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CONTAMINATION OF BUFFALO MILK WITH RESIDUES OF DIAZINON INSECTICIDE AFTER SPRAYING ANIMALS

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SUMMARY

An experiment was conducted to assess level and duration of milk contamination as a side effect of spraying dairy buffalo with diazinon. The experiment was carried out at the experimental dairy farm, Animal Production Department, Faculty of Agriculture, Cairo University. Four dairy buffaloes were sprayed with diazinon solution (1 ml diazinon/litter of water) using a motorized sprayer. Three milk samples were collected from each animal two hours after spraying, thereafter the collection was repeated two times at a 24-hour interval.

The results showed that residues level was the highest (0.586/ppm) in the first day after spraying. Differences among means were only significant between the 1st day and 2nd and 3rd days. Difference between mean diazinon levels in the 2nd and 3rd days was not significant. Body weight of the cows was a significant source of variance on its milk content of diazinon. As body weight of the animal increase, its milk is expected to contain more diazinon residues.

Key words:
Diazinon, buffalo, dairy, residues, body weight

INTRODUCTION

Diazinon is used to control and kill cockroaches, silverfish and some other insects on the body surface of large animals. It is known to be rapidly absorbed from the gastrointestinal tract and excreted in the urine within 12 hours (Muck et al., 1970). The metabolism of diazinon was investigated in vitro in rat liver microsomes (Nakatsugawa et al., 1969). It was found that diazinon underwent a dual oxidative metabolism consisting of

activation to diazinon and degradation to diethyl phosphorothioicacid. Therefore, the pathways are similar to parathion, malathion and these results emphasized the importance of microsomal oxidation the degradation of organophosphate esters indicating that many of the called phosphate products or hydrolysis products may actually be oxidative metabolites (Menzer and Dauterman, 1970 and Yang et al., 1971). Battu et al. (1989) reported that bovine (Buffalo, Bubalus bubalis) that had been sprayed with DDT and HCH to control malaria and mosquito showed a substantial increase of the residue levels in milk samples collected from rural houses.

The main purpose of this study was to assess level and duration of milk contamination as a side effect of spraying diazinon on dairy buffalo.

MATERIALS AND METHODS

This experiment was carried out at the experimental dairy farm of the Animal Production Department, Faculty of Agriculture, Cairo University. Four dairy buffaloes were tied side to side and sprayed using a motorized sprayer consisting of a sprayer gun connected to a tank containing a pump to press the diazinon solution (1 ml diazinon per each litter of water). Body surfaces of the animals were completely sprayed.

Collection of Milk Samples: Three milk samples were collected from each animal two hours after spraying. The collection was repeated two times at a 24-hour interval.

Extraction of Diazinon from Buffalo Milk: Diazinon was extracted from buffaloes milk according to the method described by Toyda et al. (1990) as: A volume of 50 ml acetonitrile was added to 25 ml milk and the mixture was vigorously shaken for 10 minutes using a mechanical shaker. The acetonitrile layer was then transferred to 300 ml erlenmeyer flask by decantation. The residue was extracted two times with 50 ml 70% acetotrile+water and each extract was filtered through filter paper. One hundred ml water and 2 g zinc sulphate was added to the combined extract. The mixture was then vigorously shaken for 10 minutes and filtered. The filtrate was added to 200 ml 3% sodium chloride and 100 ml dichloromethane. The dichloromethane phase was dried under anhydrous sodium sulphate for 30 minutes, concentrated to 2.5 ml and analyzed by G.C. method. The G.C. analysis was carried out using Hewlett Packard series P 5890 with NB detector at nitrogen mode with column 30 m, 320 mm, and the samples were injected using autosampler (Hewlett Packard 7613).

Statistical Analysis: Data were analyzed according to the General Linear Model Procedure of the statistical analysis system (SAS, 1990). The following model, including the treatment and body weight as main effects, was used to analyze diazinon residues in the milk produced:

$$Y_{ijk} = m + Ti + b1\ (W_{ij} - W) + e_{ijk}$$

where, Y_{ijk} = the observation on the kth animal in the *jth* treatment of the *ith* animal body weight class,

μ = the overall mean,

T_i = the effect of treatment, i = 1,2,3,4

1 = blank (the day before spraying),

2 = residues in 1st day after spraying,

3 = residues in 2nd day after spraying,

4 = residues in 3rd day after spraying,

b_1 = the linear regression coefficient of the diazinon level on body weight,

W_{ijk} = the body weight of the animal in the jth treatment,

W = the average body weight of the four buffalo cows,

ε_{ijk} = the error term.

RESULTS

Least square means of diazinon residues in buffalo milk after spraying with 0.1% diazinon are shown in table 1, and analysis of variance of diazinon level in milk is shown in table 2. Day of sampling showed highly significant effect on diazinon level ($P < 0.0118$). Residual level was highest in the first day after spraying (0.5861 ppm) and thereafter gradually decreased to reach the lowest level in the third day (0.0232 ppm). Differences among means were only significant

Table 1: Least square means [1] ($\bar{X} \pm SE$) of diazinon residues (ppm) in milk after spraying buffalo cows with 0.1% diazinon

Sampling day	Animal				Overall ($\bar{X} \pm SE$)
	1	2	3	4	
Blank	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0000 ± 0.0000
1st day	0.0637 ± 0.5500	2.0457 ± 0.1400	0.0749 ± 0.0450	0.0299 ± 0.0250	0.5861 ± 0.1260 a
2nd day	0.0146 ± 0.0290	0.3993 ± 0.0660	0.1600 ± 0.0350	0.0737 ± 0.0380	0.1294 ± 0.1260 b
3rd day	0.0000 ± 0.0000	0.0928 ± 0.0600	0.0000 ± 0.0000	0.0000 ± 0.0000	0.0232 ± 0.1260 b

[1] Means followed by different superscripts are significantly different ($P<0.05$).

Table 2: Analysis of variance of diazinon in milk of buffalo cows

Sources of variation	df	Mean squares	F value	P
Sampling day	3	0.785	4.12	0.0118
Regression on BW	1	1.078	5.66	0.0219
Residual	43	0.190		

between the first day and the 2nd and 3rd days. While differences between mean diazinon level in the 2nd and 3rd days were insignificant.

The individual differences were very obvious. Animal number 2 scored the highest estimates throughout the three days and was the only animal that have diazinon residues in its milk in the third day. Animal number 4 showed the second highest diazinon levels during the first and second days.

Body weight of the cow was a significant source of variance in its milk content of diazinon. The regression coefficient of diazinon level on body weight was 0.0016 ± 0.0007 ppm/kg ($P<0.0219$), indicating that as body weight of the animal increases its milk is expected to contain more diazinon residues.

DISCUSSION

The metabolism of diazinon was investigated in vitro in rat liver microsomes and cow (Robbins et a., 1957 and Nakatsugawa et al., 1969). It wa found that diazinon underwent to a dual oxidatio consisting of activation of diazinon to diethyl phosphorothic acid or diethylphosphoric acid The diethylphosphorothioic acid is formed by enzymatic hydrolysis of diazinon and the diethyl phosphoric acid could be formed either by enzymatic oxidation of the parent thionophosphate to its phosphate O,O diethyl 2-isopropyle-6methyle-4-pyrimidyl,phosphate.

The enzymatic oxidation process of diazinon has been reported to occur in both insects and mammals with other thionophosphate as a substrate with the production of a more potent antichlonesterase agent (Metcalf and March, 1953 and March et al., 1955).

Although low level of unchanged diazinon were found in the 6-24 hr in milk sample and represent 0.01% of the original dose (Robbins et al., 1957), the concentration of the toxicant diazinon present in various milk samples particularly in the first and second days should be under consideration to

avoid any harmful side effects on human especially that could be occurred from the acute toxic action on inhibiting acetylcholinesterase.

CONCLUSION

The present study reveal that in spite of the necessity of spraying diazinon insecticides to protect dairy animals, dairy producers should be aware of the possibility of milk contamination especially during the first day of spraying.

REFERENCES

Battu R.S., Singh P.P., Joia B.S., Karlt R.L., 1989. Contamination of bovine (buffalo, Bubalus bubalis L.) milk from indoor use of DDT and HCH in malaria control programs. Science of the total environment, 86:281-288.

March R.B., Metcalf R.L., Fukuto T.R., Maxon M.G., 1955. Metabolism of systox in the white mouse and American cockroach. J. Econ. Entomol., 48:355-363.

Menzer R.E., Dauterman W.C., 1970. J. Agric. and Food Chem., 5,601, cited by: Yang .

R.S.H., Hodgson E., Dauterman W.C., 1971. Metabolism in vitro of diazinon and aiazoxon in rat liver. J. Agric. and Food Chem., 19:10-13.

Metcalf R.L., March R.B., 1953. Ann. Entomol. Soc. Amer., 46:63-74, cited by: Robbins W.E., Hopkins T.L., Eddy G.W., 1957. Metabolism and excretion of phosphorus 32- labeled diazinon in a cow. J. Agric. and Food Chem., 5:509-513.

Mucke W., Alt K.O., Esser H.O., 1970. Degradation of 14C-Labeled diazinon in the rat. J. Agric. and Food Chem., 18:208-212.

Nakatsugawa T., Tolman N.M., Dahm P.A., 1969. Oxidative degradation of diazinon by rat liver microsomes. Biochem. Phranacol., 18:685-688.

Robbins W.E., Hopkins T.L., Eddy G.W., 1957. Metabolism and excretion of phosphorus 32-labeled diazinon in a cow. J. Agri. and Food Chem., 5:509-513.

SAS Institute, 1990. Statistical Methods. SAS Institute. Inc., Cary, NC, USA.

Toyda M., Adachi K., Iaa T., Noda K., Minagawa N., 1990. Simple analytical method for organophosphorus pesticide results in milk. J. Assoc. of Anal. Chem., 73:770-772.

Yang R.S.H., Hodgson E., Dauterman W.C., 1971. Metabolism in vitro of diazinon and aiazoxon in rat liver. J. Agric. and Food Chem., 19:10-13.