J Food Sci Technol (May–June 2010) 47(3):325–329
DOI: 10.1007/s13197-010-0052-y

ORIGINAL ARTICLE

Detection of cyclodiene pesticide residues in buffalo meat and effect of cooking on residual level of endosulfan

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Revised: 21 September 2009 / Accepted: 28 September 2009

Abstract Levels of cyclodiene pesticides (aldrin, α-endosulfan, β-endosulfan, endosulfan sulfate and heptachlor) residues in muscle, liver and kidney tissues of buffalo were estimated. The effects of common cooking methods (microwave cooking, boiling, broiling and pressure cooking) on the levels of endosulfan were determined. Aldrin and total endosulfan (α-endosulfan, β-endosulfan, endosulfan sulfate) residues were found in 42.86 and 64.29% of buffalo tissue samples, with overall mean residual concentration of 0.013 and 0.055 ppm, respectively. However, the levels of these residues were well below the maximum residue limit (MRL: aldrin 0.2 ppm ; endosulfan 0.1 ppm) specified by national and international regulatory bodies. Cooking of endosulfan (Endoin 35 EC) spiked meat resulted in 58.33–64.59% reduction in α-endosulfan and 55.93–61.60% reduction in β-endosulfan. Among the cooking methods, pressure cooking was most effective in reducing both α- and β-endosulfan.

Keywords Buffalo meat · Cyclodiene · Pesticide residues · Endosulfan · Cooking methods

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Introduction

The powerful insecticidal properties of organochlorine pesticides promoted their widespread usage for the control of agricultural pests and invertebrate vectors of animal diseases. Higher stability and persistence of these chemicals in the environment led to the contamination of foodstuffs, especially those having high fat content such as milk and meat products (Kannan et al. 1992). Presence of pesticides residues in food materials has caused concern due to their association with endocrine dysfunction, birth defects, carcinomas, neurological disorders and weakened immune system (Brody and Rudel 2003) and their estimation has been regarded as an essential component of food quality control programme. Though there are some reports available on organochlorine pesticides like dichloro diphenyl trichloroethane (DDT) and hexachlorocyclohexane (HCH) pesticide residues in meat and meat products, little information is available on the cyclodiene group of organochlorine pesticides (aldrin, α-endosulfan, β-endosulfan, endosulfan sulfate and heptachlor).

Generally, risk assessments due to chemical residues are based on residues levels in uncooked food, even though a large proportion of food consumed is either cooked or processed before consumption. To properly assess the risks of pesticide residues to consumer, it is necessary to consider the effects of cooking on the residues (Holden et al. 2001). Reports indicate that cooking causes considerable destruction of pesticide residues in food commodities (Mirna and Coretti 1979, Dejonckheere et al. 1996, Wani et al. 1998, Rajashekar et al. 2007). The presence of residues above the permissible levels is a major hindrance in the acceptance of meat by importing countries, besides it is necessary to protect the health of domestic consumers as well. Hence, the present experiment was undertaken to estimate the levels of cyclodiene pesticides residues in tissues of buffaloes and also to study the effect of different cooking methods on the levels of endosulfan, a routinely used cyclodiene pesticide.

Materials and methods

Muscle, liver and kidney samples (14 numbers) of buffaloes (*Bos bubalis*) aged 5 years and above were collected from Greater Hyderabad Municipal Corporation slaughterhouse, Hyderabad and stored at –20°C until analysis.

Fat extraction and clean up: A 15 g portion from each of the minced samples were ground along with 30 g of anhydrous sodium sulphate in a pestle and mortar. Resultant tissue material was extracted for cyclodiene residues by Soxhlet apparatus (Soxplus, Chennai, India) for 6 h in petroleum ether at 50–55°C as outlined by Tonkabony et al. (1981). The fat extracted from the samples was subjected to clean-up as per the procedure of AOAC (1995) with slight modification. The extracted fat diluted with 15 ml petroleum ether was extracted thrice with 30 ml of acetonitrile saturated with petroleum ether in 125 ml separator. Each time, the acetonitrile portion was drained into a 1 l separator containing 650 ml water, 40 ml saturated NaCl solution and 100 ml petroleum ether. The one litre separator containing acetonitrile extract was shaken thoroughly. The aqueous layer separated was drained into another one litre separator to which 100 ml petroleum ether were added and shaken thoroughly with care (back extraction into petroleum ether). After discarding the aqueous layer, the petroleum ether portion was combined with that of the first 1 l separator, washed with two 100 ml portions water and the washings were discarded. The clean up of samples to remove the residual fat was performed by column chromatography using activated anhydrous sodium sulphate and Florisil. Elution was carried out with 200 ml of 6% eluting solvent at 40–45 drops per min and the elute was concentrated in a vacuum evaporator (Lab Tech, Korea).

Estimation of cyclodiene residues: One µl of the reconstituted sample was injected into a gas chromatograph (Shimadzu, Japan) equipped with an electron capture detector. Instrumental settings were: injection port, column and detector temperature 80–220, 260 and 300°C, respectively with N_2 gas flow rate of 5 ml/ min. This injection mode was splitless. The retention time along with the height and areas of peak were recorded. The cyclodiene pesticides were quantified from individually resolved peak heights with corresponding peak heights of standards. For every set of 10 samples, a procedural blank consisting of all reagents and glassware used during analysis was run to detect interference and cross contamination.

Recovery experiment: Two meat samples were fortified with working standards (0.01 and 0.1 ppm) of cyclodiene compounds to estimate the recovery by the procedure described above to ascertain the efficiency of extraction. Residue levels of pesticides were corrected according to their recoveries and expressed as ppm.

Effect of cooking on the level of endosulfan residues: About 1 kg of buffalo meat was procured from local market, packed in polyethylene bags and transported in ice to the laboratory in 30 min. After removing the connective tissues and separable fat, the lean portion was ground (through 8 mm plate) using a meat mincer (model TC 22 RIO INOX, Sirman, Italy). The ground meat was spiked with commercially available endosulfan (Endoin 35 EC, Insecticides India Ltd, Bhiwadi, Rajasthan) at 10 ppm and divided into 5 parts of 200 g each. One part of spiked ground meat sample without cooking was used as control. Remaining 4 spiked ground meat samples were subjected to commonly used cooking methods like boiling in water bath (100°C), pressure cooking (121°C), broiling (100°C) and microwave cooking. For boiling, meat samples were cooked in boiling water for 30 min in low-density water impermeable polyethylene bags. Pressure cooking was done by wrapping meat samples in aluminum foil and cooked in a domestic pressure cooker for 15 min. For broiling, meat samples were made into small patties of about 1.5 cm thicknesses using mold and cooked in a preheated hot air oven for ~30 min to obtain an internal temperature of 80°C. The patties were turned upside with every 10 min interval. In microwave cooking, meat samples were placed in a glass bowl and cooked in microwave oven at 2450 MHz for 5 min. The drip collected in each cooking method was mixed with cooked samples.

The concentration of α and β-endosulfan isomers in the commercial preparation was determined before spiking the meat samples. A 15 g portion from each group was utilized to estimate the change in the level of endosulfan in a gas chromatograph as per the procedure described earlier. Residue levels of endosulfan was calculated according to their height and areas of the specific peak and expressed as ppm. The experiment was repeated 6 times.

Statistical analysis: Data were subjected to analysis of variance to determine the differences in the concentration of cyclodiene compounds among the different tissues of buffalo and also in the level of spiked endosulfan between different cooking methods. Significant differences among the means were determined by Tukey honestly significant difference test. All statistical computations were performed using the SPSS 10.0 for Windows software (SPSS Inc., Chicago, IL, USA).

Results and discussion

Cyclodiene pesticide residues: The recovery of various cyclodiene compounds from spiked meat samples ranged from 79.7 to 101.8% (Table 1). The extraction procedure employed in this experiment was efficient in recovering the maximum amount of residues present in samples as a recovery of 75–102% is considered acceptable (Solymos et al. 2001). Several workers reported similar recovery of pesticide residues from various meat samples (Doong and Lee 1999, Bedi et al. 2005).

The results indicated that 42.9% buffalo tissue samples were positive for aldrin and the residual concentrations ranged from 0.012 to 0.027 ppm (Table 2). Presence of residues of aldrin in cattle feed (Dikshith et al. 1989), buffalo

Table 1 Retention time and recovery percentage of cyclodiene pesticides in spiked meat samples

Pesticide	Retention time, min	Recovery, %
Heptachlor	34.088	101.8
Aldrin	37.586	79.7
α-Endosulfan	44.545	89.7
β-Endosulfan	48.359	80.0
Endosulfan sulphate	52.145	82.4

Heptachlor below detectable level was also reported in pork from Spain (Hererra et al. 1994) and fish samples from Punjab (Kaur et al. 2008). However, Kannan et al. (1992) reported a very low level of heptachlor (0.09 ppb) in meat and animal fat in India. Similarly, very low level of residues of heptachlor were also reported in chicken (0.0033 ppm), beef (0.0001 ppm) and pork (0.0013 ppm) by Madarena et al. (1980) and in poultry tissues by Jevsnik et al. (2004).

Table 2 Mean residual levels (ppm) of cyclodiene pesticides in tissues of buffalo

Name of Pesticide	Muscle	Liver	Kidney	Overall mean	Per cent occurrence
Aldrin	0.012 ± 0.003	0.010 ± 0.005	0.017 ± 0.004	0.013	42.86
α-endosulfan	0.038^{b} ± 0.004	0.012^{a} ± 0.004	0.008^{a} ± 0.002	0.023	57.14
β-endosulfan	0.021 ± 0.002	0.015 ± 0.004	0.012 ± 0.003	0.016	57.14
Endosulfan sulfate	0.025 ± 0.005	0.015 ± 0.004	0.018 ± 0.009	0.020	50.0
Total endosulfan	0.084	0.042	0.038	0.055	64.29
Heptachlor	BDL	BDL	BDL	-	-

BDL- Below detectable level

n=14; Means bearing different superscripts between columns (a, b) differ significantly ($P< 0.05$)

milk (Saxena and Siddiqui 1982) and meat and fat (Kannan et al. 1992) were already reported in India. However, the reported data on aldrin residues in buffalo tissues are rather sparse. Aldrin levels in the samples analyzed in the present study were much lower than the recommended maximum limit of 0.2 ppm specified by national (MFPO 1973) and international (Codex 2006) regulatory bodies.

The residues of endosulfan (sum of α-endosulfan, β-endosulfan and endosulfan sulfate) were present in 64.3% tissue samples of buffaloes and the overall mean residual level ranged between 0.038 and 0.084 ppm. Contamination of feed, feed materials (oil cakes and bran) and water with endosulfan as reported by Dikshith et al. (1989) and Shukla et al. (2006) might be the reason for detection of residues in buffalo tissues in the present study. This is in agreement with Indraningsih et al. (1993), who also observed accumulation of endosulfan residues in tissues of animals fed with endosulfan spiked feed. The higher incidence might be due to the extensive use of endosulfan to control insects, as it has wider spectrum of biological activities and higher stability in the environment as reported by Kathpal and Dewan (1975). However, the residual concentrations of endosullfan in buffalo tissues recorded in the present study were very low and in all cases the levels were below the MRL of 0.1 ppm specified by Codex (2006) and EU (2006).

Presence of endosulfan residues in milk (Kumari et al. 2005), and fish (Amaraneni 2002) were also reported in India. Residues of endosulfan were also reported in milk in Canada (Frank et al. 1985) and meat in Spain (Lazaro et al. 1996).

None of the samples screened contained heptachlor residue above detectable level (Table 2). Existence of ban on this pesticide since 1992 might be the reason for the absence of heptachlor residue in the analyzed animal samples.

Effect of cooking on endosulfan residues: Endosulfan is available in a formulation comprising mixture of α- and β- endosulfan isomers in an approximate concentration of 2:1 (Awasthi 2007). In the present study, buffalo meat was spiked with endosulfan at 10 ppm level. This has resulted in 7.464 ppm (74.6%) of α-endosulfan and 2.692 ppm (27%) of β-endosulfan in spiked samples.

A highly significant ($p< 0.01$) difference in the levels of endosulfan was observed between spiked raw and cooked buffalo meat samples (Table 3). Cooking of meat resulted in 58.3–64.6% and 55.9–61.6% reduction in the level of α- and β-endosulfan, respectively. This significant reduction in endosulfan in meat during cooking could be attributed to the volatility of these compounds and the elimination of these compounds with fat rendering induced by high temperature as suggested by Wani et al. (1998) and Sallam and Morshedy (2008). However, as reported by Lane et al. (1979) that reduction in pesticide residues level varies between naturally contaminated and artificially spiked samples due to variation in bonding of xenobiotic to some compounds of medium.

The effect of cooking in the reduction of endosulfan is in accordance with the findings of Ramesh and Balasubramanian (1999) and Rajashekar et al. (2007), who also reported 53 to 100% reduction in endosulfan residues in different food materials due to various cooking methods. Similarly, reduction in the levels of various organochlorine pesticides after thermal processing of meat is also reported. These include 44% of DDT in lamb meat cuts (Bayarri et al. 1994), 65% of lindane in rabbit meat (Mirna and Coretti 1979) and 29.2 to 55.0% of various organochlorine pesticides in beef (Sallam and Morshedy 2008).

Among cooking methods, more reduction in both α- and β-endosulfan was observed in pressure cooking,

Table 3 Effects of cooking methods on the levels of endosulfan in buffalo meat

Cooking methods	α-Endosulfan		β-Endosulfan	
	Residue, ppm	Reduction, %	Residue, ppm	Reduction, %
Control	$7.464^{z} \pm 0.125$	-	$2.692^{y} \pm 0.10$	-
Boiling	$3.120^{y} \pm 0.125$	58.3	$1.186^{x} \pm 0.10$	55.93
Pressure cooking	$2.643^{x} \pm 0.125$	64.6	$1.034^{x} \pm 0.10$	61.60
Microwave cooking	$2.848^{xy} \pm 0.125$	61.8	$1.116^{x} \pm 0.10$	58.54
Broiling	$2.799^{xy} \pm 0.125$	62.5	$1.094^{x} \pm 0.10$	59.34

Means bearing different superscripts in the same column differ significantly ($p < 0.01$) ($n = 6$)

followed by broiling, microwave cooking and boiling. Higher temperature due to pressure might have resulted in more volatility of these compounds in pressure cooking. This is in agreement with Nath and Agnihotri (1984), who noticed more loss of endosulfan residues in steam cooking than open cooking. The dissipation was higher for β-endosulfan, when compared to α-endosulfan. Rajashekar et al. (2007) also noticed higher loss of β-endosulfan than α-endosulfan in milk processing. This might be due to variation in susceptibility to heat by different chemical compounds as stated by Dejonckheere et al. (1996) that the effects of cooking and steaming in reducing the pesticide concentration varied depending on the type of pesticide and food.

Conclusion

Residues of cyclodiene group of organochlorine pesticides like aldrin, and endosulfan were found in the majority of analyzed meat and organs of buffaloes. However, the levels of contamination were quite low and well below the maximum residue limit and may not present any threat to public health. Further, cooking of meat resulted in significant reduction in the residue level of endosulfan. Among cooking methods, pressure cooking resulted in highest reduction in pesticides level. This study calls for a continuous monitoring of foods especially of animal origin for the presence of various pesticide residues and attempts should be made to define the source of contamination as a means to correct the situation.

References

Amaraneni SR (2002) Persistence of pesticides in water, sediment and fish from fish farms in Kolleru Lake, India. J Sci Food Agric 82:918–923

AOAC (1995) Official methods of analysis, 16th edn, Association of Official Analytical Chemists, Arlington, VA

Awasthi VB (2007) Chemical control. In: Agricultural insect pests and their control. Scientific Publ (India), Jodhpur, India. p 76–79

Bayarri S, Conchello P, Arino AA, Lazaro R, Herrera A (1994) DDT, DDT metabolites, and other organochlorines as affected by thermal processing in three commercial cuts of lamb. Bull Environ Contam Toxicol 52:554–559

Bedi JS, Gill JPS, Aulakh RS, Joia BS, Sharma JK (2005) Contamination levels of DDT and HCH residues in different goat tissues. Indian J Anim Sci 75:11–13

Brody JG, Rudel RA (2003) Environmental pollutants and breast cancer. Environ Health Perspect 111:1007–1019

Codex (2006) Maximum residue limit for pesticide residue in food. http://www.codexalimentarius.org (Accession on 20 Aug 2006)

Dejonckheere W, Steurbaut W, Drieghe S, Verstraeten R, Braeckman H (1996) Pesticide residue concentrations in the Belgian total diet, 1991-1993. J Assoc Off Anal Chem Int 79:520–528

Dikshith TSS, Kumar SN, Tandon GS, Raizada RB, Ray PK (1989) Pesticide residues in edible oils and oil seeds. Bull Environ Contam Toxicol 42:50–56

Doong R, Lee C (1999) Determination of organochlorine pesticide residues in foods using solid phase extraction clean up catridges. Analyst 124:1287–1289

EU (2006) Maximum residue limits for pesticide residues in foods prescribed by European Union. http:// www.europa.eu.org (Accession on 20 Aug 2006)

Frank R, Braun HE, Sirons GH, Rasper J, Ward GG (1985) Organochlorine and organophosphorous insecticides and industrial pollutants in milk supply of Ontario, 1983. J Food Prot 48:499–504

Herrera A, Agustin A, Arino A, Conchello MP, Lazaro R, Bayarri S, Perez C (1994) Organochlorine pesticide residues in Spanish meat products and meat of different species. J Food Prot 57: 441–444

Holden AJ, Li Chen, Shaw IC (2001) Thermal stability of organophosphorus pesticide triazophos and its relevance in the assessment of risk to the consumer of triazophos residues in food. J Agric Food Chem 49:103–106

Indraningsih, McSweeney CS, Ladds PW (1993) Residues of endosulfan in the tissues of lactating goats. Aust Vet J 70:59–62

Jevsnik M, Flajs VC, Doganoc DZ (2004) Evidence of organochlorine pesticide and polychlorinated biphenyl residues in Slovenian poultry tissues from 1997 to 1999. J Food Prot 67: 2326–2331

Kannan K, Tanbe S, Ramesh A, Subramaniam A, Tatsukawa R (1992) Persistent organochlorine residues in food stuff from India and their implications on human dietary exposure. J Agric Food Chem 40:518–524

Kathpal TS, Dewan RS (1975) Improved clean up technique for the estimation of endosulfan and endrin residues. J Assoc Off Anal Chem 58:1076–1078

Kaur M, Sharma KJ, Gill JP, Aulakh RS, Bedi JS, Joia BS (2008) Determination of organochlorine pesticide residues in freshwater fish species in Punjab, India. Bull Environ Contam Toxicol 80:154–157

Kumari B, Singh J, Singh S, Kathpal TS (2005) Monitoring of butter and ghee (clarified butter fat) for pesticidal contamina-

tion from cotton belt of Haryana, India. Environ Monit Assess 105:111–120

Lane LG, Ammerman GR, Lane H, Muir WM (1979) A comparison of the influence of thermal processing and broiling on naturally occurring and spiked residues of 1,1,1–Trichloro–2,2-bis (p-chlorophenyl) ethane and its metabolites in ground beef. J Agri Food Chem 27:1156–1159

Lazaro R, Herrera A, Arino A, Conchello MP, Bayarri S (1996) Organochlorine pesticide residues in total diet samples from Aragon (northeastern Spain). J Agric Food Chem 44: 2742–2747

Madarena G, Dazzi G, Companini G, Maggi E (1980) Organochlorine pesticide residues in meat. Meat Sci 4:157–166

MFPO (1973) Meat food products order, The Gazette of India Nr 348, dated 28-03–1973

Mirna A, Coretti K (1979) Influence of processing on the degradation of pesticides in meat products. Meat Sci 3:97–108

Nath G, Agnihotri S (1984) Removal of endosulfan from bitter gourds by home processing. Pesticides 18:13–15

Rajashekar K, Kondal Reddy K, Narasimha Reddy K, Sudhakar Reddy K (2007) Effect of processing milk into products on the residue levels of certain pesticides. J Food Sci Technol 44: 551–552

Ramesh A, Balasubramanian M (1999) The impact of household preparations on the residues of pesticides in selected agricultural food commodities available in India. J Assoc Off Anal Chem Int 82:725–737

Sallam KI, Morshedy MAAE (2008) Organochlorine pesticide residues in camel, cattle and sheep carcasses slaughtered in Sharkia Province, Egypt. Food Chem 108:154–164

Saxena MC, Siddiqui MKJ (1982) Pesticide pollution in India: Organochlorine pesticides in milk of woman, buffalo and goat. J Dairy Sci 65:430–434

Shukla G, Kumar A, Bhanti M, Joseph PE, Taneja A (2006) Organochlorine pesticide contamination of ground water in the city of Hyderabad. Environ Int 32:244–247

Solymos M, Eva Visi, Karoly G, Beke Berezi, Gyorfi L (2001) Comparison of extraction methods to monitor pesticide residues in surface water. J Chromat Sci 39:325–331

Tonkabony SEH, Afshar A, Ghazisaidi K, Langaroodi FA, Messchi M, Ahmadi Z (1981) Chlorinated pesticide residues in meat and fat from animals raised in Iran. J Am Oil Chem Soc 58:89–91

Wani SA, Kowale BN, Rao VK (1998) Effect of cooking on pesticide residues in spent hen tissues. Indian J Poult Sci 33: 61–66