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ACCUMULATION OF DDT RESIDUES IN SLAUGHTERED RABBIT TISSUES

(With 3 Tables and 2 Figures)

By

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تراكم متبقيات ال د. د. ت. في أنسجة الأرانب

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تم دراسة أل د. د. ت. في دهن، كبد، كلاوى ، ولحوم الأرانب المطهية والغير مطهية والتي تم حقنها عن طريق الفم بجرعة ٥مجم/كجم لمدة عشرة أيام متتاليه حيث تـــم فحـــص هــذه العينات أسبوعيا في أيام ١،٨،١٥،٢١، بعد انتهاء التجربة باستخدام جهاز كرومـــاتوجرافى الطبقة الرقيقة ذو الكفاءة العالية لتقدير كمية أل د. د. ت. في الأنسجة المختلفة. دلت النتـــائج على وجود زيادة في كمية المتبقي من المبيد في المجموعة الثانية والتي تم فحصها في اليـــوم الثامن ثم لوحظ نقص كمية المتبقي من المبيد في المجموعتين الثالثة والرابعـــة والتـــي تـــم فحصها في اليوم ١٥،٢١. تبين أن طهي لحوم الأرانب بالغليان أدى إلى نقص كمية المتبقـــي من المبيد أما بالنسبة للتجميد فلم يكن له أي تأثير.

SUMMERY

Accumulation of DDT in fat, liver, kidney, cooked and uncooked muscles of rabbits orally administrated 5mg/kg dose of the pesticide darly for 10 days was followed weekly after 1, 8, 15 and 21 days post treatment employing high performance thin layer chromatography (HPTLC). Variation of DDT content was demonstrated in the different tissues. An increase in the pesticide concentration in the fat was shown in the second group 8 days Post-administration then decreased in the third and fourth groups. Cooking of muscles resulted in diminishing the

concentration of the pesticide residues in the four groups examined. Freezing of muscles resulted in undomenostrable change in the pesticide content.

Key words: *DDT, rabbit, muscles, fat liver, kidney, HPTLC.*

INTRODUCTION

Pesticide pollution is the cause of several health problems and economic damage. Contaminants uptaken by animals are either accumulated within their tissues if they possess an appropriate and efficient detoxification mechanism or released (Simkiss et al., 982). The fate of organochlorine pesticide residues within the tissues of slaughtered animals has been well documented (Jerome, 1958, Hayes, 1959, Claborn et al., 1960, Moubry et al., 1968, Haufe and Morley, 1970, Kaphalia and Seth, 1981, Crocker et al., 1985, Falandysz and Kannan, 1992, Heaton et al., 1996 and Waliszewski et al., 1998).

High Performance Thin Layer Chromatography (HPTLC) was successfully employed to perform a rapid and efficient qualitative and quantitative determination of the trehalose sugar in the insects and freshwater snails compared to a standard (Fell, 1990).

The present investigation aims to determine the fate of DDT pesticide residues in liver, kidney, fat and muscles. Additionally, the effect of cooking on pesticide residues in the muscles of slaughtered rabbits will be discussed.

MATERIALS and METHODS

Sampling:

Thirty two rabbits (1-1.5 kg) were placed in cages and fed ad-libitum a balanced ration for one week. The animals were classified into four groups each contained 8 rabbits. Five rabbits in each group were orally administrated DDT daily in a dose of 5 mg/kg body weight for a period of 10 days, the remaining three were kept as control. Groups of five rabbits and their control were slaughtered at 11, 19, 24 and thirty days after the last treatment. The slaughtered rabbits were dissected and the kidney, liver, muscles and fats were isolated.

Extraction method:

Pesticide residues were extracted according to Gergis (1983) using the following reagents; acetone, methanol, petroleum ether, sodium sulphate anhydrous and sulphuric acid.

Five grams of the tissues were placed in a 500ml conical flask and 150 ml of petroleum ether was added. The mixture was allowed to stand overnight with frequent shaking. The solubilized tissue was then filtered through Whatman filter paper No. 1 and washed by 25 ml concentrated sulphuric acid in a separatory funnel. The latter was shaked for one minute vigorusly and an acid lower layer with a brown color containing fatty material and other impurities are formed. The acid layer was isolated and ashed with concentrated sulphuric acid 5 times till turned clear. The remaining petroleum ether was washed by distilled water and neutralized using phenolphthalin as indicator. Then, it will be transferred into a beaker, and a spoonful of sodium sulphate was added for dehydration. The mixture was filtered through Whatman filter paper and stand to allow evaporation of petroleum ether, then one ml of acetone was added to the residue.

Determination of DDT in tissues of slaughtered rabbits:

DT residues in the isolated organs of slaughtered rabbits were determined qualitatively and quantitatively using high performance thin layer chromatography (HPTLC) according to Fell (1990).The effect of freezing at -20C and boiling on DDT content of these organs will also be examined.

Apparatus and reagents:

Merk precoated silica gel plates (10x10 cm) were used for present chromatographic separation. The HPTLC plates were prewashed in methanol and dried with hair dryer. The plates were activated in a drying oven at 100 C for one hour. Then, the plates were transferred to a dessiccator where they were stored for the time of use. N-heptane-acetone 98:2 (v:v) was used as developing solvent. The chromatographic agent was consisted of 40 ml AgNo3 (0.1N), 10ml Methanol, 160 ml distilled water. Standard pesticide (DDT) was obtained from Central Pesticide Laboratory, Agricultural Research Center, ministry of Agriculture. 50 mg of the pesticide was dissolved in 5ml acetone in volumetric flask and then completed to 25ml mark with distilled water.

Chromatographic procedures:

5 µl of digested samples of liver, kidney, fat, cooked and uncooked muscles in addition to DDT standard solution were spotted by a micropipette in the preadsorbant area of the activated HPTLC plate and dried throughly using hair dryer. Then, the plate was

transferred into a Camag chamber saturated with the developing solvent. The samples were developed in 5 ml of the developing solvent (N-heptane-acetone) three times at ambient temperature. The solvent was allowed to run up 7cm distance from the bottom of the plate. After each developing, the plate was dried for one minute with hair dryer. Following the final development and drying process, the plate was allowed to stand and sprayed with chromogenic agent till the plate turned translucent. Then, the plate was dried in the oven at 100 C for thirty minutes. The separated samples appeared as spots on the HPTLC plate. The spots with the same RF of DDT standard solution are compared in the examined organs quantitatively with each other and with the standard DDT sample by absorbance scanning employing pro-gel analysis Computer Software Program.

Statistical analysis:

Two-way ANOVA were conducted to examine the effect of slaughtered time on DDT content in different organs using Super-ANOVA Computer Software Program, Abacus, California. The least square means of the different pairs were compared employing Student's t-test.

RESULTS

Chromatographic analysis:

HPTLC Chromatogram of the different samples was diagrammatically represented in Fig. (1).

Means $\pm$ standard error of DDT residues in different studied organs at different slaughtered times were shown in table (1) and represented graphically in figure (2). Generally, the accumulation of DDT residues in different organs were ranked in the following order: Liver>fat>kidney>muscle.

Variation in the amount of DDT content was shown at the four selected slaughtered times in the different organs. Table (2) represents two-way ANOVA for the effect of different slaughtered times on pesticide content in examined tissue organs. Only significant difference of DDT residues content in the organs $P<0.01$ was found. Comparison of least square of means of DDT residues in different examined organs (Table 3) showed significant differences in DDT content in group one between fat and liver, liver and cooked muscles $P<0.01$and between kidney and cooked muscle, liver and raw muscle ($P<0.05$), between fat and cooked muscles ($P<0.01$)in group two.In group three, only

significant difference was shown between DDT concentration in fat and cooked muscles ($P<0.01$). Also, significant difference was demonstrated between DDT residues of liver of slaughtered group one and slaughtered group three ($P<0.01$).

Boiling of the examined organs resulted in marked reduction in DDT content in percentage of 76.25, 82.9, 69.42 and 77.64% at one, eight, fifteen and twenty one days respectively. Freezing of treated rabbit's meat and other examined organs at -20 C for up to twelve weeks had no effect on their DDT residues

DISCUSSION

The present finding demonstrated that DDT-orally administrated rabbits accumulated the pesticide residues in a variable manner within their organs over the time. DDT residues were decreased in liver, kidney and muscles from 1.12, 0.7 and 0.42 mg/kg on day one to 0.33, 0.28 and 0.16mg/kg on day twenty one indicating their capability to release the pesticide residues from their tissues over a time. On the contrary, fat samples showed storage capability of pesticide residues where DDT content increased from 0.48mg/kg at day one to 0.96 mg/kg at day 15 and reached 0.53mg/kg at day twenty one.

Boiling of the examined organs resulted in marked reduction in DDT content in percentage of 76.25, 82.9, 69.42 and 77.64% at one, eight, fifteen and twenty one days respectively. The results are in accordance with Gergis (1983) who found a reduction percentage of 100% in treated rabbit meat after boiling for twenty minutes and frying for fifteen minutes and with Zhulenko (1982) who observed that cooking of beef meat as well as heart, liver and lung for one hour at 100 C resulted in a pronounced reduction in DDT content.

Freezing of treated rabbit's meat and other examined organs at -20 C for up to twelve weeks had no effect on their DDT residues. This finding is concomittent with the results of Gergis (1983) who found no effect of freezing of rabbit meats up to eight weeks at -20C on DDT residues concentration. Also, this result agrees with Abbasov (1983) who pointed out that freezing and long time storage didn't cause pesticide degradation.

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Table 1: Mean ± standard error of DDT residues (mg/kg) in different organs of slaughtered rabbits.

Groups of slaughtered rabbits	Fat (F)	Kidney (K)	Liver	Raw muscle (RM)	Cooked muscle (CM)
Group 1	0.48+ 0.183	0.70+ 0.167	1.12+ 0.363	0.42+ 0.124	0.10+0.030
Group 2	0.96+ 0.339	0.66+ 0.318	0.72+ 0.261	0.47+ 0.289	0.08+0.028
Group 3	0.75+ 0.307	0.49+ 0.285	0.68+ 0.284	0.33+ 0.126	0.10+0.048
Group 4	0.53+ 0.237	0.28+ 0.139	0.33+ 0.152	0.16+0. 088	0.03+0.020

Table 2: Two way ANOVA analysing the effect of slaughtered time on DDT content of different organs

Source	df	Sum of squares	Mean squares	F-value	P-value
Slaughter	3	1.315	0.438	1.828	0.1488
Organ	4	5.098	1.274	5.314	0.0008
Slaughter x organ	12	1.721	0.143	0.598	0.8378
Residual	80	19.187	0.240		

Dependent: DDT

FIGURE LEGENDS

Fig. 1: Diagrammatic representation of HPTLC chromatogram of DDT content in fat (F), liver (L), kidney (K), raw (RM) and cooked muscles (CM) of slaughtered rabbit.

Fig. 2: DDT residues in fat, liver, kidney and muscle of treated rabbits.

Table 3: Student's t-test comparing least square of means values of DDT residues in the examined organs of different slaughtered rabbit groups.

		Group I					Group II				
		F	K	L	RM	CM	F	K	L	RM	CM
GP I	F	--	0.73	2.05**	0.17	1.2	1.59	ND	ND	ND	ND
	K	--	--	1.32	0.9	1.92*	ND	0.53	ND	ND	ND
	L	--	--	--	2.22*	3.25**	ND	ND	1.53	ND	ND
	RM	--	--	--	--	1.029	ND	ND	ND	0.19	ND
	CM	--	--	--	--	--	--	--	--	--	0.07
		Group III					Group IV				
GP I	F	0.89	ND	ND	ND	ND	0.19	ND	ND	ND	ND
	K	--	0.67	ND	ND	1.92*	ND	1.35	ND	ND	ND
	L	--	--	1.56	ND	3.25**	ND	ND	2.49**	ND	ND
	RM	--	--	--	0.38	.ND	ND	ND	ND	0.84	ND
	CM	--	--	--	--	0.003	--	--	--	--	0.22
		Group 1					Group II				
GP 2	F	--	--	--	--	--	--	1.393	1.075	1.547	2.86***
	K	--	--	--	--	--	---	--	0.318	0.18	1.47
	L	--	--	--	--	--	--	--	--	0.498	1.788
	RM	--	--	--	--	--	--	--	--	--	1.289
	CM	--	--	--	--	---	--	--	--	--	--
		Group III					Group IV				
GP 2	F	0.69	ND	ND	ND	ND	1.401	ND	ND	ND	ND
	K	ND	0.137	ND	ND	ND	ND	0.816	ND	ND	ND
	L	--	--	0.032	ND	ND	ND	ND	0.957	ND	ND
	RM	--	--	--	--	1.03	ND	ND	ND	1.03	ND
	CM	--	--	--	--	--	ND	ND	ND	ND	0.145

F; fat, K; kidney, L; liver, RM; raw muscle, CM, cooked muscle. Significant difference; P<0.05, ** High significant difference; P<0.01.

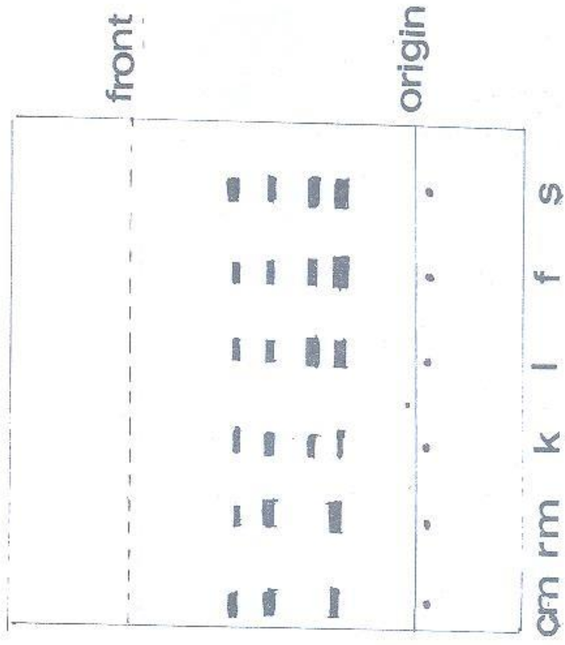

fig 1

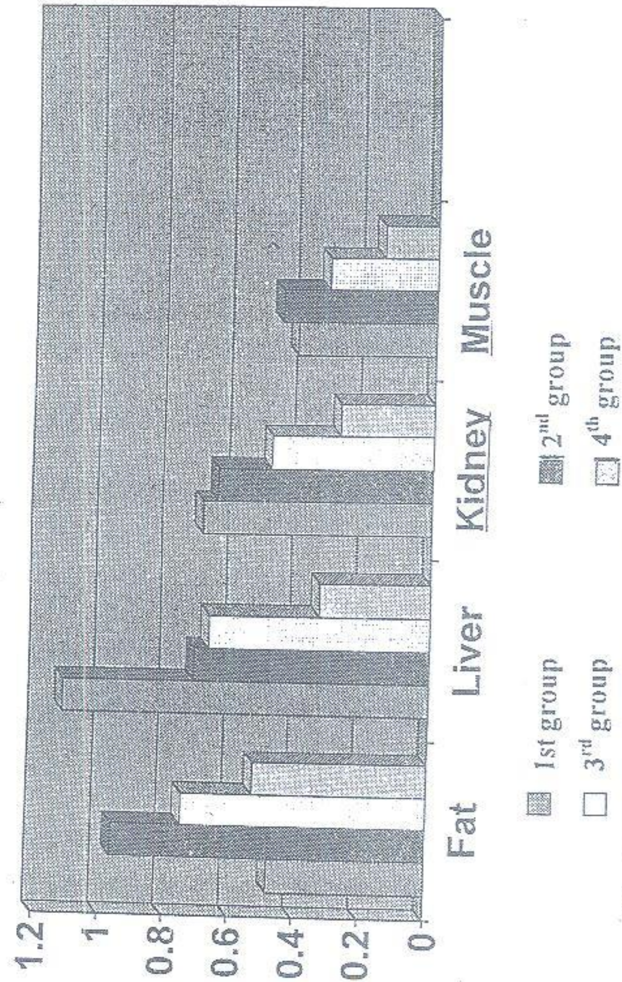

Fig. 2: DDT residues in fat, liver, kidney and muscle of treated rabbits