Bull Environ Contam Toxicol (2009) 82:251–254
DOI 10.1007/s00128-008-9609-0

Determination of 15 Organophosphorus Pesticides in Italian Raw Milk

Teresa Gazzotti · Patrizia Sticca · Elisa Zironi · Barbara Lugoboni · Andrea Serraino · Giampiero Pagliuca

Received: 29 July 2008 / Accepted: 29 October 2008 / Published online: 15 November 2008

Abstract A study was conducted on raw cow's milk to measure the residues of 15 organophosphorus pesticides used as dairy cattle ectoparasiticides or as insecticides in crops used for animal feed. For this purpose a previously devised method was improved and validated. The samples were collected directly from tank trucks during delivery of 3,974 tonnes of raw milk at nine Italian dairy plants. Approximately 4.4% of the 298 samples analyzed contained residues only in traces. The main pollutant was chlorpyriphos.

Keywords Organophosphorus pesticides · Milk · Gas chromatography

Organophosphorus pesticides (OPPs) are esters of phosphoric acid. They are widely used in agriculture and for household plant care, mainly as insecticides, due to their broad spectrum of action and shorter persistence in the environment than organochlorine pesticides. The European Union currently accounts for a quarter of the whole world trade in these pesticides, with 320,000 tonnes of active substances sold each year. The use of plant protection products has lead to major profits in terms of greater harvests and improved crop quality.

However, OPPs can become concentrated throughout the food chain. Milk cows can absorb these compounds by all routes (inhalation, ingestion and dermal absorption) and thus can secrete contaminated milk. This is favored by possible OPPs interactions with lipids and proteins and may be influenced by the season (Pagliuca et al. 2006). The presence of OPPs residues in milk has already been reported (Cardeal and Dias Paes 2006; Pagliuca et al. 2006; Salas et al. 2003; Mallatou et al. 1997; Juhler 1997).

Since consumption of dairy products can be a significant dietary source of contaminants, it is important to update or develop analytical methods for milk monitoring, as new substances and new regulatory limits are introduced. To determine all 15 insecticides with a maximum residue limit (MRL) set by European Union in milk in a single analysis, a previously devised method (Pagliuca et al. 2005) was improved, validated and used to monitor OPPs contamination in Italian raw milk collected in nine Italian dairy plants.

Materials and Methods

The sampling procedure and sample management procedures were performed according to quality assurance criteria set by the International Organization for Standardization (ISO 9001:2000) and all the analyses were carried out in a certified laboratory.

Standard pesticides of acephate (98.5%), azinphos-ethyl (99.0%), chlorpyriphos-ethyl (99.5%), chlorpyriphos-methyl (99.5%), diazinon (97.6%), disulfoton (92.0%), methacrifos (94.3%), methamidophos (99.0%), methidathion (97.5%), oxidemethon-methyl (83.0%), parathion-ethyl (99.0%), parathion-methyl (98.5%), phorate (94.5%), pirimiphos-methyl (99.0%) pyrazophos (97.0%), triazophos (70.0%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Acetone, 2-propanol and anhydrous sodium sulfate were of analytical grade from Merck (Darmstadt,

T. Gazzotti · P. Sticca · E. Zironi · B. Lugoboni · A. Serraino · G. Pagliuca (✉)
Department of Veterinary Public Health and Animal Pathology, Alma Mater Studiorum – University of Bologna, via Tolara di Sopra, 50 Ozzano Emilia, Bologna I 40064, Italy
e-mail: giampiero.pagliuca@unibo.it

Germany), while acetonitrile and dichloromethane were for pesticide residues analysis (BDH, Dorset, UK).

Two hundred and ninety eight samples of raw milk were collected in nine Italian dairy plants (six in northern, one in central and two in southern Italy). The aliquots (800 mL) were collected during milk delivery at the dairy plant. The sampling was carried out directly from the tank trucks with an automatic sampler to obtain homogeneous samples representative of the whole incoming mass of raw milk (3,974 tonnes).

The milk was collected six times during three periods (October 2004, January 2005 and May 2005). The samples were maintained under frozen storage conditions at −20°C before the analysis.

Before extraction, 200 μL of 5 ppm parathion-methyl standard solution in acetone was added to the samples. Sample preparation was carried out according to a previous method (Pagliuca et al. 2005) by extraction and centrifugation of 20 g of raw milk with 25 mL of acetone:acetonitrile (1:4) followed by partition with dichloromethane. After desolvatation the extracted material was reconstructed with 1 mL acetonitrile and directly eluted through a SPE C18 Monofunctional cartridge (500 mg/3.0 mL) (Phenomenex, Torrance, USA). The elution was completed with 2 mL of acetonitrile and 1 mL of 2-propanol. The extract was then concentrated to dryness and redissolved in 200 μL of acetone before GLC analysis.

All analyses were performed on a HRGC Mega 2 8560, equipped with two NPDs (Nitrogen–Phosphorus Detector). Two columns were used: Zebron ZB-5 (30 m × 0.32 mm i.d., 0.25 μm d.f.) and Zebron ZB-50 (30 m × 0.32 mm i.d., 0.25 μm d.f.). The columns were both connected to the injector by a glass dual column adapter to split the sample mixture. The samples were injected into the GLC system in splitless mode (30 s). The injector temperature was set to 260°C. The NPD system used nitrogen as make up gas and a temperature set point of 320°C. Hydrogen (1.5 cc/min for each column) was used as carrier gas.

The chromatographic temperature program was first rated from 80 to 100°C at 2°C/min, followed by a second rate of 4°C/min up to 300°C, then this temperature was maintained for 5 min.

The parathion-methyl peak area was used to quantify each OPP as equivalent of internal standard. The recovery and precision for each compound were determined using 18 aliquots of blank milk. The aliquots were divided into three groups of six and fortified using 0.5, 1 and 1.5 times the permitted limit each, according to Commission Decision 2002/657/EC.

Result and Discussion

The new analytical conditions permit all 15 OPPs listed in Table 1 to be measured with a single extraction procedure and a single injection. Compared with the previous method (Pagliuca et al. 2005), although there was an increase in the number of analytes (from 8 to 15) the GLC conditions were further simplified without any decrease in the analytical performances.

The analyte identifications were obtained by comparing the retention times in the two columns with different polarity and the programmed conditions were chosen to avoid interference of the matrix.

The ZB50 column was chosen as reference column for the quantitative determination due to its better separation

Table 1 Summary of the validation results

OPPs	MRL (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	Rec. % (CV%)
Acephate	20	10	1	103.3 (17.4)
Azinphos-ethyl	50	25	5	167.0 (15.6)
Chlorpyriphos-ethyl	10	5	1	95.9 (17.3)
Chlorpyriphos-methyl	10	5	1	92.7 (13.0)
Demethon-S-methyl sulfoxide	20	10	5	79.9 (22.0)
Diazinon	10	5	1	100.0 (15.2)
Disulfoton	20	10	1	59.8 (29.3)
Methacrifos	10	5	1	82.7 (14.1)
Methamidophos	10	5	1	90.0 (15.2)
Methidation	20	10	1	122.0 (13.8)
Parathion-ethyl	50	25	1	101.2 (10.3)
Parathion-methyl (internal standard)	–	25	1	109.3 (17.7)
Phorate	20	10	1	88.1 (19.8)
Pirimiphos-methyl	50	25	1	92.7 (12.6)
Pyrazophos	20	10	1	144.1 (14.2)
Triazophos	20	10	1	139.7 (17.2)

Table 2 Positive milk samples in the three collection periods

Sampling	Positive samples	Acephate	Chlorpyriphos ethyl	Disulphoton	Parathion ethyl
October	6 (6%)	–	5	1	–
January	5 (5%)	–	4	–	1
May	2 (2%)	1	–	–	1
Total	**13**	**1**	**9**	**1**	**2**

performances. Therefore ZB5 was used as confirmation column.

Table 1 reports the validation data. Precision and accuracy were measured by spiking blank milk samples with three levels of each OPP. The values of average relative standard deviation (RSD%) ranged between 10% and 29%, while recoveries were more than 80%, except for disulfoton with a value of about 60%.

As already reported (Schenck and Donoghue 2000), values above 100% for some OPPs were due to sample matrix enhancement.

The limit of detection (LOD) was estimated as the concentration of analyte yielding a signal-to-noise (S/N) ratio of at least 3/1. Azinphos-ethyl and demethon-S-methyl sulfoxide showed an LOD in milk of 5 μg/kg; for all the other OPPs the LOD was 1 μg/kg.

After its validation the procedure was used to monitor OPPs contamination in Italian raw milk (see Table 2). Among the 298 samples analyzed, none showed concentrations above the limit of quantification and 13 (4.4%) were positive in traces ($LOD < x < LOQ$). The pesticide most frequently found was chlorpyriphos ethyl (nine positive samples), followed by parathion ethyl (two samples) and disulphothon and acephate (one sample each). None of the samples showed the concomitant presence of more than one pesticide. The presence of chlorpyriphos ethyl can be explained by its wide use as an insecticide and ectoparasite on livestock.

Comparing these results with a previous study monitoring eight OPPs in milk (Pagliuca et al. 2006), the progressive decrease of the positive samples from Autumn to Spring was confirmed. In 2006 among the samples analysed, 27% were positive in traces and 7% showed an OPP contamination ranging from 5 to 18 mg/Kg. In that study, the main pollutants detected were acephate and chlorpyriphos. It is remarkable that the present work noted a near total disappearance of acephate residues in milk. This demonstrates compliance with the withdrawal of authorization for plant protection products containing acephate because of its possible impact on non-target organisms (Commission Decision 2003/219/EC).

In conclusion, the present study disclosed a positive trend compared to the 2006 data (Pagliuca et al. 2006), although almost twice the number of the analytes were monitored. The proposed method provides a good straightforward clean-up and allows the detection of all OPP insecticides with MRL fixed by the European Commission (DG SANCO 2008) (see Table 1), in spite of their wide range of polarity shown in Table 3 (Tomlin 1994).

Table 3 Properties of the OPPs studied (Tomlin 1994)

OPPs	Solubility in water mg/L (°C)	log Pow
Acephate	790,000 (20)	−0.9
Azinphos-ethyl	4–5 (20)	3.2
Chlorpyriphos-ethyl	1.4 (25)	4.7
Chlorpyriphos-methyl	4 (24)	4.2
Demethon-S-methyl sulfoxide	22,000 (20)	−0.7
Diazinon	60 (20)	3.3
Disulfoton	12 (20)	4.0
Methacrifos	400 (20)	≥3.0
Methamidophos	>200,000 (20)	−0.8
Methidation	200 (25)	2.2
Parathion-ethyl	11 (20)	3.8
Parathion-methyl	55 (20)	3.0
Phorate	50 (25)	3.9
Pirimiphos-methyl	8.6 (30)	4.2
Pyrazophos	4.2 (25)	3.8
Triazophos	30–40 (20)	3.3

In addition to the moderate stability of OPPs, the very limited contamination measured in Italian raw milk could reflect the correct compliance with withdrawal times for plant protection products and/or veterinarian drugs and the observance of good farming and breeding practices.

References

Cardeal ZDL, Dias Paes CM (2006) Analysis of organophosphorus pesticides in whole milk by solid phase microextraction gas chromatography method. J Environ Sci Health Part B 41:369–375. doi:10.1080/03601230600634745

Commission Decision 2002/657/EC (2002) Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. OJ Eur Comm L 221:8–10, 17 August 2002

Commission Decision 2003/219/EC (2003) Commission Decision of 25 March 2003 concerning the non-inclusion of acephate in Annex 1 to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance. OJL 82:40–41, 29 March 2003

DG SANCO (2008) Pesticide EU-MRLs database. http://ec.europa.eu/sanco_pesticides/public/index.cfm. Accessed 7 November 2008

Juhler RK (1997) Optimized method for the determination of organophosphorus pesticides in meat and fatty matrices. J Chromatogr A 786:145–153. doi:10.1016/S0021-9673(97)00690-0

Mallatou H, Pappas CP, Kondyli E, Albanis TA (1997) Pesticide residues in milk and cheeses from Greece. Sci Total Environ 196:111–117

Pagliuca G, Gazzotti T, Zironi E, Sticca P (2005) Residue analysis of organophosphorus pesticides in animal matrices by dual column capillary gas chromatography with nitrogen-phosphorus detection. J Chromatogr A 1071:67–70. doi:10.1016/j.chroma.2004.08.142

Pagliuca G, Serraino A, Gazzotti T, Zironi E, Borsari A, Rosmini R (2006) Organophosphorus pesticides residues in Italian raw milk. J Dairy Res 73:340–344. doi:10.1017/S0022029906001695

Salas JH, Gonzàles MM, Noa M, Pèrez NA, Dìaz G, Gutièrrez R, Zazueta RH, Osuna I (2003) Organophosphorus pesticide residues in Mexican commercial pasteurized milk. J Agr Food Chem 51:4468–4471. doi:10.1021/jf020942i

Schenck FJ, Donoghue DJ (2000) Determination of organochlorine and organophosphorus pesticide residues in eggs using a solid phase extraction cleanup. J Agr Food Chem 48:6412–6415. doi:10.1021/jf000142c

Tomlin C (1994) The pesticide manual. Royal Society of Chemistry, Cambridge