ed procedure is more precise and at least as specific as the tedious manual method for a broad spectrum of food products. This procedure appears to be the method of choice when sample through-put is a prime consideration and when specificity no better than the fluorometric method is required.

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COMMUNICATIONS

Method to Determine Ronnel and Its Oxygen Analog in Eggs

A simple method to determine ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) and its oxygen analog (dimethyl 2,4,5-trichlorophenyl phosphate) in chicken eggs is presented. With this method, lower limits of detectability of ronnel and its oxygen analog were found to be 0.5 and 2.0 ppb, respectively. Recoveries of the compounds from eggs fortified at 1–80 ppb were in the range of 70–75%.

Ronnel (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) is an organophosphorus insecticide that is used to control external parasites such as ticks, flies, lice, and mosquitoes on livestock. One of the more recent methods developed to determine ronnel and its oxygen analog (dimethyl 2,4,5-trichlorophenyl phosphate) in tissues of cattle was by Ivey and Claborn (1971). Their method works well on a variety of tissues of other species also, but consistent recovery is not obtained when this method is used to determine residues of ronnel and its oxygen analog in chicken eggs. The purpose of this present research was to develop a method adequate for the consistent extraction, cleanup, and analysis to determine ronnel and its oxygen analog in eggs.

EXPERIMENTAL SECTION

All chemicals were reagent grade. All solvents used—acetonitrile, dichloromethane, and hexane (Skellysolve B)—were redistilled in glass. The silicic acid (100 mesh analytical reagent grade powder obtained from Mallinckrodt) used in the cleanup columns was heated 16 hr at 225°, cooled to room temperature, had 20% water added, and then was allowed to equilibrate prior to use (Ivey and Claborn, 1971). The gas chromatograph, Micro-Tek Model 160, was equipped with a flame photometric detector and a 4 mm i.d. × 1.22 m borosilicate glass column packed with Gas-Chrom Q (80–100 mesh) coated with 5% DC-200. Carrier gas was prepurified nitrogen at 75 ml/min. The column was operated at 200°, injector at 240°, and detector at 170°, operating in the phosphorus mode. A Polytron Homogenizer was used to blend the egg samples with the drying agent and the extraction solvent.

Extraction of Ronnel and Its Oxygen Analog from Eggs. Egg samples used in the study were from pooled samples (yolk and albumin) that had been mixed and frozen until analysis. While still frozen, the outer part of the pooled eggs was shaved off to reduce the possibility of obtaining freezer-burned samples. Five-gram samples of the eggs (semi-thawed) were weighed in duplicate into 100-ml beakers. After the samples were allowed to thaw, 5.0 g of anhydrous sodium sulfate was added to each beaker and mixed well. A 25-ml aliquot of acetonitrile was added, and the mixture was blended for 2 min with the Polytron Homogenizer at medium speed (setting of 3); care was taken to loosen the mixture from the bottom or sides of the beaker. An additional 25 ml of acetonitrile was added, and the mixture was blended for 2 more min. After the egg residue was allowed to settle, the supernatant liquid was filtered through folded Whatman No. 1 filter paper into a 125-ml erlenmeyer flask. Another 25-ml aliquot of acetonitrile was added to the egg residue, and it was blended for an additional 1 min. The entire contents of the beaker were transferred to the filter paper, and the generator of the homogenizer and the beaker were rinsed several times with small aliquots of acetonitrile. These washings were poured over the egg residue on the filter paper. After filtration was complete, a three-ball Snyder column was attached to the flask containing the supernatant liquid, and the acetonitrile was evaporated to ca. 5–10 ml. Twenty-five milliliters of hexane was added to the flask and the solution was again evaporated to a low volume. [Hexane and acetonitrile form an azeotropic mixture. The mixture boils at 54.4° when the quantity of acetonitrile is about 26% of total (Horsley, 1947).] The addition of hexane followed by evaporation was repeated twice more or until all traces of acetonitrile were removed.

Cleanup and Quantitation. The concentrated sample was then passed through a cleanup column as described by Ivey and Claborn (1971). The chromatographic column was prepared by adding, in order, a glass wool plug, 2.5 cm of sodium sulfate, 12 g of silicic acid, another 2.5 cm of sodium sulfate, and a glass wool plug. The column was prewashed with hexane. The ronnel was eluted with hexane and the oxygen analog with a mixture of dichloromethane–hexane (3:1). The solvents were again evaporated to about 3 ml with a Snyder column and hot plate. The rest of the solvent was removed at ambient temperature by a filtered dry air stream. The residues of ronnel and its oxygen analog were dissolved in an appropriate volume of hexane (0.5–2.0 ml) and were subjected to separate gas chromatographic analyses. The gas chromatographic conditions have been described previously (Ivey and Claborn, 1971). Extracts of unknowns were compared with known standards for accurate quantitation.

RESULTS AND DISCUSSION

The recovery of ronnel from eggs fortified with the pure compound at levels of 1–40 ppb averaged 74.5% with this method. The detection limit for ronnel in eggs was 0.5 ppb.

The recovery of the oxygen analog of ronnel from eggs fortified with pure analog at levels of 4–80 ppb averaged 72.1% with this method. The detection limit for the oxygen analog by this method was 2.0 ppb. One of the reasons for a detection limit this high is the inability of the cleanup column to completely remove all residue from the oxygen analog extract. This residue causes a masking effect and excessive background when subjected to analysis by gas chromatography.

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Antidotes Protect Corn from Thiocarbamate Herbicide Injury

N,N-Diallyldichloroacetamide and related compounds added in small amounts to EPTC (*S*-ethyl dipropylthiocarbamate) or other thiocarbamate herbicides prevent the onset of herbicide injury to corn plants and greatly increase crop yields. Antidotes of this type provide a novel method to obtain greater selectivity and new crop uses for the nonpersistent thiocarbamate herbicides.

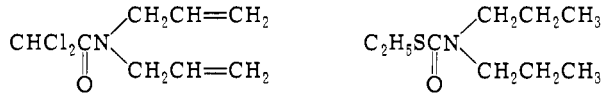
A new concept in weed control involves the use of antidotes to protect crops from herbicide injury (Hoffman, 1962, 1969). We have recently found a new class of herbicide antidotes (Stauffer Chemical Co., 1972) which are superior to previously described compounds for protecting corn from injury by EPTC (*S*-ethyl dipropylthiocarbamate) and other thiocarbamate herbicides.

EPTC, an established herbicide, has many desirable characteristics including low toxicity to mammals and wildlife. It also undergoes rapid degradation in the environment so it is appropriate for use in a crop rotation sequence. Yet, at the levels applied for weed control, it is frequently phytotoxic to corn plants limiting its usefulness on this major crop.

Antidote screening tests were carried out in the greenhouse by incorporating EPTC into loamy sand soil at an excessive rate of 6.7 kg/ha, so that corn planted in the soil was severely injured. The soil was placed in 21 × 31 × 9 cm metal flats to a depth of 7 cm. Hundreds of compounds were synthesized and tested for antidotal activity by coating the corn seeds with up to 0.5% of the compound by weight of the seeds and planting ten seeds per flat 2 cm deep in the soil containing EPTC. The coating was done by placing 50 mg of the compound in a glass vial with 10 g of corn seeds, sealing, and shaking the vial. After growing in the greenhouse for 2 weeks, corn plants were evaluated for injury. The crop injury was rated as follows: the number of plants which showed leaf-rolling and stem-twisting injury symptoms in the treatment were multiplied by 100 and divided by the number of plants in the treatment. Table I gives the results of these tests with 16 compounds chosen from several hundred known active structures.

The most active EPTC antidotes are the N,N-disubstituted dichloroacetamides. The monochloroacetamides are generally less active than the dichloroacetamides. A variety of substituents on the nitrogen atom including alkyl, haloalkyl, alkenyl, and heterocyclic groups impart various degrees of protective activity. Usually compounds having two substituents on the nitrogen atom are more active than those with only one substituent.

Further tests at lower rates showed that *N,N*-diallyl-2,2-dichloroacetamide (R-25788) is the most active in the group and well suited for practical application. When applied at a rate of only 0.1% by weight of the corn seed, it provided complete protection from EPTC at 6.7 kg/ha. Of even greater interest was the discovery that a mixture of EPTC and R-25788 applied to the soil before the seeds were planted still gave complete protection of corn without affecting the control of weeds.



R-25788 antidote

EPTC herbicide

1 part R-25788
+
12 parts EPTC

Eradicane
new corn herbicide

In a loamy sand soil, 0.035 kg/ha of R-25788 was sufficient to protect corn from 3.4 kg/ha of EPTC, while two- and fourfold larger doses of R-25788 were needed for complete protection when the herbicide level was increased two- and fourfold, respectively. Thus, a linear relationship exists between the amount of herbicide applied and the amount of antidote required. Only about 1 part of antidote is needed per 100 parts of EPTC to protect corn plants in the greenhouse. When applied alone, R-25788 has no effect on corn even at 5.6 kg/ha.

While the antidote protects corn from the action of EPTC, it affords no such protection to 24 different species of weeds even at the ratio of 1.1 kg/ha of antidote to 2.2 kg/ha of EPTC. These weeds include johnson grass (*Sorghum halepense*), nutsedge (*Cyprus spp.*), and wild cane (*Sorghum bicolor*), which are of great economic importance worldwide.

The results of tests carried out to determine if R-25788 protects corn from other thiocarbamate herbicides are reported in Table II. The results show that two other commercial thiocarbamates behave like EPTC but antidote protection to the corn plant is incomplete with one herbicide of the same chemical class.