acid. But, in the absence of the interfering sulfate, the concentrated phosphoric acid attacks the rock with vigor and releases fluosilicic acid in concentrations that undergo substantial decomposition before it has an opportunity to recombine with the phosphate rock.

The influence of relatively small concentrations of sulfuric acid in phosphoric acid has a practical bearing on fluorine evolution in the manufacture of superphosphate with wet-process phosphoric acid. For example, an acid containing 2.6% sulfur trioxide and 52.5% phosphorus pentoxide corresponds to an 8% replacement of phosphoric acid with sulfuric acid. This could cause an appreciable reduction in fluorine volatilization, according to the results shown in Figure 7.

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INSECTICIDE EVALUATION

Dermal and Oral Treatments of Cattle with Phosphorus-32–Labeled Co-Ral

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Only small amounts of phosphorus-32 were absorbed through the skin and eliminated in the urine following dermal application of the compound to cattle. High levels of the unchanged toxicant were found on the hair several weeks after treatment. The compound was ineffective as a systemic against stable flies and screwworm larvae but highly effective against these insects by contact. On oral treatments, at 10 and 20 mg. per kg., approximately 38% of the dose was excreted in urine as polar degradation products and about 35% in the feces 7 days after treatment.

Co-Ral *O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate, has been shown by several workers (*7, 8*) to be a promising insecticide for the control of second- and third-instar cattle grubs when applied dermally to cattle. Brundrett, McGregor, and Bushland (*1*) have found this compound to be effective against grubs prior to their encystment. To obtain information on the absorption, excretion, and systemic action of this compound, several cattle were treated both dermally and orally with phosphorus-32–labeled Co-Ral at Kerrville, Tex.

Materials and Methods

The phosphorus-32–labeled Co-Ral used in these studies had a specific activity of 426 to 923 counts per minute per microgram and a radiochemical purity greater than 97.5%.

The compound was applied dermally to three Hereford steers as an emulsion prepared from 20% xylene–Triton X-100 concentrate, using a 2-gallon pressure sprayer equipped with a brush on the nozzle.

To prevent ingestion of the insecticide and contamination of the urine, the head and underside of the animals were not treated. The runoff was collected and assayed radiometrically. A summary of dermal treatment data is given in Table I. Precautionary measures were taken throughout the experimental period to

Table I. Dermal Treatment Data with P^{32}-Labeled Co-Ral on Three Hereford Steers

1956–7	Weight of Animal, Kg.	Active Ingredient, %	Intended Dose		Applied Dose		Runoff	
			Grams	Mg./Kg.	Grams	Mg./Kg.	Grams	%
November	152	2	11.4	75	9.8	64.5	1.7	14.9
February	156	2	11.7	75	7.6	48.8	4.1	35.0
March	147	1	4.4	30	4.0	27.2	0.4	9.1

Table II. R_f Values of Co-Ral and Possible Degradation Products Determined by Three Paper Chromatographic Systems

Compound	Normal	Reverse	Pyridine–Ammonium Hydroxide
Co-Ral	0.82	0.07	0.88
Oxygen analog	0.40	0.50	0.87
Diethylphosphoric acid	0.00	0.82	0.45
Diethylthiophosphoric acid	0.00	0.81	0.67

Table III. Radioactivity (μeq./Ml.) of Blood of Three Animals Treated Dermally with P^{32}-Co-Ral

Days	64.5 Mg./Kg.	48.7 Mg./Kg.	27.2 Mg./Kg.
1	0.05	0.03[a]	0.06
2	0.17	0.03[a]	0.08
3	0.21	0.04[a]	0.07
4	0.10	0.03[a]	0.06
5	0.05	0.03[a]	0.06
6	0.07	0.03[a]	0.06
7	0.01[a]	0.02[a]	0.06
8	0.01[a]	0.02[a]	...
9	Trace	0.01[a]	...
10	Trace	0	...
11	0.03[a]	0	...
12	0.02[a]	0	...
13	0.03[a]	0	...
14	0.02[a]	0	...

[a] Standard error greater than ±5%.

Table IV. Radioactivity (μeq./Ml.) of Blood of Two Animals Treated Orally with P^{32}-Co-Ral

Time, Hours	20 Mg./Kg.	10 Mg./Kg.
2	0.08	0.00
4	0.34	0.14
6	0.52	...
8	0.69	0.36
10	0.88	0.44
12	0.71	0.40
16	0.62	...
18	...	0.30
20	0.57	...
24	0.46	0.26
30	0.52	0.12
36	0.46	0.12
42	0.36	0.08[a]
48	0.34	0.09[a]
54	0.28	0.09[a]
60	0.33	0.05[a]
66	0.27	0.02[a]
72	0.27	0.01[a]
84	0.23	0
96	0.16	0
108	0.19	0
Days		
5	0.14	0
6	0.10	0
7	0.08	0

[a] Standard error greater than ±5%.

prevent the animals from ingesting the dermally applied insecticide.

Co-Ral was also administered orally, by capsule, to two Hereford steers. One animal, which weighed 143.3 kg., was treated at the rate of 20 mg. per kg. (2.87 grams), and the other, which weighed 144.2 kg., at 10 mg. per kg. (1.44 grams).

Following treatment the animals were held in a metabolism cage (*2*) and provided with food and water throughout the experiment.

Heparinized blood samples (12 ml.) were taken from the jugular vein. The blood was transferred to a 15-ml. glass-stoppered centrifuge tube, chilled, and held under refrigeration until analyzed. Assays of total radioactive compounds in the blood were made on plated whole blood samples. To determine whether any of the radioactive material in the blood was soluble in organic solvents, 5 ml. were extracted with an equal volume of hexane. Following centrifugation, aliquots of the extract were plated, air-dried, and radioassayed. Control samples in which known quantities of phosphorus-32–labeled Co-Ral were added and extracted resulted in 92 to 94% recoveries.

The urine was collected in a urinal attached solidly to the underside of the abdomen and leading to a 1- or 5-gallon container. It was held under refrigeration until analyzed. Samples of urine were plated for counting.

The feces from the oral treatments were wet-digested by the procedure of Robbins, Hopkins, and Eddy (*5*). Each sample was thoroughly mixed in a polyethylene bag. Two grams were weighed into a gelatin capsule and wet-digested in a calibrated blood sugar tube with nitric acid and hydrogen peroxide. The digests were adjusted to known volumes and aliquots were plated for radioassay. Digestion of three control samples containing known amounts of the phosphorus-32 Co-Ral gave a mean recovery of 86%.

Daily, throughout the experimental period, three samples of hair weighing 100 mg. were taken from different parts of the treated area, close to the skin. The hair was wet-digested with nitric acid and hydrogen peroxide. The digests were then adjusted to known volumes, and thoroughly shaken and aliquots were plated for the determination of total radioactivity. The addition of known quantities of the parent compound to control hair samples and digestion by the same procedure resulted in recoveries above 98%. For chromatographic analyses 100 mg. of hair were extracted four times with 25 ml. of acetone. The extracts were concentrated, and aliquots were applied to paper strips.

To determine the systemic activity of Co-Ral against newly hatched screwworm larvae [*Callitroga hominivorax* (Cqrl.)], the animals were wounded on both shoulders and larvae were placed in the wound. The wound on one shoulder was protected with gauze and adhesive tape, but that of the other was left unprotected. Stable flies [*Stomoxys calcitrans* (L.)] were fed on the hip of the animals after thorough decontamination of this area.

Radioassays were performed with Geiger-Müller tube or a gas flow proportional counter. All samples were plated in duplicate or triplicate. The mean counting rates were corrected for decay and self-absorption and were expressed in micro- or milliequivalents of Co-Ral.

Three paper chromatographic systems were used for the separation of Co-Ral and possible degradation products.

Normal System. The stationary phase consisted of Whatman No. 1, 1-inch strips coated with a 5% (v./v.) solution of Carbowax 400 in acetone. The mobile phase was a mixture of 6.5 parts of Skellysolve B, 2.4 parts of benzene, 1 part of methanol, and 0.1 part of ammonium hydroxide.

Reverse System. The stationary phase consisted of Whatman No. 1, 1-inch strips coated with a 5% (v./v.) solution of Silicone 550 in hexane. The mobile phase was a mixture of 54 parts of water, 43 parts of absolute ethyl alcohol, and 3 parts of chloroform.

Pyridine–Ammonium Hydroxide (*6*). Uncoated strips of Whatman No. 1 were developed with a solvent consisting of 8 parts of pyridine and 2 parts of water (v./v.).

The ascending method was used for all systems. Co-Ral and related compounds were detected radiometrically and colorimetrically. Co-Ral, its oxygen analog, and diethylphosphorothioic acid were detected with acidified potassium permanganate, and the diethylphosphoric acid was detected with the Hanes-Isherwood reagent (*3*). For radioassay the developed chromatograms were cut into 1-cm. sections. The R_f values for Co-Ral and related compounds in the systems utilized are summarized in Table II.

Results

Blood. The blood of all three dermally treated animals showed very low levels of radioactivity (Table III). The maximum concentration of 0.21 μeq. per ml. occurred on the third day in the animal that received the highest dosage. None of the radioactive compounds present in the blood samples of any of the treated animals, at the higher concentrations (0.05 and 0.21 μeq. per ml.) was soluble in organic solvents.

The peak of radioactivity in the blood of the animals subjected to the oral treatments (Table IV) occurred at 10

Table V. Radioactivity Eliminated in Urine of Three Animals Treated Dermally with P^{32}-Co-Ral

Days	Mg./Kg. 64.5	48.7	27.2	Mg./Kg. 64.5	48.7	27.2
	μeq./Ml.			Cumulative % of Dose Eliminated		
1	5.0	9.6	20.6	0.21	0.30	0.78
2	4.6	11.7	19.5	0.41	0.80	1.41
3	6.6	10.4	11.3	0.58	1.09	1.83
4	4.3	6.1	8.8	0.70	1.34	2.16
5	3.6	4.4	7.8	0.82	1.51	2.46
6	1.4	3.5	7.1	0.93	1.65	2.74
7	1.2	2.4	6.9	1.03	1.80	2.96
8	1.2	2.9	...	1.12	1.91	...
9	1.1	2.1	...	1.18	2.01	...
10	1.4	1.6	...	1.23	2.06	...
11	1.1	1.5	...	1.27	2.11	...
12	0.9	1.5	...	1.30	2.15	...
13	0.9	1.0	...	1.34	2.18	...
14	0.6	0.9	...	1.38	2.21	...

Table VI. Radioactivity Eliminated in Urine of Two Animals Treated Orally with P^{32}-Co-Ral

Time, Hours	μeq./Ml. 20 mg./kg.	10 mg./kg.	Cumulative % of Dose Eliminated 20 mg./kg.	10 mg./kg.
1.5	8.4	...	0.04	...
2	...	1.3	...	0.02
4	68.8	3.6	0.64	0.06
6	191.6	...	3.13	...
8	...	95.8	...	2.03
10	322.0	119.0	6.56	3.27
12	...	120.8	...	...
16	289.6	...	10.29	...
18	...	112.8	...	8.28
20	257.2	...	13.40	...
24	216.0	124.8	16.36	10.66
30	201.6	158.4	18.97	16.48
36	185.2	120.0	21.38	20.08
42	159.8	102.9	25.90	24.96
48	117.4	62.7	28.97	26.92
54	96.0	55.4	30.88	28.09
60	84.4	46.4	31.52	29.49
66	87.2	23.3	33.03	30.53
72	52.4	15.9	34.38	30.87
84	22.6	12.7	35.63	31.66
96	14.0	13.2	36.36	32.29
108	12.4	13.2	36.83	32.73
Days				
5	7.0	12.6	37.05	33.06
6	3.8	10.0	37.30	34.03
7	2.8	6.2	37.54	34.92

Table VII. Metabolites in Composite Urine (4 to 72 Hours) of Two Animals Treated Orally with P^{32}-Co-Ral by Pyridine–Ammonium Hydroxide System

Metabolites	Per Cent 20 mg./kg.	10 mg./kg.
Unknown(s)	25.1	26.7
Diethylphosphoric acid	19.9	20.2
Diethylphosphorothioic acid	55.0	53.1

Table VIII. Radioactivity of Feces of Animals Treated Orally with P^{32}-Labeled Co-Ral

Time, Hours	μeq./Gram 20 mg./kg.	10 mg./kg.	Cumulative % of Dose Eliminated 20 mg./kg.	10 mg./kg.
4	...	1.2	...	0.04
6	2.9	...	0.02	...
8	...	13.0	...	0.63
10	57.2	21.1	0.20	1.57
12	99.1	...	0.39	...
16	100.0	...	6.41	...
18	...	38.7	...	2.78
20	100.0	...	11.08	...
24	115.6	50.7	11.15	9.13
30	132.9	58.7	15.11	11.54
36	144.2	64.5	17.33	17.44
42	128.6	41.1	22.79	21.21
48	78.5	31.5	27.51	26.87
54	47.0	26.7	29.62	28.30
60	34.8	21.8	30.84	31.20
66	32.0	12.4	32.18	32.58
72	17.4	7.4	32.91	33.16
84	17.0	5.6	34.03	33.31
96	14.5	4.9	34.86	33.50
108	3.3	3.9	35.13	33.65
Days				
5	1.9	1.6	33.27	33.71
6	1.4	0.6	35.48	33.87
7	1.1	...	35.63	...

hours. On the seventh day the concentration in the animal treated with 20 mg. per kg. was 0.08 μeq. per ml. Only the samples taken from this animal at 6, 8, 10, and 12 hours contained material soluble in organic solvents. The extractable concentrations were 0.01 at 10 hours and 0.02 p.p.m. at 12 hours.

Urine. Relatively low levels of radioactivity were detected in the urine (Table V) for the dermal treatments. The maximum concentration of 19.4 μeq. per ml. occurred on the second day with the animal receiving the smallest dosage. This animal also eliminated the highest percentage of the applied dose (2.96%) over a 7-day period. Because of the low radioactivity levels, no chromatographic analyses were performed with the urine.

Table VI shows close agreement with the oral treatments on the per cent of the dose eliminated in the urine of these two animals. Chromatographic analyses of urine samples from the fourth through the 72nd hour employing the pyridine ammonium hydroxide system (Table VII) demonstrated the known major metabolites to be diethylphosphoric acid and diethylphosphorothioic acid. About 25 to 26% of the radioactivity represented unknown or more polar degradation products.

Feces. In the fecal samples Table VIII) both orally treated animals showed high levels of radioactivity. Radioactivity was initially detected 4 hours after treatment, and the peak occurred after 36 hours. About 34 and 36% of the administered material was accounted for by this route for the 10 and 20 mg. per kg. treated animals, respectively, at the end of 7 days. Chromatographic analyses of chloroform extracts (10 to 120 hours) of the 20 mg. per kg. treated animal demonstrated the presence of Co-Ral (56%), its oxygen analog (32%), and polar degradation products (12%). Because of external contamination, the feces of the dermally treated animals were not analyzed.

Hair. Assay of hair of the animal treated dermally with 64.5 mg. per kg. demonstrated a high retention of the insecticide over a 6-week period. The weekly average readings of radioactivity were 22.2, 19.9, 17.7, 17.2, 17.6, and 15.1 meq. per gram of hair. Chromatography of hair samples taken from the animal at the end of the second, third, and sixth weeks showed the radioactive material present to be unchanged Co-Ral.

Systemic Activity. Co-Ral did not show systemic activity against screwworm larvae infecting the protected wound. Larvae infesting the initially decontaminated but unprotected wound were killed a short time after implantation because of external contamination by the insecticide. Stable flies fed on decontaminated areas were not affected, whereas those fed on contaminated areas were killed within 24 hours.

Discussion

The differences in the levels of radioactivity detected in the blood and urine of the animals treated dermally with phosphorus-32–labeled Co-Ral may be due, in part, to differences in hair thickness as well as structure of the dermis. The low levels in the blood and urine and the high levels of activity on the hair, however, were characteristic for all the dermally treated animals. Co-Ral was less efficiently absorbed from the bovine digestive tract than several other organophosphorus compounds (*5*, *6*), as evidenced by the relative per cent recovered in the urine and feces. The unidentified metabolites excreted in the urine following oral administration may represent a dealkylated metabolite (*4*).

Co-Ral, although systematically ineffective against newly hatched screwworm larvae and stable flies when applied dermally, appeared to be an effective residual insecticide.

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INSECTICIDE RESIDUES

Microdetermination of TDE in Spray Residues

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TDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane, has been used to protect crops from damage by insects. A method is described for its determination as a residue in plant extracts. After partial purification by solvent partitioning and treatment with adsorbent, the residue is dehydrohalogenated to 1-chloro-2,2-bis(p-chlorophenyl)ethylene, in a rapid, selective manner using sodium ethylate in dimethylformamide. Treatment of this alkene with sulfuric acid yields a colored carbonium ion complex with a maximum absorption at 502 mμ. Extraction and cleaning procedures are described, with a discussion of the method.

THE INSECTICIDE 1,1-dichloro-2,2-bis (*p* - chlorophenyl)ethane (TDE, DDD, or Rhothane) is used as an economic poison to protect a variety of crops against insects. The study, as presented here, represents endeavors to develop an analytical method to determine microamounts of spray residues on raw agricultural products.

TDE spray residues are difficult to determine in the presence of certain other chlorinated pesticides, as the residue methods now employed—Schechter-Haller (*9*) and total organic chlorine (*4*)—do not have a high degree of specificity for TDE.

Earlier work in this laboratory had indicated the possibility of a method applicable to dehydrohalogenated TDE using sulfuric acid. Researchers in this field had already pointed out the usefulness of such a procedure with DDT (*1, 3*), methoxychlor (*2*), and Perthane (*8*). Adaptation of existing procedures to TDE failed, because the conventional cleanup sequences gave poor recovery of TDE, and the dehydrohalogenation step could not be carried out reproducibly by boiling in alcoholic potassium hydroxide.

Reagents

All materials are reagent grade, unless specified otherwise.

Alumina, Merck reagent grade.

n-Hexane, technical grade, 95 mole % minimum. This is used for extraction and stripping of fruits and vegetables.

n-Hexane, purified, technical grade, 95 mole % minimum, passed through activated alumina. Using a column 4 cm. in diameter, 1 pound of alumina will clean up 2 gallons of solvent.

Acetonitrile, purified, technical grade, distilled or reagent grade, passed through activated alumina (see *n*-hexane, purified).

Equilibrated solvents. Saturate purified *n*-hexane and purified acetonitrile with each other.

Rhothane purified, technical material, recrystallized from methanol twice. Melting point 110–10.5° C.

Sodium ethylate, 0.1*N*. Store and disperse from an automatic buret protected from the atmosphere by silica gel and Ascarite.

Absorbent mixture.

77 parts sodium sulfate (anhydrous)
5 parts Attasol
5 parts Filter Cel
2 parts charcoal, activated (Nuchar)

Mix well and dry for 24 hours at 110° C. Keep tightly stoppered until used.

Extraction

Because of the multiplicity of problems presented by the variety of samples it is difficult to outline definite extraction procedures. The main purpose is to achieve complete removal of TDE from the sample. Ordinarily, hexane is used and the solvent to sample ratio can be varied according to needs.

Depending on the nature of the analysis, the sample can be macerated before extraction and then a methanol-hexane extraction may be necessary. Extracts are dried over anhydrous sodium sulfate prior to storage. Such extracts can be stored for several months without loss of TDE.

Separation

A 5- to 15-ml. aliquot of the extract, or an amount containing 10 to 50 γ (optimum 30) of TDE, is placed in a 60-ml. cylindrical separatory funnel. Enough *n*-hexane (saturated with acetonitrile) is added to make a total volume of 25 ml., followed by 25 ml. of acetonitrile (saturated with *n*-hexane). The phases are then shaken for 2 minutes for thorough equilibration. After the phases have separated, the acetonitrile layer (lower) is withdrawn into a tube of Type 2 (Figure 1). Four grams of absorbent mixture are added to the tube, which is shaken for 2 minutes. The solution is filtered with suction through a fritted-glass filter funnel (Type 3, Figure 1, funnel only with suction attachment) into a dehydrochlorination tube of Type 1 (Figure 1).

Dehydrochlorination

The acetonitrile solution is evaporated almost to dryness in a water bath at 50° to 55° C., under a gentle stream of nitrogen, with the last bit of solvent being removed at room temperature under nitrogen. Three milliliters of dimethylformamide are added to dissolve the residue, followed by 1 ml. of 0.1*N* sodium ethylate. The mixture is swirled for 1