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Monitoring Methyl Parathion Residues in Milk and Yogurt, and Fate of [^{14}C] Methyl Parathion During Milk Processing

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Methyl parathion (MP), also known as "Cotton poison" is a highly toxic organophosphorus (OP) ester insecticide, that should only be used in open fields to control insects. Today, it is commonly used in current animal feed cultivations such as cotton, soybeans, vegetables and grains including corn and wheat (WHO 1993). Thus animals usually become contaminated either by free grazing on contaminated pastures or by eating contaminated hay or cereals. Although it is relatively less stable and persistent than the organochlorines, there is a number of ways in which it can reach milk (Lino and Da-Silveira 1992 and WHO 1993).

Because (MP) finds widespread agricultural and public health use, particularly in situations where direct exposure to humans and domestic animals may occur, it was selected for the present study. Moreover, due to the few numbers of published reports on residues of (MP) in food throughout the world. Therefore, the aim of this study was to investigate the presence of (MP) and its metabolite residues in milk and yogurt and the distribution of [^{14}C] MP during pasteurization, boiling and yogurt processing using high-performance liquid chromatography (HPLC) followed by liquid scintillation counter.

MATERIALS AND METHODS

[^{14}C] methyl parathion, with a specific activity of 1 μCi/10 mg, was used. Non-radioactive high purity methyl parathion (99 %) and methyl paraoxon (98.4 %) were purchased from Chemservice, INC. (West Chester, PA; USA), while p-nitrophenol from Sigma Chemical Co. (St.Louis, MO; USA). Carbo-sorb, Permafluor and Combustaid were provided by Packard Instrument Co., INC. (Downers Grove, IL; USA). Florisil was purchased from Fisher Scientific Co. (Raleigh, NC; USA). All solvents used for HPLC analysis were HPLC grade and all other chemicals used in this study were obtained in the highest purity available.

Twenty samples (10 each of cow's milk and yogurt) were randomly collected from different regions of Kafr EL-Sheikh Governorate, Egypt; cooled to about 4°C and as soon as possible, brought into laboratory to determine (MP) residues and its metabolites.

Raw (MP) free cow's milk (6 kg) was divided into three portions. Every portion was fortified with radio-labelled methyl parathion (MP) to obtain the following concentrations 1, 2 and 3 μg / ml (0.05, 0.1 and 0.15 μCi / ml) for each of the following treatments: a) Heat treated milk: pasteurization at 63 °C for 30 min., and boiling at 100 °C for 10 min.
b) Yogurt manufacture (according to Egyptian Organization for Standardization, EOS, 1970). Three replicates were done for every treatment to study the fate of [^{14}C] MP during milk processing. Yogurt samples were examined at zero time, 24h, 48h and 72 h after processing.

Samples of milk and yogurt were oxidized, after being weighed in duplicate (100 μl or mg each) onto absorbent combustion caps held in combustion cones, by combustion in a Packard Tri-Carb sample oxidizer Model 306 B, using 10 ml of the trapping solution Carbosorb and 12 ml of the scintillation cocktail Permafluor. Total [^{14}C] radioactivity was determined with a Beckman Model LS 6500 liquid scintillation counter. Recovery of radioactivity in the oxidized samples was 99 %.

Milk and yogurt samples were extracted and cleaned up for (MP) and its metabolites by a modification of the previously described method (Toyoda *et al.* 1990). Each sample was homogenized with 0.1% HCl in acetonitrile five times its weight using the Polytron homogenizer (Brinkman Instruments, Westbury, N.Y.) for 20 sec. at a speed of 5. The extract was centrifuged at 2500 rpm for 25 min. The supernatant was dried over anhydrous sodium sulphate (Heated at 500 °C for 4 h and stored in desicator until used). The combined solvent extract was cleaned up from interfering materials by passing through florisil column (Activated overnight at 105 °C), length of sterile syringe 5 ml, (Mills 1968 and walters 1990).The solvent extract was put under N_2 gas at 35 °C to evaporate the organic solvent. The tube was washed two more times with acetonitrile each time with 0.5 ml. All samples were filtered two times through a 0.2 μm sterile Whatman filter unit. Samples were assayed within three hours or kept at (-20 °C) until used for HPLC analysis. Analysis of each sample was repeated three times. (MP) and its metabolites were separated by reverse-phase high-performance liquid chromatography (HPLC) (Waters-600 Multisolvent Delivery system) using a C_{18} Radial-Pak Cartridge 4 μ. (8MMI.D. type 8) Waters Associates, INC. NC.) and a guard column filled with C_{18} bondapak (E-Merk, Darmstadt, Germany) (De Lima et al. 1996). The mobile phase was acetonitrile – water gradient elution (5: 95, v/v, 100: 0, v/v, in 40 min. following 10 min. isocratic delay); at a flow rate of 1.5 ml/min. Compounds were detected by monitoring the UV absorbance of the column eluates at 286 nm. A mixture of authentic standards of (MP) and its metabolites was injected (100 μl) into the HPLC along with various samples to be analyzed. For quantitative determination of (MP) and its metabolites, the HPLC was equiped with a fraction collector for collection of elution solvents after the separation of radioactive compounds. The radioactive effluents were collected in glass scintillation vials every 1 min. The scintillation fluid (Ultima Gold) (Packard Co.) was added to each vial in ratio 1: 5 and shaken vigorously. The radioactivity was matched with the UV detector trace of scintillation counter to identify and quantify each compound. Analysis of each sample was repeated three times.

RESULTS AND DISCUSSION

The recovery rates for (MP) from fortified milk and yogurt samples at 1 ppm level (average ± S.D. of 3 trials) were 70.6 ± 2.1 and 69.1 ± 2.3 respectively, for methyl paraoxon were 83.6 ± 4.9 and 70.7 ± 4.8 respectively and for p-nitrophenol were 102.9 ± 2.5 and 68.1 ± 4.4 respectively. Nearly similar recoveries of (MP) and methyl paraoxon from milk samples were reported by Lino and Da-Silveira (1992).

Figure 1. shows a representative chromatograms of the 1 μg / ml (MP), methyl paraoxon and p-nitrophenol standards with retention times (Average ± S.D. of three chromatograms) of 25.816 ± 0.04, 20.152 ± 0.02 and 19.106 ± 0.03 minutes respectively.

All the collected milk and yogurt samples were free from (MP) and its metabolites. These results agree with those reported by Lino and Da-Silveira (1992) and Baynes and Bowen (1995) who concluded that, dosages of (MP) not causing overt signs of toxicity are not associated with excretion of (MP) or its metabolite methyl paraoxon in milk. Moreover, a metabolism study was conducted by Van Dijk (1988) on a lactating goat dosed with radiolabelled(MP) in the feed for 3 days.Neither (MP) nor methyl paraoxon were detected in milk. On the other hand, Mallatou et al. (1997) detected (MP) in bovine milk in 2 samples at a mean concentration of 161 ng/g. of fat. Downey (1971) attributed the low persistence of (OP) to the rapid decomposition by physio-chemical process in the environment and enzymatic process in the animal body.

The Joint FAO/WHO reported that, the ADI (Acceptable Daily Intake) of (MP) is 0.02 mg/kg b.w./day (FDA 1993). The Australian MRL (Maximum Residue Limit) for (MP) is 0.05 mg/kg for milk and milk products (FAO/WHO 1994).

Methyl paraoxon was not detected in any of the examined samples. It is the oxygen analogue of (MP) and is a strong cholinestrase inhibitor which is very poisonous to mammals (Eto 1977). The Joint FAO/WHO reported that the MRL of methyl paraoxon in milk is 0.01 mg / kg (IDF 1997).

Data presented in Table 1 and Figure 2 indicated that, pasteurization of milk fortified with 1, 2 and 3 ppm (MP) resulted in quantitative reduction of the total [^{14}C] radioactivity by 39, 35 and 33.3 % of the three treatments used respectively. Concerning (MP), the corresponding reductions were 80, 75 and 83.7 % respectively. Moreover, p-nitrophenol was the only metabolite detected. It was detected in 0.06, 0.1 and 0.15 μg/ml respectively. (MP) was not detected in pasteurized milk samples examined by Lino and Da-Silveira (1992), while methyl paraoxon was detected.

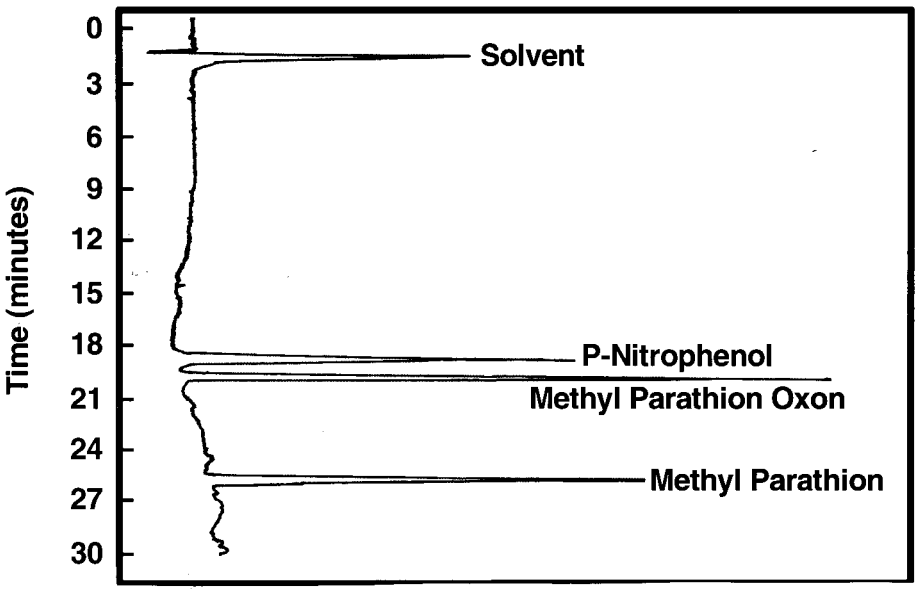

Figure 1. Chromatograms of the 1 μg / ml methyl parathion, methyl paraoxon and p-nitrophenol standards.

Boiling of milk fortified with 1, 2 and 3 ppm (MP) yielded a decrease in the total [^{14}C] radioactivity where about 27, 25 and 23.3 % respectively were destroyed. Concerning (MP) the corresponding reductions were 68, 70 and 74.3 % respectively (Table 1 & Figure 2). Moreover, p-nitrophenol was detected in 0.05, 0.12 and 0.15 μg/ml respectively.

It is noteworthy of mention that, pasteurization caused a considerable reduction in (MP) more than that of boiling. The heat-induced reduction in (OP) insecticide residues in milk were observed by Leshchev et al. (1972) who concluded that pasteurization and boiling of milk reduced diazinon residues by 16.5 and 52.5 % respectively.

Data on the role of processing of milk fortified with radiolabelled (MP) (1, 2 and 3 ppm) on the degradation of (MP) residues in resultant yogurt are tabulated in (Table 1 & Figure 2). The obtained results indicate that, there was severe decrease in levels of total [^{14}C] radioactivity by 50, 61 and 50.3 % of the three treatments used respectively. The degradation increased by time and reached the maximum reduction after 72 h. The corresponding reduction of (MP) increased by time course and reached the maximum after 72 h. (94, 92.5 and 95 % respectively).

Table 1. Effect of pasteurization, boiling and yogurt processing on [^{14}C] methyl parathion.

Treatment	Time course	Total [^{14}C] after heat treatment and yogurt processing*						Methyl parathion **						p-nitrophenol **		
		A		B		C		A		B		C		A	B	C
		µg/ml	%	µg/ml	%	µg/ml	%	µg/ml	%	µg/ml	%	µg/ml	%	µg/ml	µg/ml	µg/ml
Pasteurization		0.61	39	1.3	35	2.0	33.3	0.20	80	0.5	75	0.49	83.7	0.06	0.1	0.15
Boiling		0.73	27	1.5	25	2.3	23.3	0.32	68	0.6	70	0.77	74.3	0.05	0.12	0.15
Yogurt																
	Zero time	0.60	40	1.1	45	1.89	37	0.155	84.5	0.32	84	0.42	86	0.07	0.13	0.19
	24 hr	0.54	46	0.95	52.5	1.68	44	0.1	90	0.21	89.5	0.24	92	0.07	0.13	0.18
	48 hr	0.51	49	0.93	53.5	1.49	50.3	0.08	92	0.18	91	0.21	93	0.08	0.15	0.18
	72 hr	0.50	50	0.78	61	1.49	50.3	0.06	94	0.15	92.5	0.15	95	0.07	0.14	0.17

A, B, C : A: 1 µg / ml; B: 2 µg / ml; C: 3 µg / ml
%: % of reduction
Pasteurization at 63 °C for 30 min. and boiling at 100 °C for 10 min.
*Total [^{14}C] radioactivity was measured by oxygen combustion.
**Methyl parathion and p-nitrophenol were detected by HPLC analysis following by Scintillation counting of effluent from each peak.

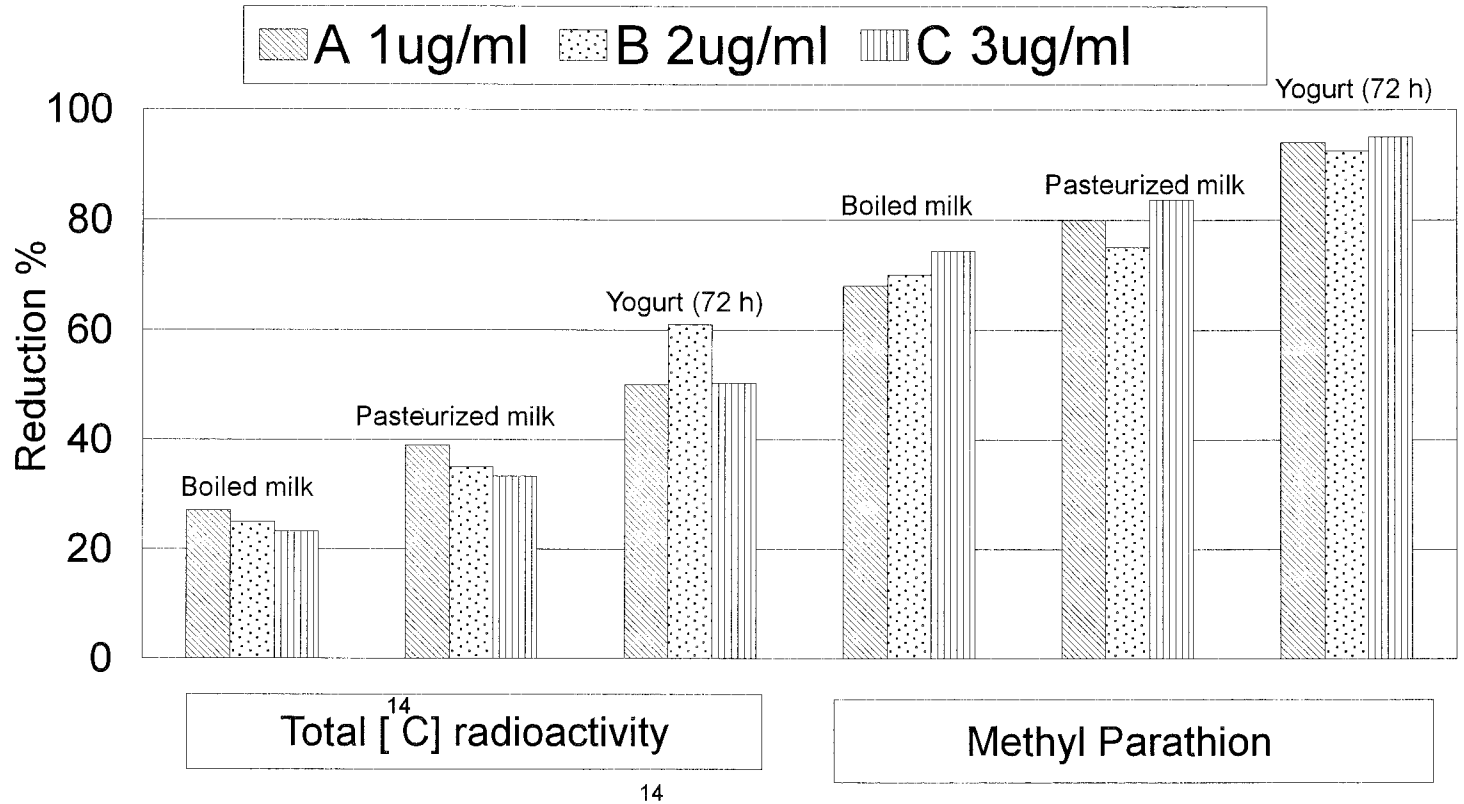

Figure 2. Persistence of [^{14}C] methyl parathion during milk processing

The production of yogurt yielded a decrease in the (MP) level that can be attributed to two factors. First heat degradation of (MP) during processing. The second factor may be due to the growth of yogurt starter culture. The obtained results nearly agree with those reported by Ali et al. (1993) who reported that yogurt processing reduced the natural contaminated pesticides content of milk by 85 % due to the effect of heating and starter bacteria.

Moreover, p-nitrophenol was detected in 0.07, 0.14 and 0.17 μg/g respectively after 72 h. storage. Although methyl paraoxon peak could not be detected, the presence of p-nitrophenol may be an indirect measure of paraoxon formation (De Lima et al. 1996).

The foregoing data concluded that, the manufacturing of yogurt resulted in the highest reduction in the (MP) contaminating the initial milk. That is hardly followed by pasteurization of milk and then closely followed by boiling. Therefore, (MP) present in milk would transfer to the processed products.

Exposure of the general population to (MP) residues occurs predominantly via food. If good agricultural practices are followed, the ADI (0 –0.02 mg/kg b.w.) established by FAO / WHO, will not be exceeded.

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