting the loss solely due to extraction.

As a further investigation for sensitivity, 200 ml. of milk were analyzed, with the same 85% average recovery. It was expected that higher blank values might develop. However, absorbance readings for the controls showed the same magnitude as obtained with 100-ml. samples and no complications were experienced with this larger quantity of milk.

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INSECTICIDE RESIDUES

Diazinon Residues in Treated Silage and Milk of Cows Fed Powdered Diazinon

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THIS STUDY is a part of a continuing program to find insecticides which may be used to control forage insects without imparting harmful residues to the forage. Harmful residues are those present in amounts which are toxic to livestock ingesting the forage or those which may cause meat or milk contamination when ingested by livestock. The present study concerns the stability of Diazinon in silage and the residues of Diazinon excreted in the milk of cows fed the insecticide in the diet. Diazinon [*O,O* - diethyl - *O* - (2 - isopropyl-4 - methyl - 6 - pyrimidinyl)phosphorothioate] was studied because of its effectiveness against a wide variety of insects. To find the persistence of Diazinon in silage a 22-day, small scale study designed to follow the rate of insecticidal breakdown was undertaken. The insecticide was also administered in various amounts in capsules to dairy cattle to find the maximum permissible dosage.

Experimental Procedure

Immature rye grass of 19.6% dry

This study was initiated to determine the stability of Diazinon in treated silage and the residues of Diazinon excreted in milk of cows fed this insecticide. Diazinon, sprayed on chopped rye grass at the rates of 10 and 100 p.p.m., disappeared rapidly in the silage during fermentation with only about 3% remaining after 22 days of storage. Residues of this insecticide were not detectable in butterfat of cows fed daily doses of as much as 500 p.p.m. of dry matter intake.

matter was sprayed with Diazinon at the rate of 10 and 100 p.p.m. of fresh chopped (3/4-inch cut) forage for examination at 0, 5, 10, and 20 days after storage. The eight treatments were replicated three times with two controls for each replicate. All samples were sealed in quart jars (500-gram size). The three replicates were harvested, treated, and stored successively at 9:00 A.M., 10:30 A.M., and 1:15 P.M. on April 5, 1961. The procedure was to treat duplicate 3000-gram subsamples of fresh chopped forage with 0.120 or 1.20 grams of 25% Diazinon emulsifiable concentrate made up to a 300-ml. volume with water and applied manually with a fly sprayer.

Diazinon recovery in milk was studied with eight milking cows (two per treatment) fed gelatin capsules of 25% Diazinon wettable powder at the rate of 0, 10, 50, and 100 p.p.m. of dry matter intake per day. Samples containing approximately 50% butterfat were separated from the combined morning and afternoon milking of each cow for residue analysis. Samples were collected prior to and on predetermined days after initial dosage. At the conclusion of this portion of the study, the two cows receiving the highest dosages were continued at the rate of 250 p.p.m. and finally 500 p.p.m. Table I indicates the number of days on each treatment and the average daily intake of feed and insecticide.

Table I. Length of Treatment Period and Average Daily Intake of Feed and Diazinon

Description	Daily Dry Matter Intake, P.P.M. 0	10	50	100	250	500
Av. daily dry matter intake (lb.)	23.2	22.3	24.7	23.4	23.4	23.4
Av. Diazinon intake per day:						
Grams	0	0.1	0.5	1.1	2.6	5.3
Mg. per kg. bodyweight	0	0.2	1.0	1.9	4.6	9.1
Days on treatment	17	17	17	17	16	7

Analytical Procedure

Samples were analyzed using the method of Geigy Chemical Co. (*1*). Briefly, this method involves extraction of samples with petroleum ether, partitioning into constant boiling hydrobromic acid, and hydrolysis to liberate hydrogen sulfide by refluxing. The hydrogen sulfide is determined colorimetrically as methylene blue by addition of *N*,*N*-dimethyl-*p*-phenylenediamine and ferric chloride solutions to the hydrogen sulfide collection tube. The absorbance readings are compared to a standard calibration curve for Diazinon. This method determines residues as low as 10 μg. giving a sensitivity of 0.2 p.p.m. in silage and 0.04 p.p.m. in milk. Although the method is nonspecific, it is adequate for an experiment where recovery of a known additive is desired.

The silage samples required no modification in the method. Untreated check samples gave absorbance readings identical to reagent blanks upon development of color. Recovery of Diazinon added to silage varied from 100 to 106%.

Preliminary work showed that Diazinon occurred only in the butterfat fraction of the whole milk. The butterfat was concentrated to about 50% in a cream separator and readily removed by the following method.

About 100 grams of cream was placed in a quart fruit jar. Enough anhydrous sodium sulfate was added to completely tie up all of the water before adding 200 ml. of pentane. The jars were sealed and shaken for 5 minutes. The pentane layer was passed through filter paper into a glass-stoppered Erlenmeyer flask. More butterfat could have been extracted by addition of more pentane if it were needed. The pentane was evaporated using three-ball Snyder columns, and the remaining butterfat was weighed into vials and refrigerated until the analysis was begun.

Further cleanup was required because of interference from the butterfat. This was accomplished by dissolving the sample in pentane and partitioning into acetonitrile. Distilled water was then added to the acetonitrile fraction and the Diazinon partitioned into petroleum ether. After drying over anhydrous sodium sulfate, the petroleum ether layer was treated exactly as the silage extract. This cleanup procedure gave untreated check sample readings which corresponded closely to the reagent blank. Recovery values from whole milk to which Diazinon was added varied from 90 to 102%.

Results and Discussion

Table II presents data on the stability of Diazinon residues during fermentation in silage. Significantly less residue remained at each successive sampling period. This was observed at both the 10 and 100 p.p.m. levels of treatment. Diazinon, sprayed on chopped rye grass at 10 and 100 p.p.m., disappeared rapidly during fermentation, with only traces of residue remaining after 22 days of storage. The highest detectable amounts at the end of this period were 0.4 and 3.0 p.p.m. for the 10 and 100 p.p.m. rates of application, respectively. There is currently no recommendation by USDA for the use of Diazinon on forage.

Table II. Diazinon Remaining in Silage after Varying Lengths of Storage

Days	Diazinon Remaining, %[a]			Mean
	10 P.P.M. Treatment			
0	100.0	100.0	100.0	100.0
5	56.8	56.4	58.0	57.1
12	37.0	33.0	29.0	33.0
22	3.2	4.0	3.2	3.5
	100 P.P.M. Treatment			
0	100.0	100.0	100.0	100.0
5	50.0	75.6	62.0	62.5
12	19.2	30.0	28.3	25.8
22	2.1	3.3	3.0	2.8

[a] Three replicate samples.

Oral administration of Diazinon in capsule form was begun on June 28, 1961. Milk from the two cows on each treatment was sampled at 0, 1, 4, 7, and 14 days after the initial dosage. No traces of residue were detected in the butterfat samples.

Beginning on July 15, 1961, the two cows fed at the highest levels were fed 250 p.p.m. daily with sampling at 5 and 12 days later. On July 31, 1961, the daily dose was increased to 500 p.p.m. and the milk sampled 1 and 3 days later.

Administration of Diazinon was discontinued on August 7, 1961. There was no apparent excretion of the insecticide in milk even at the extremely high level of intake. Diazinon is apparently altered in some manner in the digestive system of the dairy cow since the insecticide was recovered from the butterfat separated from milk to which it had been added.

A maximum single nontoxic dose of 10 mg. per kg. has been reported by Radeleff (*2*) for adult cattle. The two cows at the highest level received 8.2 and 10.0 mg. per kg. daily for a period of 7 days with no apparent symptoms of toxicity.

It appears that forage treated with Diazinon at reasonable rates might be ensiled and fed to dairy cows without concern for residue contamination of the milk or toxic effects on the cow.

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SOIL ADSORPTION OF HERBICIDES

The Adsorption of Monuron and Diuron by Hawaiian Sugarcane Soils

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Chemical analysis of monuron and diuron in aqueous solutions shaken with Hawaiian sugarcane soils has been used to study the equilibrium adsorption and desorption of these chemicals. All soils show greater adsorptive capacity and bond strength for diuron than for monuron; both chemicals exhibit normal isothermal adsorption equilibria. Binding energy, measured as the resistance to desorption, was found to be directly related to adsorptive capacity.

Two pre-emergence herbicides, 3-(*p*-chlorophenyl)-1, 1-dimethylurea (monuron) and 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (diuron), have been used extensively in sugarcane in Hawaii since 1951 and 1953, respectively. Total soil residues from rates of 4 to 5 pounds of active chemical per acre decreased to negligible quantities in about 12 months. Soil residues from normal use are essentially zero during the entire second year of the 2-year crop cycle, and no residual buildup has occurred. Effective control of annual weeds may vary from zero to 90 days, with the average around 40 to 50 days.

Considerable variation in weed control and crop injury, not associated with weed species or climatic factors, has occurred over the approximately 75,000 acres treated each year. Analysis of cane tissues (total plant above ground) and soil samples for monuron or diuron has consistently shown that cane injury could be closely related to plant residue levels in young cane, but that the same injury had little or no apparent correlation with total soil residue from a 0- to 6-inch soil profile sample. Thus, a uniform application rate was producing variable results which appeared to be associated with soil factors.

This article describes experiments designed to measure the adsorptive capacity of a variety of Hawaiian sugarcane soils for monuron and diuron. A series of experiments is also described to support the conclusion that the adsorptive bond strength of soils for monuron and diuron is directly related to the adsorptive capacity. Additional work with other soil active herbicides is in progress.

Hawaiian soils, except for small coral areas, are derived from volcanic basalts or andesites. Although the parent material was fairly uniform, wide variations in climate and vegetation, and in the age of successive lava flows, account for the major differences. Sugarcane is grown on four islands at elevations ranging from near sea level to about 2500 feet, in areas of rainfall from 10 to 150 inches per year, and at average air temperatures of from 78° F. at sea level to about 72° F. in the higher regions with little summer-winter variation. In rainfall areas of less than 60 inches per year, cane is irrigated. Soil types (great soil groups) on which sugarcane is grown include mainly Low Humic Latosols, Humic Latosols, Hydrol Humic Latosols, Humic Ferruginous Latosols, Alluvial, Gray Hydromorphic, and Dark Magnesium Clays with smaller amounts of numerous others. In general, soils are acidic (pH 4 to 8), high in iron, titanium, and aluminum oxides, and low in silica. Clays are principally kaolinite or oxides in the Latosols and montmorillonite in the darker gray soils. Weathering has reduced the soils in most areas mainly to clays (*6*). Carbonaceous residues vary from 0 to 15% with no peat or muck soils.

Experimental Procedure

Adsorption of Monuron and Diuron on Soils. A 50-gram sample of air-dry soil, ground to pass a 2-mm. mesh screen, was mixed with 100 ml. of an aqueous solution containing 6.25 mg. of monuron and 2.5 grams of K_2SO_4 per liter and shaken for 15 minutes at an ambient temperature of 26° ± 2° C. The liquid was decanted through Whatman No. 12 filter paper, and the clear filtrates were analyzed to determine the amount of unadsorbed chemicals by the colorimetric micromethod of Young and Gortner (*8*).

The procedure used for most of the determinations utilized 12.5 μg. of monuron per gram of soil. Other measurements, especially for the Freundlich plots, were made at levels from 3.9 to 312 μg. of monuron per gram of soil. Values given are averages of two or more determinations; blank determinations were run to correct for chemical residue in the soil.

Diuron adsorption was determined similarly on 50 grams of soil with 100 ml. of an aqueous solution containing 5.76 mg. of diuron and 2.5 grams of K_2SO_4 per liter.

Phosphate determinations for comparison of adsorptivity and fixation were made on 50 grams of soil with 100 ml. of an aqueous solution containing 500 mg. of P from KH_2PO_4 and 2.5 grams of K_2SO_4 per liter.

The K_2SO_4 prevented serious de-