

## Organochlorine Pesticides in Swine Tissues from Abattoir Material Collected in Nairobi, Kenya

R. Kotonya, 1,\* G. M. Mutungi, 1 L. W. Kanja2

<sup>1</sup>Department of Animal Physiology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 30197, Nairobi, Kenya <sup>2</sup>Department of Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya

Received: 7 June 1993/Accepted: 1 November 1993

Subsequent to the discovery of DDT in the early 40's, other organochlorine pesticides have been introduced. These compounds generally resist microbial and chemical degradation and therefore persist in the environment. Despite the fact that the use of organochlorine pesticides has been banned or restricted, environmental contamination remains the main source of organochlorine pesticides in food animals (Raisbeck et al. 1989). Studies on organochlorine pesticide residues carried out on different tissues of various animal species in Kenya, have indicated varying levels of environmental contamination. (Kanja et al.; 1992, Mitema and Gitau 1990; Mugachia 1992 a; b). Organochlorine pesticides found in follicular fluid of infertile women have been implicated as the cause of infertility (Bauklouh et al. 1985).

Due to the fact that swine are polytocous, the large number of follicles and corpora lutea available makes it a suitable animal model for the study of the possible effects of organochlorine pesticides on reproduction. In this study, swine fat, muscle, liver, corpus luteum and follicular fluid samples from abattoirs were analysed for organochlorine pesticide residues. The tissues were obtained from two groups of gilts; one group came from farms that used only commercial feed; the other originated from farms that used commercial feed and swill interchangeably. The objectives of this study were to establish the levels of organochlorine pesticide levels in various swine tissues and to compare the levels of the pesticides found in swine tissues from two slaughter houses obtaining pigs from different backgrounds.

## MATERIALS AND METHODS

Samples for the survey were collected from female pigs (mainly gilts) slaughtered at the Farmers' Choice and the Ndumbuini abattoirs, both in Nairobi. Pigs from the Farmers' Choice abattoir originated from two commercial farms and had been fed on commercial pig feed only. Those from the Ndumbuini abattoir originated from various small scale farmers and had been fed on a combination of commercial

<sup>\*</sup>Present address: Lancaster University, Lancaster LA1 4YR, UK Correspondence to: R. Kotonya

pig feed and pig swill. The tissues were separately wrapped in aluminium foil and sorted according to individual animal in labelled plastic bags. These were then transported to the laboratory and frozen as they awaited analysis. Prior to freezing, depending on the stage of the estrous cycle, the corpora lutea were carefully excised from the rest of the ovarian tissue or optimum follicular fluid aspirated from as many follicles as possible and placed in sampling vials.

A slight mordification of the methods described by Bjerk and Sundby (1970) and Brevik (1978) was adopted for extraction of the solid tissues (corpus luteum, muscle, and liver) and follicular fluid respectively. Extraction of the solid tissues involved homogenizing 3 g of the tissue with 4.5 g of acid washed sand and 4.5 g of anhydrous sodium sulphate. Diethyl ether (15-20 ml) was used in extraction of fat from 4.0 g of the homogenate. The follicular fluid was extracted by ultrasonic disintegration with acetone (15 ml) and hexane (20 ml). The extraction was repeated using 5 and 10 ml of acetone and hexane respectively. In extraction of fat, 10 ml of water and 3 ml of 2% NaCl were added to 1 g of macerated fat sample and extracted by ultrasonic disintegration using hexane and acetone (as was the case for follicular fluid).

A modification of the method described by Bjerk and Sundby (1970) was used in the 'clean up'. It involved redissolving the fat extract in hexane (0.05 g fat/ml hexane), and treating two aliquots of the fat with concentrated H<sub>2</sub>SO<sub>4</sub> and KOH, respectively. 1-3 µl of these clean up extracts were analysed using a Packard gas liquid chromatograph (GLC), with a <sup>63</sup>Ni electron capture detector (ECD). Nitrogen of 99% purity was used as the carrier gas. The packing materials used were GP 1.5% SP-2250/1.95% SP and GP 4%, SE-30/6%, SP-2401 both on Supelcoport 100/120 in the analytical and confirmatory columns, respectively. During pesticide residue determination the GLC was set at the following operating conditions: Injection port temperature, 230°C; Detector temperature, 250°C; Oven temperature, 210°C; Nitrogen flow rate, 60 ml/minute. The percentage recovery rates of the samples analysed were as follows (mean  $\pm$  S.E.M.):- fat - 92.9  $\pm$  2.8, muscle -  $92.0 \pm 2.9$ , liver -  $82.7 - \pm 4.0$ , corpus luteum  $90.9 \pm 3.3$  and follicular fluid -  $93.3 \pm 2.5$ . The data does not include corrections for recovery. The limit of pesticide residue detection was 0.001mg/kg. The levels of pesticides in tissues from the two groups of pigs in the survey were compared using the unpaired Student's t-test.

## RESULTS AND DISCUSSION

Seven different pesticides were detected in the swine tissues analysed. The percentage of samples with detected toxicant were p,p'-DDE - 81%, p,p'DDT - 70%, o,p'-DDD - 17% and o,p'-DDT - 7%. Trace amounts of aldrin, dieldrin and heptachlor were detected only in fat tissue.

On wet weight basis, the mean sum DDT (p,p'-DDT + 0,p'-DDT + 1.11 [p,p'-DDD + p,p'-DDE]) ranged from 0.03 mg/kg in muscle to 0.56 mg/kg in fat tissue and on fat weight basis from 0.07, in the corpus luteum to 0.38 in the liver on a fat weight basis. All comparisons were made using the levels of pesticides residues

Table 1. Levels of organochlorine pesticide residues in swine tissues samples obtained from the Farmers' Choice abattoir

	Fat (n=21)	=21)	Musc	Muscle (n=28)	Liver (n=28)	-28)	Corpus luteum (n=9)		Follicular fluid (n=9)	id (n=9)
	No. positi	No. mg/kg fat positive mean ± sd	No. posit	No. mg/kg fat positive mean ± sd	No. positive	No. mg/kg fat positive mean ± sd	No. positive	No. mg/kg fat positive mean±sd	No. positive	No. mg/kg fat positive mean ± sd
p,p'-DDE	21	$0.18 \pm 0.10$	21	$0.22 \pm 0.30$	24	$0.13 \pm 0.11$	6 0.	$0.04 \pm 0.02$	0	
p,p'-DDT	18	$0.27 \pm 0.24$	20	$0.36 \pm 0.74$	7	$0.39 \pm 0.79$	1 0.	80.0	0	
p,p'-DDD	-	90.0	-	0.09	6	$0.13 \pm 0.15$	1 0.	0.03	0	
o,p'-DDT	0		0		9	$0.37 \pm 0.37$	0		0	
Sum DDT	21	$0.42 \pm 0.29$	23	$0.53 \pm 0.76$	25	$0.32 \pm 0.49$	6 0.	$0.07 \pm 0.06$	0	
p,p'-DDT ratio	19	$1.36 \pm 0.21$	18	$1.49 \pm 0.95$	7	$2.99 \pm 3.48$	-	1.21	0	
H. epoxide	0		0		0		0		0	
Heptachlor	4	+	0		0		0		0	
Aldrin	7		0		0		0		0	
Dieldrin	7	$0.09 \pm 0.10$	0		0		0		0	
α-НСН	0		0		0		0		0	
<b>β-нсн</b>	0		0		0		0		0	
Lindane	0		0		0		0		0	
Endrin	0		0		0		0		0	

Table 2. Levels of organochlorine pesticide residues in swine tissues samples obtained from the Ndumbuini abattoir

	Fat (n=19)	=19)	Muscle (n=20)	ri.	Liver (n=20)	Corpus luteum (n=8)		Follicular fluid (n=11)	id (n=11)
	No. positi	No. mg/kg fat positive mean ± sd	No. mg/kg fat positive mean±sd		No. mg/kg fat positive mean±sd	No. positive	mg/kg fat mean ± sd	No. positive	No. mg/kg fat positive mean±sd
p.pDDE	19	$0.22 \pm 0.17$	17 0.45 ± 0.39	36	$18  0.35 \pm 0.28$	9	0.21	0	
P,p'-DDT	17	$1.04 \pm 0.59$	$12 0.82 \pm 0.24$	4	$12  1.20 \pm 0.48$	$8   3   0.66 \pm 0.38$	0.38	0	
DDD-d'd	S	$0.09 \pm 0.07$	1 0.09		$10  0.25 \pm 0.20$	0 0		0	
o,p'-DDT	_	1.51	1 2.05		_	_		0	
sum DDT	19	$1.26 \pm 0.66$	$18  1.23 \pm 0.23$	23	$18  1.38 \pm 1.76$	9	69.0	0	
p.pDDT ratio	17	$2.10 \pm 3.06$	$12  2.77 \pm 1.78$	8/	$12  2.98 \pm 2.80$	$0$ 3 $1.42 \pm 1.27$	1.27	0	
p,p'-DDE									
Heptachlor	0		0		0	0		0	
H. epoxide	0		0		0	0		0	
Aldrin	0		0		0	0		0	
Dieldrin	0		0		0	0		0	
α-НСН	0		0		0	0		0	
в-нсн	0		0		0	0		0	
Lindane	0		0		0	0		0	
Endrin	0		0		0	0		0	
n: no of samples	10000	n: no of samples	H. epoxide: Heptachlor epoxide	otachlor e	poxide				
i. uace (<0.001)	, ucieri	ed but not quantity	÷						

42

expressed on fat weight basis. Tables 1 and 2 show the levels of the pesticides in mg/kg fat weight from the two abattoirs, indicating the mean ± standard deviation (s.d). These levels were much lower than the ERL (Extraneous Residue Limit) levels or the maximum concentration of a pesticide residue that is recommended by the Codex Alimentarius Commission to be legally permitted in food. The Codex Alimentarius Commission limit for DDT in carcass meat is 5 mg/kg (Smith 1989).

Higher mean sum DDT levels of 5.91 mg/kg fat, than those found in this study have been detected in adipose tissue of mothers (18-30 years old) living in Nairobi (Kanja et al. 1992). The variations in pesticide concentrations observed in the two studies can be attributed mainly to the age and species variations (Matsumura 1982; Raisbeck et al. 1989). Differences in the metabolism and excretion of the pesticides between the two species may have an effect on the differences in the pesticide residue levels. The comparatively low levels of sum DDT observed in the survey could also be attributed to the fast growth rate in pigs. Fast growth rate may result in fat deposition in the body leading to dilution of pesticides stored in adipose tissue. Fat tissue had the highest occurrence rate of p,p'-DDT an its metabolite p,p'-DDE. DDE is the main degradation product of DDT in aerobic conditions (De Kock and Simmons 1988) and like DDT it is stable and lipophillic. This explains the high occurrence rate of DDT and DDE in fat. In the survey, p,p'-DDE and p,p'-DDT also had the highest rate of occurrence in all the tissues with an exception of the liver. Similar results have been observed in Kenya, in studies on organochlorine pesticide residues in eggs (Mugambi et al. 1989), human milk (Kanja et al. 1986), human adipose tissue (Kanja et al. 1992), fish (Mitema and Gitau 1990; Mugachia 1992 a;b) and crocodile eggs (Skaare et al. 1992).

The liver had an exceptionally higher occurrence of p,p'-DDD compared to the other tissues, 68% of the total DDD. This could be explained by the fact that DDT is metabolised to DDD by enzymes in the vertebrate liver (Hassell 1990). Similar observations have been made in rats where the presence of DDD in the liver but not in the body fat has been reported in rats which had not consumed any DDD (O'Brein 1967). Although DDD is stored in adipose tissue, it is eventually biodegraded to DDA which is excreted in urine.

No pesticide residues were detected in the follicular fluid samples analysed. The age and maturity status of the pigs, and the low lipid level of the follicular fluid could be contributing factors to the absence of organochlorine pesticides residues in the follicular fluid. Organochlorine pesticides including DDT, dieldrin and HCH have been detected in the follicular fluid of humans (Bauklouh et al. 1985). Dieldrin and DDT have been detected in the corpus luteum of mice by radioautographic technique (Backstrom et al. 1965).

The sum DDT levels between obtained from the two abattoirs were analysed to detect differences in pesticide levels in similar tissues between the two groups of pigs. Significant differences (p < 0.05) were observed between the liver and the muscle tissue obtained from gilts fed on commercial feed only and commercial feed and swill, respectively. In both cases the tissues from gilts fed on swill in addition to commercial feed had a higher mean sum DDT. No significant differences were observed in the fat and corpus luteum (p > 0.05). (See Figure 1.)

The p,p'-DDT to p,p'-DDE ratio was determined and more than 50% of the ratio in all the tissues was greater than one. The ratio in the pigs which had been fed on a combination of commercial feed and swill ranged from 1.42 in the corpus luteum to 2.98 in the liver, while that found in the pigs which had been fed on commercial feed only ranged from 1.21 in corpus luteum to 2.99 in the liver (Tables 1 and 2). There was a higher mean p,p'-DDT to p,p'-DDE ratio found in the pigs which had been fed on commercial feed and swill interchangeably than those which had been fed on commercial feed alone. Significant differences in the p,p'-DDT to p,p'-DDE ratio were observed between the muscle (p < 0.01), fat and liver (p < 0.05) from the two groups of pigs. This implies that there was a higher proportion of DDT in the pigs which had been fed on commercial feed and swill interchangeably which could indicate a more recent exposure compared to the tissues obtained from pigs fed on commercial feed only. This could also imply that the pigs with a higher mean p,p'-DDT to p,p'-DDE ratio had a more direct exposure to p,p'-DDT than the ones with a lower ratio, which acquired it after environmental conversion to p,p'-DDE. On visitual examination the hogs fed on swill seemed to have a higher body weight than the other pigs. This could be due to the different management practices between the two types of farmers one striving for quality and the other quantity. Therefore the time on feed might have been longer for the swill hogs allowing more time for DDT to build up. There is therefore a need to determine the levels and sources of chlorinated hydrocarbon residues in varying types of swine feed.

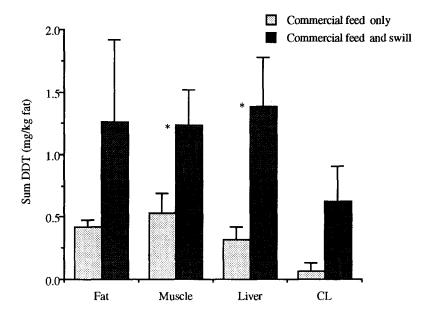


Figure 1. Levels of sum DDT in mg/kg fat obtained from the tissues analysed from pigs fed on commercial feed alone and commercial feed and swill. The number of positive samples is indicated at the bottom of each bar. Significant difference (p < 0.05) in tissues between the two groups of samples analysed is indicated by an asterisk.

Acknowledgments. The authors are grateful to the Farmers' Choice and Ndumbuini Slaughter Houses for providing samples for the project, Mr. F. Gitau for his technical assistance and to NORAD and the Norwegian Veterinary Association for funding the project.

## REFERENCE

- Backstrom J, Hansson E, Ullbergh J (1965). Distribution of C<sup>14</sup>- DDT and C<sup>12</sup>-dieldrin in pregnant mice determined by whole body autoradiography. Toxicol Appl Pharmacol 7: 90-96.
- Bauklouh V, Bohnet HG, Trapp M, Heeschen W, Feichtinger W, Kemeter P. (1985). Biocides in human follicular fluid. Ann NY Acad 442: 240-250.
- Bjerk JE, Sundby (1970). Residues of Organochlorine insecticides and PCBs in terrestrial and aquatic organisms. Norwegian part of OECD programme 1966/68. Norsk Vet Tidssr 82: 241-246.
- Brevik EM. (1978). Gas Chromatographic methods for the determination of organochlorine pesticides in human milk. Bull Environ Contam Toxicol 19: 281-286.
- De Kock AC, Simmons R (1988). Chlorinated hydrocarbon residues in African Marsh harrier eggs and concurrent reproductive trends. Ostrich 59: 180-181.
- Hassell KA (1990). The Biochemistry and Uses of Pesticides: structure, metabolism, mode of action and uses in crop protection, 2nd Ed., Macmillan Press, London.
- Kanja LW, Skaare JU, Nafstad I, Maitai CK, Lokken P. (1986). Organochlorine pesticides in human milk from different parts of Kenya 1983-1985. J Toxicol Environ Hlth 19: 449-464.
- Kanja LW, Skaare JU, Ojwang SBO, Maitai CK. (1992). A comparison of organochlorine pesticide residues in maternal adipose tissue, maternal blood, cord blood, and human milk from mother/infant pairs. Arch Environ Contam Toxicol 22: 21-24.
- Matsumura F (1982). Toxicology of insecticides. 2nd Ed., Plenum Press, New York
- Mitema ES, Gitau FK (1990). Organochlorine residues in fish from Lake Victoria, *Kenya*. Afre J of Ecol 28: 234-239.
- Mugachia JC, Kanja L, Gitau F (1992) Organochlorine pesticide residues in fish from Lake Naivasha and the Tana River, Kenya. Bull Environ Contam Toxicol 49: 199-206.
- Mugachia JC, Kanja L, Maitho TE (1992) Organochlorine pesticide residues in estuarine fish from the Athi River, Kenya. Bull Environ Contam Toxicol 49: 207-210.
- Mugambi JM, Kanja L, Maitho TE, Skaare JU, Lokken P (1989). Organochlorine residues in domestic fowl (Gallus domesticus) eggs from central Kenya. J Sci Food Agric 48: 165-176.
- O'Brein RD. (1967). Insecticides: action and metabolism. Academic Press, London.
- Raisbeck MF, Kendall JD, Rottinghaus GE (1989) Organochlorine insecticide problems in livestock. Clin Toxicol. 5: 391-410.
- Skaare JU, Ingebrigtsen K, Aulie A, Kanui TI (1991). Organochlorines from crocodile eggs in Kenya. Bull Environ Contam Toxicol 47: 126-130.
- Smith BL (1989). Codex Alimentarius Abridge Version, Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission, FAO, Rome.