Bull Environ Contam Toxicol (2010) 84:582–586
DOI 10.1007/s00128-010-9984-1

Effect of Processing on ^{14}C-Chlorfenvinphos Residues in Maize Oil and Bioavailability of its Cake Residues on Rats

F. M. Mahdy · S. I. El-Maghraby

Received: 12 May 2009 / Accepted: 7 April 2010 / Published online: 20 April 2010

Abstract Maize seeds obtained from ^{14}C-chlorfenvinphos treated plants contained 0.12% of the applied dose. The insecticide residues in crude oil, methanol and cake amounted to 10%, 6% and 69%, respectively of original residues inside the seeds. The ^{14}C-activity in the crude oil could be a gradually reduced by the refining processes. The alkali treatment and bleaching steps are the most effective steps in these processes. The refined oil contained small amount of the ^{14}C-residues originally present. The major residues in processed oil contain the parent compound, in addition to five metabolites of the insecticide. When rats fed the extracted seeds (cake), the bound residues were found to be considerably bioavailability. After feeding rats for five days with the cake, a substantial amount of ^{14}C-residues was eliminated in the urine (59.5%), while about 20% excreted in the feces. About 15% of the radioactive residues were distributed among various organs.

Keywords ^{14}C-Chlorfenvinphos · Maize oil · Refining processes · Residues · Bioavailability

Pesticides are of interest as residues because of their widespread use in a variety of crops for field and post-harvest protection of their degradation products, they could be a potential health hazard. Several investigations suggest that most organochlorine and organophosphorus pesticides in edible oils can be reduced considerably by chemical refining processes, but pyrethroid pesticides remain to a certain extent (Fukazawa et al. 1999; Hilbert et al. 1998).

F. M. Mahdy · S. I. El-Maghraby (✉)
Department of Applied Organic Chemistry,
National Research Center, Dokki, Giza, Egypt
e-mail: somiaibrahem@yahoo.com

Chlorfenvinphos, (2-chloro-1-(2,4dichlorophenyl) vinyldiethylphosphate I), is one of the most important members of the vinyl phosphate insecticides family and is marketed under the trade names, Birlane, Dermatone and Sapecron.

It is a widely used organophosphorus insecticide of low mammalian toxicity. It is used against soil-borne and foliage insects, in agricultural to control a wide range of pests on potatoes, rice, maize, sugar cane, oil plants and in carrots during storage (Jebakumar et al. 1998; Uygun 1997). Chlorfenvinphos is a wide spread organophosphorus insecticide which can cause structural and functional changes in the liver (Dahamna et al. 2004), neuro behavioral effects to rats (Lutz et al. 2006) and it is a reported hazardous chemical for aquatic non target organisms (Sismeiro-Vivas et al. 2007). Intravenous, intraperitoneal (I.P) and oral treatment of rats with lethal doses of chlorfenvinphos exhibited typical signs of anti-cholinesterase poisoning along with marked inhibition of brain and erythrocyte cholinesterase activity (Takahashi et al. 1991). The purpose of the present work was to determine the effect of different simulated commercial processing procedures on the elimination of insecticide and its residues, from corn oil obtained from plants treated with ^{14}C-chlorfenvinphos as well as the bioavailability to rat of the residues cake were also studied.

Materials and Methods

^{14}C-Chlorfenvinphos, labeled at carbon atoms of vinyl groups was obtained from Izinta Isotope Trading Enterprise of the Institute of Radioisotopes. It had a specific activity of 1 MBq and radiochemical purity 98%. Pure non-labeled chlorfenvinphos was synthesized by the reaction of triethylphosphite with 2, 4-dichlorophenacylidene dichloride

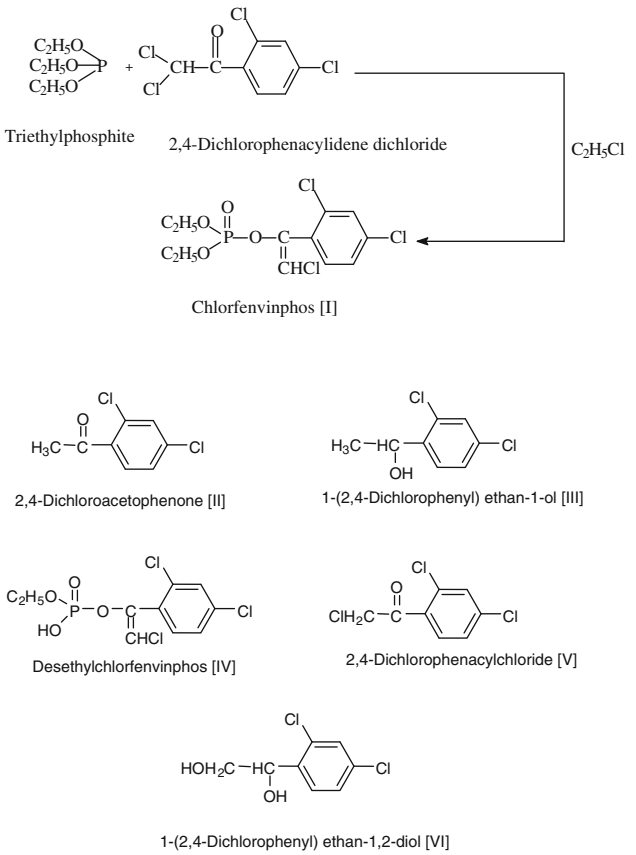

Fig. 1 Synthesis and degradation products of chlorfenvinphos in maize oil (Hutson and Hathway 1967)

according to known method (Hutson and Hathway 1967), as shown in Fig. 1. It is an amber liquid with a boiling of 168–170°C at 0.5 mmHg. For identification purposes, substances of chlorfenvinphos and metabolites have been synthesized according to the previous procedure (Fig. 1).

Pesticide-free Zea mays seeds (Var. Giza. 2 hybrid) were obtained from Agricultural Research Centre (Cairo). The seed were cleaned from any dockage and impurities before cultivation. Sound whole seeds of maize were cultivated under normal field conditions in controlled, isolated field area. Irrigation, fertilization and soil management were conducted as practiced in the field. Shortly before blooming, leaves of plants were treated twice, 23 days a part, with ^{14}C-chlorfenvinphos at a concentration of 5 mg/plant. The weight of radioactive insecticide is negligible, so each plant was treated with a solution of labeled insecticide which diluted with the non-labeled insecticide in water (total spray: 5 mg/2.5 μCi/plant). At maturity seeds were collected and dried for preparation of oil and cake.

Seeds were crushed and soxhlet extracted with n-hexane for 6 h. After evaporation of hexane under reduced pressure the oil was analyzed for ^{14}C-activity. The remaining residues were further extracted with methanol. Both methanol extract and cake were determined for their radioactivity. Oil Processing takes place throughout four steps, alkali refining, bleaching, winterization and deodorization.

The crude oil (20 g) was stirred vigorously with 2 N sodium hydroxide solution for about 30 min at 27°C. The mixture was then centrifuged to remove the soap and excess alkali, washed several times with warm water until pH 7.0 and centrifuged to obtain the clear oil.

The alkali refined oil was treated with 0.5% by weight of a factory grade Fuller's earth (Tonsil), the mixture stirred vigorously at 80–100°C for 20 min, and then centrifuged. The bleached oil was filtered.

The clear dry oil was winterized at 5°C for 3 days and the separated high saturated glycerides were removed by centrifugation.

Superheated steam was passed into the heated oil at about 200°C under reduced pressure, for 3 h, to remove steam-distillable substances including odors. Triplicate samples of oil, after each refining process, were analyzed for their radioactivity.

Oil gained from individual refining processes was partitioned between acetonitrile and hexane. The oil was extracted with hexane and the insecticide residues remained in the acetonitrile layer. Residues were characterized by TLC on silica gel plates 20 × 20 cm, 0.25 mm thickness with fluorescent indicator (Kiesel gel 60 F_{254}, Merck, Germany) using two solvent system (system A: Ethanol: Benzene 1:9 and system B: Acetone: Hexane 1:4). Authentic samples were run along side as reference material for location of spots. Plates were seen under UV-light at 254 nm and spots were made visible by spraying with Hanes–Ischer wood reagent (Hanes and Isherwood 1949) gave blue and yellow color.

The radioactivity in the solutions was measured by direct liquid scintillation counting. Radioactivity in solids and oils was determined by combustion in a Harvey Biological Oxidizer (Model OX-600) followed by liquid scintillation counting. For quench correction an internal standard was used. Thin layer plates were divided in 1 cm increments, scraped in vials, covered with scintillator and counted. Radioactivity levels were all corrected for quenching using an internal standard, ^{14}C n-hexadecane (0.2 μCi/mL or 7.4 KBq/mL), with known amount of radioactivity and recounting after adding the sample. The difference in count is corrected for samples, separately.

For bioavailability to rats, the adult male albino rats (Rattus spp.) weighing 100–150 g each, were obtained from the National Research Centre, Dokki, Cairo. The rats were placed individually in metabolism cage, which allowed the separation and collection of urine and feces. The animals were conditioned for 2 days to a daily standard diet, then were left without feed for 24 h after which they were fed a known amount of extracted maize seeds

(25 mg insecticide/rat). Urine and feces were collected daily for 120 h and radioactivity was determined.

Results and Discussion

The ^{14}C-residues in dry maize seeds obtained from ^{14}C-chlorfenvinphos treated plants amounted to 0.12% of the originally applied radioactivity. The insecticide residues in crude oil (hexane extract), methanolic extract and cake amounted to 10%, 6% and 69% respectively Table 1. The same trend of foliar application of ^{14}C-chlorfenvinphos on maize plant led to appearance of a trace of the insecticide residues in maize seeds (Beynon and Wright 1968). Dry seeds of soybean from ^{14}C-carbofuran treated plants contained about 1% of the originally applied radioactivity (Zayed et al. 1998). The foliar treatment of soybean plants with ^{14}C-pirimiphos-methyl lead to appearance of ^{14}C-residues in the mature seeds. These amounted to 0.37% of the applied dose (Zayed et al. 2003).

Simulated commercial processing procedures led to a gradual decrease in the total amount of ^{14}C-residues in oils with aged residues Table 2. As seen from Table 2, the alkali treatment and bleaching processes proved to be the most effective steps in reducing ^{14}C-chlorfenvinphos residues as their eliminated about 63% of the residues originally present in crude oil. The insecticide residues retained in the final refined oil was 17% of the originally present residues. The major part of aged residues could be eliminated by oil refinement in a stepwise manner simulating actual commercial processing procedures. Table 2 shows that the alkali treatment and bleaching give the highest reduction of ^{14}C-residues in refined oil. Similar results, after post-harvest treatment of soybeans and corn grains with ^{14}C-chlorpyrifos were reported by other authors (Tayaputch et al. 1995). Mendez et al. 2005 reported that in olive oil the simazie residues are eliminated during bleaching and deodorizing stages. On the other hand, the refining olive oil, obtained from treated olive tree with dimethoate reduced 50% of the total ^{14}C-activity in oil by neutralization step. Bleaching and deodorization processes further reduced the ^{14}C-residues and the refined oil (Gozek et al. 1999). Hilbert et al. 1998, found in fish oil that the deodorizing step seems to cause a decrease in the amount of organochlorine contaminates to about half the concentration in the crude fish oil. Working on cotton oil grown with ^{14}C-aldicarb, 63% of the residues in crude oil were also found to be lost during the refining processes (Tuncbilek et al. 1997). Miyahara and Saito 1993 found that, in some organophosphorus insecticide such as Malathion, dichlorvos and chlorpyrifos residues in soybean oil the deodorizing process is effective in removing pesticide residues due to combination of decomposition and volatilization. Hegazi et al. 2003 found that the complete refined sunflower oil obtained from treated sunflower plants lost about 64% of the total ^{14}C-zineb residues in crude oil. Zayed et al. 2005 reported that the commercial processing techniques used for refining cotton seed oil, which was obtained from treated cotton seed plant with ^{14}C-malathion removed 80% of ^{14}C-activity in crude oil. The refining of soybean oil which was obtained from treated soybean plant with ^{14}C-pirimiphos-methyl led to the reduction of 75% of ^{14}C-activity in crude oil. The refined oil had a residues level of 0.4 ppm of insecticide.

Chromatographic analysis of the crude oil and refined oil showed the presence of the parent compound in addition to five degradation products. The percentage of chlorfenvinphos was decreased with progressive refining processes as shown in Table 3. The refined oil contained small amount of these degradation products. The formation of these products indicated that chlorfenvinphos is degraded via cleavage of the P–O–C (alkyl) to give desethylchlorfenvinphos and the hydrolysis of P–O-vinyl aster to give other degradation products. Such degradation has also been observed in vinyl phosphates such as tetrachlorfenvinphos when applied to stored faba and soybeans and other grains (Hutson and Hathway 1967). The residues in the methanol extract constituted about 6% of the total residues in seeds. These were hydrophilic and insoluble in organic non-polar

Table 1 Distribution of ^{14}C-chlorfenvinphos residues in 100 g maize seeds

Fraction	Weight (g)	^{14}C-Residues (mg)	%
Maize seeds	100 ± 0.05	170 ± 0.40	100
Hexane extractable	9 ± 0.06	17 ± 0.02	10
Methanol extractable	5 ± 0.03	10 ± 0.06	6
Cake	80 ± 0.20	118 ± 0.10	69
Total recovery	94	145	85

Data are means of 3 replicates

Total ^{14}C-in seeds = 100%

Table 2 Effect of commercial processing procedures on ^{14}C-chlorfenvinphos residues in crude oil

Treatment	^{14}C-Residues in oil with aged residues	
	Mg	%
Crude oil	1.70 ± 0.95	100
Alkali refining	0.96 ± 0.21	56
Bleaching	0.62 ± 0.08	37
Winterization	0.49 ± 0.09	29
Deodorization	0.29 ± 0.01	17

ppm = mg insecticide/gm oil

Data are means of 3 replicates

Table 3 R_f values of main degradation products of ^{14}C-chlorfenvinphos and its metabolites in maize oil after subjecting to commercial processing procedures

Compound	R_f value		% ^{14}C-Residues*				
	System A	System B	A	B	C	D	E
Chlorfenvinphos [I]	0.63	0.25	75	40	20	10	–
2,4-Dichloroacetophenone [II]	0.70	0.55	–	15	20	25	30
1-(2,4-Dichlorophenyl) ethan-1-ol [III]	0.56	0.36	–	10	20	30	10
Desethylchlorofenvinphos [IV]	0.01	0.0	5	10	10	15	20
2,4-Dichlorophenylchloride [V]	0.80	0.50	–	5	10	15	20
1-(2,4-Dichlorophenyl) ethan-1,2-diol [VI]	0.38	0.12	10	10	15	10	10

System A: Ethanol: Benzene (1:9), System B: Acetone: Hexane (1:4)

Coloring reagent: Hanes-Ischerwood reagent

A = Crude oil, B = Alkali refined oil, C = Bleached oil, D = Winterized oil, E = Deodorization

* Total ^{14}C-products in oil = 100%

solvents Table 1; presumably a mixture of conjugated metabolites. No attempt was made to separate the individual glucosides. The products were liberated by heating the mixture with 2 N HCL, extracted with chloroform and identified by TLC. Analysis showed the presence of chlorfenvinphos (I), 1-(2, 4-Dichlorophenyl) ethan-1-ol (III), and Desethylchlorfenvinphos (IV). This is an indication that these compounds were present in the aged chlorfenvinphos residues in free and in conjugated forms Fig. 1.

Elimination and distribution of ^{14}C-chlorfenvinphos residues in extracted maize seeds (cake) fed to rats for 5 days are shown in Table 4, the major part of the radioactivity was extracted in the urine (59%), while, the feces contained about 20% of the released ^{14}C-bound residues. Cumulative percentage of ^{14}C-chlorfenvinphos residues detected in urine and feces of treated rats for 5 days (Table 4; Fig. 2). The total amount of distribution in different organs was 13% of the total administration dose fed to rats. The major percentages of these residues were detected in liver, fat, blood, and lung as shown in Table 4. The major part of ^{14}C-activity was detected in the urine during the first 48 h. The recovered radioactivity during 5 days accounted for about 93% of the administered dose. The elimination and distribution of ^{14}C-chlorfenvinphos residues in extracted maize seeds fed to rats for 5 days are indicate that most of the ^{14}C-chlorfenvinphos residues fed to rats were bioavailable.

This seems to be in agreement with previous results on different pesticides. A single oral dose of ^{14}C-chlorfenvinphos to rats was found to be quantitatively eliminated in 4 days. Excretion of radioactivity was mostly via the urine (87%). About 67% of the dose was excreted during the first 24 h after administration of chlorfenvinphos. In addition, 11% was eliminated in the feces and 2% via the expired air (Hutson and Hathway 1967). Chlorfenvinphos was

Table 4 Elimination and distribution of ^{14}C-chlorfenvinphos residues on extracted maize seeds (cake) fed to rats after 5 days

Sample	Insecticide equivalent*	Percentage of administrated dose (%)
Urine	14.9	59.60
	5.1	20.38
Feces	20.0	79.98
Liver	1.03	4.12
Fat	0.75	2.60
Intestine	0.63	1.50
Lung	0.42	1.38
Blood	0.42	1.05
Kidney	0.26	1.02
Brain	0.18	0.72
Heart	0.07	0.28
Testis	0.04	0.16
Spleen	0.03	0.12
	3.83	12.95
Total released	23.83	92.93

* Administrated dose = 100% (25 mg equivalent/rat)

Data are means of 3 replicates

completely metabolized in rats and dogs when administrated orally at a dose around 2 mg/kg. The present results obtained are in line with recent studies which indicate a moderate to high bioavailability of grain bound ^{14}C-pesticide residues in experimental animals (Mahdy and Taha 2007). Such high bioavailability data is an indication that about 65% of ^{14}C-pirimiphos-methyl residues contained in stored wheat grains and fed to rats were recovered in urine (Khan et al. 1992). In this connection, Qureshi et al. 1992 also reported a total elimination for about 70% during 5 days for the same pesticide residues in stored wheat

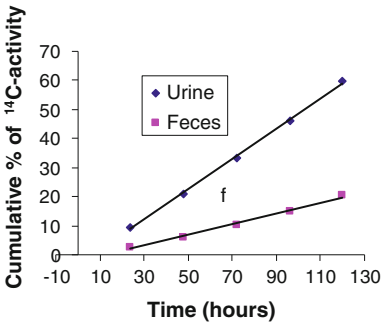

Fig. 2 Cumulative percentage extract urine and feces of rat fed extracted maize seeds containing ^{14}C-chlorfenvinphos residues through a period of 120 h

grains. In a recent study carried out on rats treated orally and intraperitoneally with ^{14}C-deltamethrin indicated that the pesticide was highly bioavailable and excreted in urine and feces (El-Maghraby 2007).

In conclusion, aged residues of chlorfenvinphos in maize seeds oil included, other than the parent compound, both free and conjugated metabolites. Refining processes led to progressive degradation of the parent insecticide. The alkali treatment was found to be different step for reduction of the insecticide residues. Bleaching leads to significant losses of the pesticide residues in oil as well. The removal efficiency during the refining processes seems to depend upon the nature of the residue present. Data obtained emphasize the importance of studying the effect of oil refinement on reduction and/or elimination of aged pesticide residues in edible oil.

Bound residues of ^{14}C-chlorfenvinphos were found to be bioavailable in rats fed the extracted cake. The main excretion route was via urine and feces, while a small amount of the radioactivity was distributed among various organs. Therefore, it should be stressed that the presence of bound pesticide residues can no longer be ignored in the evaluation of toxicological hazards.

References

Beynon KI, Wright AN (1968) Breakdown of ^{14}C-chlorphenvinphos on crops. J Sci Fd Agric 19:146–153

Dahamna S, Sekfali N, Walker CH (2004) Biochemical indicator of hepatotoxic effects of pesticides. Commun Agric Appl Biol Sci 69(4):821–828

El-Maghraby S (2007) Metabolism of deltamethrin in rats. Biomed Environ Sci 20:212–216

Fukazawa T, Aoyama M, Tsutsumi T, Tokairin S, Ehara H, Maruyama T, Niiya I (1999) Behavior of pyrethroid pesticides towards edible oils during refining. J Jpn Oil Chem Soc 48:885–889

Gozek K, Yucel U, Ilim M, Aysal P, Tuncbilek AS (1999) ^{14}C-dimethoate residues in olive oil during oil processing. J Environ Sci Health B 34:413–429

Hanes CS, Isherwood FA (1949) Separation of the phosphoric ester on the paper chromatogram. Nature 164:1107

Hegazi B, Abdel-Gawad H, Afifi LM (2003) ^{14}C-Zineb residues in sunflower seeds and oils and the effect of processing. Arab J Nucl Sci Appl 36:345–354

Hilbert G, Lillemark L, Balchen S, Schriver-Hojskov CR (1998) Eduction of organo-chlorine contaminants from fish oil during refining. Chemosphere 37:1241–1252

Hutson DH, Hathway DE (1967) The metabolism of 2-chloro-1-(2, 4-dichlorophenyl) vinyl diethyl phosphate in the dog and cat. Biochem J 102:133–142

Jebakumar SRD, Kannan N, Subramanian BR, Jayaraman J (1998) Residues of phosphamidon in rice fields. Environ Pollut 61:227–234

Khan US, Kacew S, Mattews W (1992) Bioavailability to rats of bound ^{14}C-pirimiphos-methyl in stored wheat. J Environ Sci Health B 27:355–367

Lutz P, Wiaderna D, Gralewicz S, Kur B (2006) Exposure to chlorphenvinphos, an organophosphate insecticide, prevents from behavioral sensitization to amphetamine. Int J Occup Med Environ Health 19(2):132–141

Mahdy F, Taha H (2007) Toxicity of bound ^{14}C-dichlorvos residues in stored soybeans and their bioavailability to rats. Bull NRC Egypt. 32:125–137

Mendez MVR, De La Rosa IP, Marquez AJ, Ojeda MU (2005) Elimination of pesticides in olive oil by refining using bleaching and deodorization. Food Addit Contam 22:23–30

Miyahara M, Saito Y (1993) Pesticide removal efficiencies of soybean oil refining processes. J Agric Food Chem 41:731–734

Qureshi MJ, Jamil FF, Haq A, Mujtaba SM (1992) Bioavailability and toxicity to rats of bound residues of ^{14}C-pirimiphos-methyl in stored wheat. J Environ Sci Health B 27:369–375

Sismeiro-Vivas J, Abrantes N, Pereira JL, Castro BB, Goncalves F (2007) Short-term effects on Quirlan (chlorphenvinphos) on the behavior and asetylcholinesterase activity of *Gambusia holbrooki*. Environ Toxicol 22(2):194–202

Takahashi H, Kojima Kojima T, Ikeda T, Tsuda S, Shirasu Y (1991) Differences in the mode of lethality produced through intravenous and oral administration of organophosphorus insecticides in rats. Fundam App Toxicol 16:459–468

Tayaputch N, Sitayoung R, Thangnipon W (1995) Effect of processing of crude soybean oil on ^{14}C-chlorpyriphos residues. Isotope aided studies of pesticide residues during food processing. IAEA-TECDOC. 818:9–13

Tuncbilek AS, Aysal O, Halitligil MB (1997) Loss of label during processing cotton grown with ^{14}C-aldicarb. Bull Environ Contam Toxicol 58:213–218

Uygun U (1997) Degradation of chlorfenvinphos in carrots during storage. Food Chem 60:479–487

Zayed SMAD, Farghaly M, Mahdy F (1998) Effect of commercial processing procedures on carbofuran residues in soybean oil. Food Chem 6:265–268

Zayed SMAD, Farghaly M, Mahdy F (2003) Efficiency of the refining processes in removing ^{14}C-pirimiphos-methyl residues in soybean oil. Bull NRC Egypt. 28:567–575

Zayed SMAD, Farghaly M, Mahdy F (2005) Effect of refining processes on magnitude and nature of malathion and carbofuran residues in cotton seed oil. Arab J Nucl Sci Appl 38:277–288