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Loss of Label During Processing Cotton Oil Grown with ^{14}C-Aldicarb

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The annual cotton production of Turkey in 1990 has been reported to be about 654,600 metric tonnes (Anonymous 1990). Cotton is grown in large areas, especially in the South-eastern region. Aldicarb (2-methyl-2[methylthio] propionaldehyde-O-[methyl carbamoyl] oxime; TEMIK®, Rhone-Poulenc's registered trademark), a systemic pesticide, controls a wide variety of insects, mites, and nematodes and is used world-wide on more than 40 economically important crops. The white fly, *Bemisia tabaci* Genn (Homoptera Aleyrodidae), a major insect pest, causes extensive loses to the crop in Turkey if no control measures are applied. Aldicarb is a potent acetyl cholinesterase inhibitor and is commercially available only in granular formulations containing 5% to 15% active ingredient. There are many potential uses of aldicarb on field crops and its prolonged pesticidal activity have stimulated detailed research on its metabolic fate in a variety of biological systems. The metabolism of aldicarb as well as certain of its degradation products have been studied in cotton plants by several researchers (Metcalf 1966; Coppedge et al. 1967; Bartley et al. 1970; Bull 1986).

Cotton oil, among other vegetable oils, is one of the essential foods for human nutrition and the residues after oil processing are also used for animal feed. A number studies have been conducted on the residues of several pesticides including aldicarb, in cotton plant and cotton oil (Coppedge et al, 1967; Andrawes et al. 1971a; Andrawes et al. 1971b; Andrawes et al. 1973). However, this problem has not been properly investigated for cotton produced in Turkey and it is not known whether the Turkish people are at risk from the pesticide residues which may be present in the oil. So it is important to determine the distribution of residual levels at each refining step. The present paper reports, for the first time a study undertaken by field application of ^{14}C-labelled aldicarb to cotton plants in Turkey. The radioactive insecticide was utilised to provide information on the mass balance of residual ^{14}C in cotton oil during processing.

MATERIALS AND METHODS

Chemicals: ^{14}C-Aldicarb with the specific activity 6.06 mCi/mmol was a gift from Rhone-Poulenc Co., Istanbul. Unlabeled aldicarb as standard and Temik 15G for the application were also obtained from the same firm. Rotiszint was used as the scintillation solution for Liquid Scintillation Counter (LSC). The cocktail used for trapping $^{14}CO_2$ from combusted organic samples was purchased from Harvey Instrument Corporation. Fuller's Earth (Tonsil) was obtained from a local oil factory in Afyon. All solvents used were of analytical grade.

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Apparatus: A Packard Tricarb 1550 Low Level Liquid Scintillation Counter (LSC) was used for the measurement of radioactivity. Quench corrections were determined using an external standard. A Harvey Biological Oxidizer OX 600 (BO) was used for the combustion of solid samples. A Buchi rotary evaporator was used to concentrate the samples. A steam generator manufactured by Workshop of Ankara Nuclear Research and Training Center was used to obtain super-heated steam for the deodorization of oil.

A selected area of the Research Institute of Rural Affairs in Tarsus was used for this investigation. The experiment was carried out outdoors in boxes measuring 60 x 60 x 60 cm, constructed from galvanised-steel. The bases of boxes contained holes to permit the drainage of excess water which was collected in a metal try. The boxes were covered inside with polyethylene sheets. They were placed into pits so that the upper surface of the soil would be as same as the level of the surrounding ground. The soil (silty clay loam) in the boxes was typical for the cotton growing area in Turkey. At 63 days after planting, cotton plants were treated with a predetermined quantity of ^{14}C-aldicarb. A stock solution of ^{14}C-aldicarb (specific activity 6.06 µCi mM^{-1}) diluted with cold technical material was prepared to contain 0.92 mg of aldicarb per millilitre of 50 % acetone in water. Experimental specific activity was 725.1 dpm g^{-1}. A furrow, approximately 10 cm wide and 10 cm deep, was opened around the plants. 25 millilitres of the stock solution were pipetted into each furrow, spreading the solution over a 5 cm width. The treatment represents an in-furrow application of 2.505 kg of technical aldicarb per hectare (equivalent to the recommended rate of 16.7 kg per hectare for the commercial product containing 15 % aldicarb). The boxes were irrigated as needed throughout the growing season. A summary of weather data on the farm the growing season is shown in Table 1. The cotton seeds were harvested for analysis following 113 days of treatment, at which the plants had reached maturity and bolls were fully opened.

Table 1. Summary of daily temperature and rainfall records taken during the growing season of field-grown cotton plants

Periods	Temperature (°C) High	Low	Rainfall (cm)
May	25.4	13.8	13.0
June	29.3	19.5	0.0
July	30.8	22.3	0.0
August	31.3	22.6	1.0
September	31.5	18.1	5.4

The procedure used for commercial production of crude oil was simulated in the laboratory as outlined in Figure 1. Fibres on the cotton seeds were cut and then the seeds was crushed with a hammer. The cotton seeds were extracted with hexane in Soxhlet apparatus for 12 hours. The extract was evaporated in a rotary evaporator and centrifuged (3000 rpm). Crude oil, cotton seeds and residual cake samples (100-300 mg) were combusted in a Harvey OX-600 Biological Oxidizer to determine the total ^{14}C and the $^{14}CO_2$ counted by liquid scintillation (LS). All determinations were carried out in duplicate.

The refining process followed the outline flow-chart given in Figure 2 (Cavanagh 1976; Mounts 1981). Necessary equipment was constructed or adapted to simulate the refining process in the laboratory. Residual levels of ^{14}C was determined in oil samples taken after each refining step. Samples of crude oil (30-35 g) were heated to 85°C with continuous stirring and then neutralized with a quantity of 2N NaOH

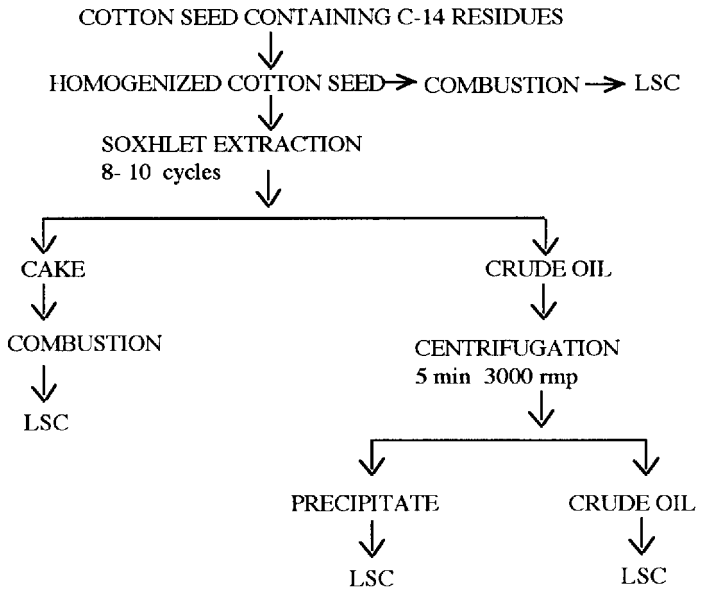

Figure 1. Processing of cotton seed into crude oil (LSC: Liquid Scintillation Counter).

solution. An excess of 2N NaOH (20 % of the amount required to neutralise the oil) was then added. Stirring was continued for 30 minutes and the oil was centrifuged at 3000 rpm. After separating from the soup solution, the oil was washed with hot distilled water until the washing was neutral.

Neutralized oil was heated to 60°C under reduced pressure and 0.15 g of Fuller's earth (tonsil) was added. The mixture was then heated to 100- 110°C for 30 minutes with continuous stirring. The oil was centrifuged and decanted. Bleached oil was mixed with 0.01 g of Perlit. The mixture was then cooled to 5°C for 2-3 days. The oil was centrifuged and decanted. The bleached oil was placed in a two-neck round bottom flask connected to a steam generator the exit line passing through an ice trap. Oil was heated to 200-220°C under a vacuum of 10 mmHg. Super-heated steam was generated by heating distilled water in a closed system at 2 atm pressure. The deodorization was carried out for 3 hours.

The oil was cooled under vacuum and the vacuum released with nitrogen. Free fatty acid (FFA) levels of crude and refined oil were determined according to the procedure given in Turkish standards (Anonymous 1983). FFA values were found to be 1.49 ±0.02 % and 0.12±0.03 % for the crude and refined oils, respectively. The cotton oil obtained in the laboratory is in the class of fine oil (maximum acidity 1.570 in terms of oleic acid).

Reference standards of aldicarb, aldicarb sulfoxide and aldicarb sulfone were applied on a 2 mm thick TLC plate (Merck, Silicagel 60 F_{254}) as spots and developed with ethyl-acetate-acetone (1: 1, v/v) hexane-benzene (2:1, v/v) solvent systems. Crude ^{14}C oil was applied as a band, developed with the same solvent systems. No distinct radioactive bands were observed on TLC. The plate was then

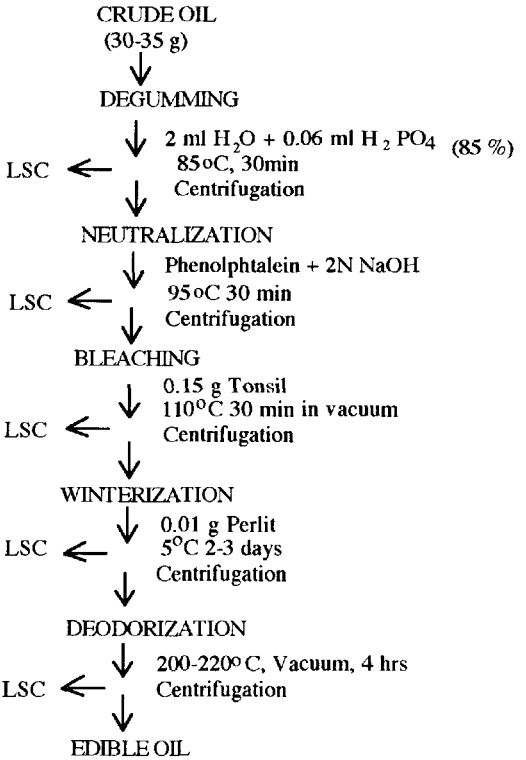

Figure 2. Flowchart of refining processes.

scraped in one centimetre bands, the scrapped material suspended in the scintillation cocktail and counted by LS.

RESULTS AND DISCUSSION

The soil received a total dose of 0.28 g aldicarb containing 90 µCi of ^{14}C before harvesting the cotton seeds. The plants yielded 155.9 g of cotton seeds containing 0.0079 % ^{14}C of the initially applied radioactivity to the soil. This is equivalent to 0.14 ppm of aldicarb in the soil. So, over the 113-days after treatment there was an effective uptake of radioactivity from the soil and systemic movement throughout the plant. A substantial residue remains in the cake, which contained 0.0056 % ^{14}C (0.10 ppm) of the total ^{14}C in cotton seeds. The water washing of the crude oil contained only a very small amount of radioactivity. The final product consisted of crude cotton oil contained 27.2 % ^{14}C (0.19 ppm) of the total ^{14}C in the harvested cotton seeds. The crude oil thus obtained as described above contained 1.49 % FFA.

Andrawes et al. (1973) reported that the lack of appreciable transfer of aldicarb or its carbamate metabolizes to cotton seed from the low level of residue in seed from fields which had been commercial y treated with Temik 15G. Due to the low level

in commercial cotton seed, methodology has not been developed to characterize the seed residues. In our study we were unable to detect any measurable ^{14}C in the aqueous phase when the oil containing ^{14}C was washed repeatedly with water; almost all the radioactivity remained in the oil phase. TLC of crude oil did not result in the separation of any distinct bands. The ^{14}C material was found to be spread with the oil over the whole area of the TLC plate.

The crude oil was further refined according to the scheme outlined in Figure 2 and the ^{14}C in the oil during the refining process is shown in Table 2. At the end of the process the refined oil from the treated seeds contained 35 % ^{14}C (0.07 ppm equivalent aldicarb) of the total in the crude oil. The tolerance limits set by Environmental Protection Agency (EPA) in the USA for aldicarb in cotton seeds has been reported to be 0.1 ppm (Sitting 1980).

Table 2. ^{14}C-Residues in crude oil during refining process *

Sample	Residue (ppm)	Loss (%)
Crude oil	0.19 ± 0.022	0 ± 0.0
Neutralized oil	0.11 ± 0.003	42 ± 1.9
Bleached oil	0.11 ± 0.031	42 ± 2.3
Vinterized oil	0.10 ± 0.029	47 ± 1.2
Deodorized (Refined) oil	0.07 ± 0.022	63 ± 4.0

* All data based on radioassey and expressed as average of duplicate analyses.

Whether the refined oil obtained from the cotton seeds treated with aldicarb in the field is a nonhazardous commodity will remain a matter of conjecture until more information is available on the toxicological nature of the unidentified ^{14}C residues.

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REFERENCES

Andrawes NR, Bagley WP, Herrett RA (1971a) Fate and carryover properties of Temik aldicarb pesticide 2-methyl-2-(methylthio) propionaldehyde 0-(methyl carbamoyl) oxime in soil. J Agric Food Chem 19:727-730

Andrawes NR, Bagley WP, Herrett RA (1971b) Metabolism of 2-methyl-2-(methylthio) propionaldehyde O-(methylcarbamoyl) oxime (Temik aldicarb pesticide) in potato plants. J Agric Food Chem 19:731-737

Andrawes NR, Romine RR, Bagley WP (1973) Metabolism and residues of Temik aldicarb pesticide in cotton foliage and seed under field conditions. J Agric Food Chem 21:379-386

Anonymous (1983) Edible Olive Oil. Turkish Standards TS 341

Anonymous (1990) Agricultural structure and production. State Institute of Statistics Prime Ministry Republic of Turkey

Bartley WJ, Andrawes NR, Chantey EL, Bagley WP, Spurr HW (1970) Metabolism of Temik aldicarb pesticide [2-methyl-2-(methylthio) propionaldehyde-O-(methyl carbamoyl) oxime] in the cotton plant. J Agric Food Chem 18:446-453

Bull DL (1986) Metabolism of UC 21149 [2-methyl-2-(methylthio) propionaldehyde O -(methylcarbamoyl) oxime] in cotton plants and soil in the field. J Econ Entomol 61:1598-1602

Cavanagh GC (1976) Miscella Refining. J Am Oil Chem Soc 53:361-363

Coppedge JR, Lindquist DA, Bull DL, Dorough HW (1967) Fate of 2-methyl-2-(methylthio) propionaldehyde O -(methylcarbamoyl) oxime (Temik) in cotton plants and soil. J Agric Food Chem 15902-910

Metcalf RI, Fukuto TR, Collins C, Borck K, Burk J, Reynolds HT, Osman MF (1966) Metabolism of 2-methyl-2-(methylthio) propionaldehyde O -(methylcarbamoyl) oxime in plant and insect. J Agric Food Chem 14579-584

Mounts TL (1981) Chemical and Physical effects of processing fats and oils. J Am Oil Chem Soc 58:51A-54A

Sitting M (1980) Pesticide Manufacturing and Toxic Materials Control Encyclopaedia p 754-755