Biological Evaluation of Residual Malathion in the Meat of Dipped Hens: Influence on Lipid Profile of Erythrocytes and Brain and Pancreatic Lipase and Amylase Activity

A. K. Pal[1]*, H. S. Kushwah, S. B. Jadhao[1] and A. B. Srivastava
Department of Animal Biochemistry, College of Veterinary Science & Animal Husbandry
Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur 482 001 (M.P.), India

ABSTRACT : Biological evaluation of residual malathion after 168 hrs of single dipping exposure of White Leghorn hens to different concentrations (0, 0.5, 1.0 and 1.5%) of pesticides was investigated. Thirty-two male albino rats divided into four groups of eight each were kept on 20% isoproteinous diet prepared from the meat of these malathion dipped hens. After 30 days feeding trial, the rats were killed by decapitation. No significant change was found in erythrocytes. However, the triglyceride concentration in brain tissue was increased significantly ($p<0.05$) when dose level of pesticide was 1% in dipping solution. Similarly, malathion exposed poultry meat failed in altering any significant change in the pancreatic amylase and lipase activities of rats. This study concludes the virtual absence of toxic accumulation of pesticide in the meat of birds after 168 hrs of exposure in usual concentration range upto 1.5%. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1050-1053*)

Key Words : Biological Evaluation, Residual Malathion, Poultry Meat, Model Rat, Pancreatic Lipase, Amylase, Lipid Profile, Erythrocytes, Brain

INTRODUCTION

Much attention is now given to hazards for public health arising from the increasing use of drugs and chemicals that can accumulate in the animal body and contaminate food supplies. Thus, food safety is a term broadly applicable to procedures designed to ensure that food quality is high and free from factors which may adversely affect human health (Lee and Ryue, 1999). Although, presently there are many advanced and precise analytical methods for quantification of toxic residues in animal products, biological evaluation is still a method with its own importance for evaluating safety of animal products.

Malathion, an organophosphorus pesticide, is commonly used in the form of dip or spray in poultry industry to control external parasites. Although it is used in safe levels for this purpose, some residues can accumulate in the tissues. Toxicites of malathion in the mammalian and avian biosystems have been well established (Varshneya et al., 1988; Pal et al., 1995a, b; Pal and Kushwah, 1998). However, limited information is available on the toxic potency of residual malathion in the meat of exposed birds. Pal and Kushwah (1995, 1997) earlier reported the toxic influence of residual malathion in regard to different biochemical constituents of test animals. The liposolubility of malathion and its consequent affinity for accumulation in the lipid rich tissues intended us to undertake the present study in albino rats (bio model) for exploring toxic impact of residual pesticide (if any in the meat of exposed chicken) on certain membrane associated lipid constituents of erythrocytes and brain tissue. The reason for using erythrocytes as one of the study model was that it serves as an excellent model for evaluating the chemical and structural damage inflicted by the pesticides *in vivo*. In view of the importance of pancreatic enzymes viz. lipase and amylase in relation to lipid and carbohydrate metabolism, the other objective was also to evaluate the toxic influence on the pancreatic lipase and amylase activity

MATERIALS AND METHODS

Experimental animals, treatment of pesticides and biological evaluation

Adult White Leghorn hens (14 months old) of K strain were obtained from All-India Coordinated Research Project on Poultry for Eggs, Jawharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur. Malathion 50% E.C. (o, o-dimethyl diphosphate diethyl mercapto succinate) containing 50% malathion and 50% solvent and auxiliaries was procured from Artee Minerals, Pesticide Division, New Delhi. Birds were caged in 4 groups, each containing 6 animals. Group I was kept as untreated (control) in which birds were dipped in fresh water. In other groups (II, III and IV), they were dipped in 0.5, 1.0 and 1.5% malathion solution for 15

* Correspondign Author: A. K. Pal. Fax: +91-22-6361573, E-mail: fishinst@bom3.vsnl.net.in.
[1] Present Address: Central Institute of Fisheries Education, Seven Bungalows, Versova, Mumbai 400061, India.
Received October 13, 1999; Accepted December 22, 1999

Table 1. Changes in lipid pattern of red blood cells (RBC) and brain of rats kept on diet containing meat of malathion dipped hens

Malathion (50% E.C.) in dipping solution	Total lipid		Phospholipid		Cholesterol		Triglyceride	
	RBC	Brain	RBC	Brain	RBC	Brain	RBC	Brain*
0%	20.23	72.76	7.59	33.97	2.35	12.15	10.28	26.64[a]
0.5%	23.94	87.84	8.30	37.74	2.97	11.99	12.69	36.88[ab]
	(+18.3)	(+20.7)	(+9.2)	(+11.1)	(+26.3)	(-1.2)	(+23.3)	(+38.4)
1.0%	20.19	95.65	7.22	36.76	2.13	14.31	10.84	44.59[b]
	(-0.1)	(+31.1)	(-4.9)	(+8.2)	(-9.4)	(+17.7)	(+5.4)	(+67.4)
1.5%	18.96	79.49	10.13	33.37	2.25	13.35	7.08	32.76[ab]
	(-6.2)	(+9.2)	(+33.3)	(-1.7)	(-4.3)	(+9.8)	(31.0)	(+22.9)
Pooled SE	3.62	6.07	1.16	3.24	0.48	1.62	2.64	12.63

Data are expressed average (mg/gm wt tissue) of eight observations; Figures having different superscripts in column differ significantly (* $p<0.05$); Figures in the parenthesis represent percentage of increase/decrease.

sec keeping the head and neck outside the solution. The hens were exsanguinated after 168 hr. The meat samples were collected and dried; and protein content was estimated by micro-Kjeldahl's procedure.

Thirty two colony bred male Albino rats (103-112 g) were divided into four groups of eight each. They were kept on 20% isoprotein diet prepared from meat of malathion dipped hens. The actual daily feed consumption was recorded. The animals were killed by decapitation after 30 days of feeding trial. The blood was collected in the heparinised tubes. Erythrocytes were separated by centrifugation of blood at 2,500 rpm for 10 min. Erythrocytes were washed three times with chilled saline, finally hemolysed in water (1:1) and then processed for lipid extraction. Brain was quickly dissected out, washed with chilled normal saline (0.85%) and blotted dry with filter paper, weighed and processed for lipid extraction.

Extraction and estimation of lipid fractions

The lipid was isolated as described by Folch et al. (1957). The total lipid was estimated by charring procedure of Marsh and Weinstein (1966). Total phospholipid was estimated as per the procedure of Wagner et al. (1962). Total cholesterol was estimated by the method of Zlatkis et al. (1953) while total triglyceride was estimated by deducting the value of cholesterol and phospholipids from total lipids as described by Khulbe et al. (1989). The pancreas was also dissected out and processed for enzymatic activity. Amylase activity was assayed by the method of Henry and Chiamori (1960) while lipase activity was estimated by the method of Cherry and Crandell (1932).

Means were tested for significance using analysis of variance for completely randomised design as per the methods described by Snedecor and Cochran (1968)

RESULTS AND DISCUSSION

Lipid profile of erythrocytes and brain

The data pertaining to biological evaluation of residual malathion on the meat of pesticide dipped hens in regard to lipid pattern of erythrocytes and brain of the bio-model rats are presented in table 1. It may be noted that in the 30 days feeding trial with meat of adult hens obtained 168 h following a single dipping exposure to malathion in the concentration range of 0.5 to 1.5% failed to alter the total lipid, phospholipids, cholesterol and triglycerides of erythrocytes. However, at 1% concentration of pesticide, the triglyceride concentration in the brain tissue was significantly ($p<0.05$) increased. No parallel reports are available to compare these findings. However, pharmacokinetic studies (Gupta and Paul, 1973) indicated that half clearance of malathion from plasma was 4 hrs and the rate of elimination of malathion was 0.17 hr^{-1}. Marion et al. (1968) reported the residual effect of malathion (25 and 500 ppm) on laying hens with no accumulation of pesticide in the tissue of these hens or in the eggs laid by them. The maximum acceptable daily limit of intake in human beings is 0.2 mg/g weight for malathion (FAO/WHO 1972). Although, organophosphorus compounds are known to be powerful inhibitors of many enzymes, under the conditions of present investigation it has been earlier reported (Pal et al., 1995b; Pal and Kushwah, 1995, 1997) that the activities of enzymes of carbohydrate and protein metabolism and also activity of acetyl cholinesterase (AchE) in different tissues and blood were unaffected in the biomodel rats.

Pancreatic lipase and amylase activity

Impact of malathion exposed chicken meat on pancreatic lipase and amylase activities in rats are

summarized in table 2. Feeding of malathion exposed poultry meat failed to show any altered significant changes in the amylase and lipase activity. Pal and Kushwah (1990) observed a highly significant ($p<0.01$) increase in pancreatic lipase activity in rat upon *in vitro* action of malathion at the concentration of 150 and 200 ppm. No comparable data was found to corroborate the present findings. However, it is a record that DDT failed to mobilize fatty acids from adipose tissue of rats when administered orally a 200 ppm dose for 14 days but it stimulated release of non-esterified fatty acid with the consequent increase in plasma concentration (Kholi et al., 1975). Therefore, absence of any significant change in the pancreatic lipase and amylase activity indicates the virtual clearance of malathion in the meat at 7th day of dipping exposure. This corroborates our earlier observation (Pal and Kushwah, 1997). Any effect on pancreas leads to abnormal carbohydrate metabolism. However, no effect on blood glucose and lactic acid found in a similar study (Pal et al., 1995) is possible explanation for no effect on pancreatic lipase and amylase activities.

Table 2. Pancreatic lipase and amylase activity of rats kept on diet for 30 days containing meat of malathion dipped hens

Malathion in dipping solution	Lipase^{NS} Activity/100 mg wet tissue	Amylase^{NS} Activity/100 mg wet tissue
0% (control)	30.7495	6.1093
0.5%	30.9157 (+0.5)	6.1341 (+0.5)
1.0%	31.1660 (+1.3)	6.6488 (+8.8)
1.5%	32.6666 (+6.2)	5.2531 (-14.0)
Pooled SE	1.2346	0.7400

Figure in parenthesis represents the percentage of increase/ decrease; NS=Not significant.

Thus, this investigation denotes the importance of use of biochemical indicators for evaluation of product safety. The study suggests that malathion did not accumulate in the meat of exposed birds to the extent which might induce perceptible alternation in lipid profile in the test animals or the pesticides was degraded relatively to non toxic products which were eliminated quickly. Therefore, these findings further attests to no risk in human following consumption of meat after 168 hr of dipping exposure of malathion at usual concentration (1-1.5%). However, for a better insight into the probable effects of pesticides and mode of action in a biological system, a parallel study investigating effects on some biochemical indices of other internal organs (liver, heart, spleen and kidney) is warranted.

ACKNOWLEDGEMENTS

The first author is grateful to Indian Council of Agricultural Research, New Delhi for financial support in the form Senior of Research Fellowship.

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