The emulsifying capacity of the extract was found to be 24.8 ± 1.1 ml per g of extract, thus indicating a relatively low value for this property. A rise in this value could probably be expected in an extracted product with a lower oil content than the present one. The extract was found not to form a gel either at 15 or 30% concentrations after being heated in boiling water for 10 to 15 min and refrigerated overnight. Through these tests it was found that the extract was highly dispersible in water at room temperature without presenting any sedimentation at all.

Milk drinks prepared with either 6 or 10 g of extract per cup of milk proved to have an acceptable flavor, texture, and general appearance, thus indicating the possibility of using the material. Cookies prepared by substituting 15 or 30% of the regular wheat flour with the extracted product proved as well to have an acceptable flavor, texture, color, and general appearance, indicating also the feasibility of using the extract in bakery product formulations. During these tests it was observed that the extract had some whippability characteristics.

The acceptability tests of the above products were carried out using ten semitrained panelists and the consumer preference test described by Kramer and Twigg (1966).

From the preceding findings and observing some relationships between chemical and functional properties with possible practical applications as recently reported by Wolf (1970), there remains little doubt that the coconut extracted material obtained through the enzymic chemical technique as reported by Molina and Lachance (1973) could be used for the manufacture of conventional food products, while concomitantly enhancing the total content and probably the quality (Lachance and Molina, 1974) of the protein in the product. Nutritionally this would be so, particularly in cereal products where lysine is the most limiting essential amino acid, since Lachance and Molina (1974) have shown the coconut protein extract to be especially rich in this amino acid. Furthermore, the total protein content of the extracted product exceeds by far that of most cereal flours or products.

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Gas Chromatographic Analysis of Oryzalin Residues in Agricultural Crops and Soil

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A method is described for determining residues of oryzalin (trademark Surflan, Elanco Products Co., Indianapolis, Ind.) [3,5-dinitro-N^4,N^4-di(n-propyl)sulfanilamide] in crops and soil. The samples were extracted with methanol and cleanup was carried out by liquid–liquid partitioning and column chromatography on alumina using 95:5 benzene–ethyl acetate as eluent. Oryzalin was converted to the N^1,N^1-dimethyl derivative for measurement by gas–liquid chromatography using an electron capture detector. Recoveries of oryzalin from crops fortified at 0.05 ppm averaged 72%. Soil samples fortified at 0.1 ppm showed an average recovery of 92%. Residues as low as 0.01 ppm may be detected.
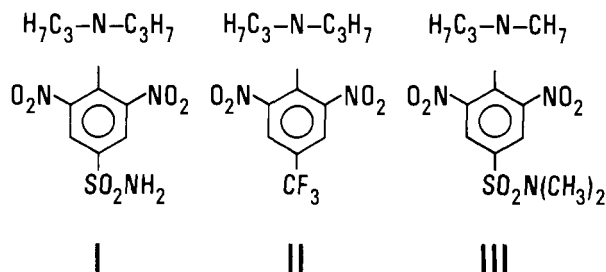
Oryzalin, 3,5-dinitro-N^4,N^4-di(n-propyl)sulfanilamide (I), is a selective, preemergence herbicide for the control of certain annual grasses and broadleaf weeds in soybeans and other selected crops (Burnside, 1970; Elmore, 1972). Unlike the herbicide trifluralin (II), oryzalin cannot be

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quantitatively estimated directly by gas chromatography. Under a wide range of operating conditions, the gas chromatographic peaks are broad and exhibit considerable tailing, making quantitative assessment uncertain. The reduction in theoretical plate efficiency shown by the gas chromatographic peak is most probably associated with the greater polarity and hydrogen bonding potential of the sulfonamide group compared with the trifluoromethyl substituent of trifluralin. When oryzalin is converted to 3,5-dinitro-N^1,N^1-dimethyl-N^4,N^4-di(n-propyl)sulfanilamide (III), the derivative exhibits excellent gas chromatographic characteristics. Sulfonamides which have hydrogen atoms on the nitrogen atom can be alkylated by

treatment with alkyl iodides or alkyl sulfates in an alkaline medium (Shriner et al., 1957). In this paper, we compare these two methods and describe the complete analytical procedure which has been developed for the determination of residues of oryzalin in agricultural crops and soil.

$$H_7C_3-N-C_3H_7 \qquad H_7C_3-N-C_3H_7 \qquad H_7C_3-N-CH_7$$

$$O_2N \underset{SO_2NH_2}{\bigcirc} NO_2 \qquad O_2N \underset{CF_3}{\bigcirc} NO_2 \qquad O_2N \underset{SO_2N(CH_3)_2}{\bigcirc} NO_2$$

$$\text{I} \qquad\qquad \text{II} \qquad\qquad \text{III}$$

EXPERIMENTAL SECTION

Apparatus. The gas chromatograph used for the majority of the determinations was a Hewlett-Packard Model 5713A equipped with a Model 18731A ^{63}Ni linear electron capture (EC) detector. The chromatographic column was 120 cm long glass tubing, 3 mm i.d., containing 5% XE-60 on 80/100 mesh Chromosorb W HP (Applied Science Laboratories, State College, Pa.). A Rinco rotary vacuum evaporator was used for removal of solvent from sample extracts. Chromatographic columns were 14 mm i.d. equipped with a stopcock.

Reagents. The reagents used were analytical reagent grade. The n-hexane, benzene, methyl iodide, dichloromethane, and chloroform were distilled prior to use. Dimethyl sulfate and Alcoa F-20 alumina were used as received. Solutions of the standards of oryzalin and its dimethyl derivative were prepared fresh weekly and stored protected from light.

Standardization of Alumina. The alumina used for the column chromatography step is standardized as follows. A glass wool plug is placed in the bottom of a 14 mm × 250 mm chromatographic column equipped with a Teflon stopcock and 250-ml reservoir. Add 30 ml of benzene to the column. Add 20 g of alumina and rinse down with benzene. Remove entrapped air by stirring and allow the alumina to settle. Gently add 5 ml of anhydrous sodium sulfate to the top of the alumina and drain the benzene to the surface of the sodium sulfate. Add 250 μg of the dimethyl derivative of oryzalin to a flask containing 5 ml of benzene. Transfer to the column and start to drain. Rinse the flask with two successive 5-ml portions of benzene, allowing each to go into the column before the next addition. Add an additional 50 ml of benzene to the column, allow to drain at a flow rate of 3–5 ml/min, and discard the effluent. Add 85 ml of 95:5 benzene–ethyl acetate to the column and observe the movement of the yellow band as the column drains. Note the volume of solvent required to move the leading edge of the band to within 1 in. of the bottom of the column and discard this amount of eluent. Continue the elution of the yellow band and record the volume required to completely remove the yellow color from the column. This volume plus an additional 20 ml will be collected in the column chromatography procedure to ensure complete removal of the compound. Normally, 60–65 ml of 95:5 benzene–ethyl acetate should be collected.

Procedure. *Extraction.* A 25-g sample of the ground and mixed crop tissue is weighed into a quart Mason jar. Methanol is added to make a total of 200 ml of liquid with allowance for water content of the sample. Dry plant material may present a rather bulky sample which absorbs considerable solvent, and therefore is more easily extracted in 400 ml of solvent. Samples are blended on an

Omni-mixer for 5 min and the insoluble matter is allowed to settle. A 10% aliquot is decanted from the supernatant liquid for purification.

Representative soil samples are mixed thoroughly and a 25-g sample is weighed into a quart Mason jar. Methanol (200 ml) is added and the sample is blended for 5 min on an Omni-mixer. The mixture is allowed to settle for a few minutes and a 20-ml aliquot is decanted for derivatization.

Sample Cleanup. Transfer the methanol aliquot from the crop extract to a separatory funnel containing 80 ml of 5% sodium chloride. Wash the aliquot container with 20 ml of methanol and add to the separatory funnel. Wash the aqueous methanol mixture twice with 30-ml portions of hexane, discarding the hexane washes. Add 10 ml of 1.25 N sodium hydroxide to the aqueous methanol to raise the pH to >12. Wash the aqueous methanol with 30 ml of carbon tetrachloride and discard the wash. Additional washes may be used if needed to obtain sufficient cleanup. Dichloromethane may be used in place of carbon tetrachloride. However, the use of dichloromethane or additional carbon tetrachloride washes will reduce the recovery obtained. Add 20 ml or more of saturated boric acid to the aqueous methanol to give a pH of 8–9. Extract the aqueous methanol twice with 20 ml of dichloromethane passing each extract through anhydrous sodium sulfate into a 125-ml boiling flask. Wash the sodium sulfate with 10 ml of dichloromethane. Remove the dichloromethane by rotary vacuum evaporation using a water bath maintained at 50 °C.

The 20-ml aliquot obtained from a soil extract is used for derivatization without prior purification when the methyl iodide procedure is used. If the dimethyl sulfate procedure is used, transfer the 20-ml aliquot of the methanol extract to a 125-ml separatory funnel. Add 40 ml of 5% sodium chloride and 20 ml of dichloromethane to the sample. Shake the funnel and pass the dichloromethane layer through sodium sulfate into a 125-ml boiling flask. Repeat the extraction with an additional 20-ml portion of dichloromethane and combine the extracts. Remove the dichloromethane by rotary vacuum evaporation on a 50 °C water bath.

Derivatization with Methyl Iodide. Add 20 ml of methanol to the dry flask containing the crop extract and swirl to dissolve. Soil extracts are already in 20 ml of methanol at this point. Add 0.5 g of anhydrous sodium carbonate and 3 ml of methyl iodide to the flask. (**Caution:** Methyl iodide is a toxic compound and skin contact or inhalation must be avoided. This chemical is also a dangerous fire hazard.) Stopper lightly and incubate overnight at 50 °C. Following incubation, add 40 ml of distilled water to the flask and transfer the mixture to a 125-ml separatory funnel. Rinse the flask with 20 ml of dichloromethane and add to the separatory funnel. Shake the funnel and pass the lower dichloromethane layer through sodium sulfate into a 125-ml boiling flask. Repeat the extraction with an additional 20-ml portion of dichloromethane and combine the extracts. Remove the dichloromethane by rotary vacuum evaporation on a 50 °C water bath.

Derivatization with Dimethyl Sulfate. Add 5 ml of dioxane to the dry flask and swirl to dissolve the residue. Add 4.6 ml of distilled water, 0.4 ml of 10 N sodium hydroxide, and 0.3 ml of dimethyl sulfate. (**Caution:** Dimethyl sulfate is highly irritating and very toxic if inhaled. Do not allow skin contact. This chemical is also very reactive and a dangerous fire hazard.) Stopper the flask. Agitate the reaction mixture for 20 min at room temperature. Transfer the mixture to a 125-ml separatory

funnel. Rinse the flask with 30 ml of chloroform and add to the separatory funnel. Shake the funnel and pass the lower chloroform layer through sodium sulfate into a 125-ml boiling flask. Repeat the extraction with an additional 30-ml portion of chloroform and combine the extracts. Remove the solvent by rotary vacuum evaporation using a 50 °C water bath with care being taken to ensure complete removal of the dioxane.

Column Chromatography. For samples methylated with methyl iodide, prepare an alumina column using Alcoa F-20 alumina which has been previously standardized. Dissolve the residue in the flask in 5 ml of benzene, transfer to the column, and start to drain. Rinse the flask with two successive 5-ml portions of benzene, allowing each to go into the column before the next addition. Add an additional 50 ml of benzene to the column, allow to drain at a flow rate of 3–5 ml/min, and discard. Add 85 ml of 95:5 benzene–ethyl acetate to the column and collect the volume of elution solvent as determined by the alumina standardization. Remove the solvent by rotary vacuum evaporation using a 50 °C water bath.

Samples derivatized by the dimethyl sulfate procedure should be chromatographed on 100/200 mesh Florisil. Prior to use determine the moisture content of the Florisil and add water to bring the moisture level to 2%. Dissolve the residue in 5 ml of toluene and proceed with the chromatography on a prepared Florisil column. The procedure described above may be followed, except that toluene and toluene–ethyl acetate, 95:5, are substituted for benzene and benzene–ethyl acetate, respectively. The volume of toluene–ethyl acetate required to elute the dimethyloryzalin is determined in a similar manner to the procedure previously described for the standardization of alumina. Remove the solvents from the boiling flask by rotary vacuum evaporation using a 50 °C water bath.

Gas Chromatography. The residue remaining in the flask following evaporation of the column eluate is dissolved in an appropriate amount of benzene for detection and measurement by gas chromatography. Crop extracts are normally dissolved in 1 ml of benzene and soil extracts are dissolved in 2 ml or more of benzene, depending on the anticipated level of oryzalin residue in the soil sample. A benzene solution containing 0.125 μg/ml of oryzalin as the dimethyl derivative is used as a direct standard to determine retention time and establish operating conditions for the gas chromatograph. The injection volume is usually 3.0–3.5 μl. A series of concentrations of standards of the dimethyl derivative (e.g., 0.025 to 0.25 μg/ml) should be prepared in benzene to determine the response curve for the electron capture detector. To facilitate standard curve interpolations, the GC standards may be prepared in terms of oryzalin equivalents (1 μg of oryzalin = 1.08 μg of dimethyl derivative). Otherwise, the mass of dimethyl derivative corresponding to a particular point on the curve must be multiplied by 0.925 to correct for differences in molecular weight of the oryzalin and its dimethyl derivative. The injection of about 0.4 ng of oryzalin as the dimethyl derivative has generally produced a recorder response of 40–60% full scale deflection (FSD) with an electrometer setting of 1.28 × 10^{-10} A full scale. Gas chromatography of samples derivatized with methyl iodide has been carried out on the 5% XE-60 column. A 150-cm glass column packed with 10% SE-30 was used for samples methylated with dimethyl sulfate. The SE-30 phase was prepared on Gas-Chrom Q, 100/120 mesh. This column was used in a Pye Model 104 gas chromatograph with a detector pulse of 150 μs and electrometer setting of 10^{-9} A full scale. Typical operating parameters for the gas

Table I

	5% SE-60	10% SE-30
Column temp	230 °C	217 °C
Injector temp	260 °C	250 °C
Carrier gas	70 ml/min	65 ml/min
Retention time	6 min	5.4 min

Table II. Recovery of Oryzalin from Crops (0.05 ppm) and Soil (0.1 ppm)

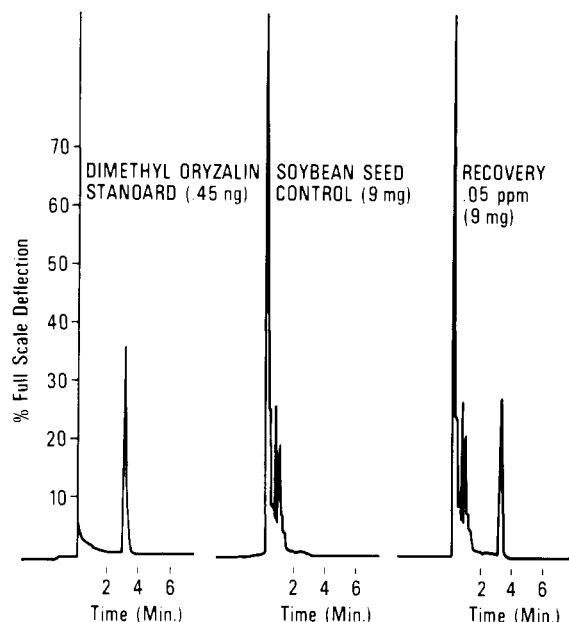| Material	μg added	Av/μg found	% recovery Range	% recovery Av	Coeff of variation
Peaches, plums, and prunes	1.25	0.89	53–85	71	14
Soybeans	1.25	0.91	49–96	73	12
Soil	2.5	2.30	64–103	92	8

Figure 1. Gas chromatograms of dimethyloryzalin standard, control soybean seed, and recovery of 0.05 ppm of oryzalin from soybean seed.

chromatograph are listed in Table I.

The carrier gas for the XE-60 column was argon–methane (90:10) and nitrogen carrier was used with the SE-30 column. The packed columns were conditioned at 250 °C for 24 h prior to use. Standard recovery samples are assayed with each set of experimental samples. Recovery samples are prepared by adding a methanol solution of oryzalin to 25 g of control crop tissue or soil to provide a fortification level of 0.5 or 0.10 ppm, respectively. The efficiency of the derivatization of oryzalin is monitored by treating an amount of oryzalin (methylation check sample) equivalent to the amount present in the fortified samples. This sample should also be passed over the alumina or Florisil column prior to gas chromatography. The residue found in an experimental sample is calculated with a correction for the recovery efficiency of the fortified samples.

RESULTS AND DISCUSSION

Recovery Experiments. The efficiency of the procedure using methyl iodide for methylation was tested by adding a known amount of oryzalin to control samples of various crops and soil. The recovery of oryzalin from fortified samples is reported in Table II. These data represent the assay of more than 20 fortified samples each

Table III. Pesticides Studied for Interference in the Detection and Measurement of Oryzalin

Captan	DCPA
Malathion	Trifluralin
EPN	Chloroxuron
Methoxychlor	Diphenamid
Toxaphene	Nitralin
DDT	Vernolate
Dalapon	CDEC
Diazinon	Alachlor
Azinphosmethyl	Prophos
Carbophenothion	Chloramben
Carbaryl	Chlorbromuron
Disulfoton	Dinoseb
Linuron	CDAA
	Naptalam

of soil and crop tissues. The results have not been corrected for methylation efficiency. Peak height measurements were used in these computations. Figure 1 shows a chromatogram for dimethyloryzalin standard, control soybean seed, and recovery at 0.05 ppm. Methylation check samples using methyl iodide show about 88% efficiency in the conversion of oryzalin to the dimethyl compound. The within day coefficient of variation for the methylation was found to be about 6%. In this laboratory, duplicate recovery samples and methylation check samples are run with each set of experiment samples.

The dimethyl sulfate procedure shows about 90% conversion efficiency for the methylation process with a coefficient of variation of 8%. The average recovery of oryzalin from fortified crops (grapes, broad beans, asparagus, potatoes, wheat) and soil was about 64%. The coefficient of variation for these determinations was 9.3%.

Sensitivity. With the gas chromatograph electrometer set at 1.28×10^{-10} A full scale, the injection of an amount of the dimethyl compound equivalent to 0.438 ng of oryzalin gave an instrument response of 44% FSD. At these conditions, 0.088 ng was readily detected and gave a response of about 10% FSD. Recovery experiments in which 0.25 μg of oryzalin was added to 25 g of soybean seed (0.01 ppm), and a volume of extract equivalent to 0.088 ng was injected into the chromatograph, showed a response of 6% FSD, or about 60% recovery. The control soybean extract showed no peaks and there was no noise in the recorder baseline. Methylation was carried out by the methyl iodide procedure.

Interference Due to Other Pesticides. The pesticides listed in Table III have been studied for interference when using the crop cleanup procedure and methylation with methyl iodide. An amount of pesticide equivalent to the tolerance allowed for soybean seed was added to control soybean seed, both with and without added oryzalin, and assayed according to the procedure. No interference was observed for any of these compounds.

Once carefully optimized, the dimethyl sulfate procedure gave satisfactory derivatization of oryzalin. However, it was found that slight changes in the purity of the reagents and solvent composition led to erratic conversions. Consequently, the method was not considered suitable for residue determinations, although the procedure afforded a rapid conversion. The methyl iodide reaction is considerably slower, but the milder reaction conditions do not lead to decomposition of the oryzalin (which may occur in highly alkaline solutions) and, therefore, this method is clearly preferable for residue determinations of oryzalin.

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Studies of the Degradation of Mirex with an Iron(II) Porphyrin Model System

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Mirex reacts with reduced hematin to yield products formed from reductive dechlorination which include mono-, di-, tri-, and tetrahydro derivatives as well as other more polar decomposition products. The decomposition is rapid with about half of the Mirex initially present being observed after ca. 13.9 min using an initial molar ratio of Mirex to hematin of 1:5. This iron(II) porphyrin system may serve as a model for understanding Mirex degradation under certain environmental conditions.

The insecticide Mirex (dodecachloropentacyclo[5.3.0.-$0^{2,6}.0^{3,9}.0^{4,8}$]decane, 1) has been used extensively throughout the Southeastern United States to control the imported fire ants Solenopsis invicta and Solenopsis richteri. A considerable amount of controversy (Alley, 1973) has evolved concerning the use of this chemical, much of which is due to its unusual stability both in laboratory tests and under environmental conditions. Extensive chemical studies on Mirex (McBee et al., 1956; Eaton et al., 1960; Dilling et al., 1967) show this chemical

to be thermally stable and resistant to most common oxidizing and reducing systems. Studies on the photochemical stability of Mirex have been carried out both in solution and on solid surfaces. Ultraviolet lamp irradiations of Mirex in aliphatic amines as solvents (Alley et al., 1974) lead to two major photoproducts identified as the monohydro and dihydro derivatives 2 and 4, whereas, in hydorcarbon solvents (Alley et al., 1973), the major photoproducts were the monohydro and dihydro derivatives 5 and 6.

The photochemistry of Mirex on silica gel surfaces and chromatoplates using sunlight (Gibson et al., 1972; Ivie et al., 1974a) shows this chemical to decompose slowly with the major photoproduct formed being identified as the

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