Irish Journal of Agricultural and Food Research **52:** 197–207, 2013

Review of studies on flukicide residues in cows' milk and their transfer to dairy products

C. Power[1,2], Riona Sayers[3], B. O'Brien[3], A. Furey[2], M. Danaher[4] and K. Jordan[1†]
[1]*Food Safety Department, Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland*
[2]*Team Elucidate, Department of Chemistry, Cork Institute of Technology, Bishopstown, Cork, Ireland*
[3]*Teagasc Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland*
[4]*Food Safety Department, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland*

Flukicides are widely used to treat infestations of liver fluke in dairy cattle. This could result in flukicide residues in milk if animals are improperly treated or if withdrawal periods are not properly observed. The purpose of this review is to summarise the results of studies on depletion of flukicides from milk and the transfer of flukicide residues to dairy products, if present in the milk. As the depletion of flukicide residues from milk of animals treated during lactation was relatively slow, the studies support the view that the dry period (when milk is not being used for human consumption) is the most suitable time for flukicide treatment. Migration of residues to product occurred at different rates, depending on the drug in question. Generally, concentration of flukicides occurred in cheese, butter and skim milk powder. Pasteurisation or heat treatment during spray drying had no impact in reducing residues.

Keywords: Dairy products; flukicides; milk; residues

Introduction

Liver fluke parasites can be frequently found in cattle on pasture environments, even on so-called 'dry' pasture, with potential uptake by grazing animals. Infestation of the animals with liver fluke can lead to loss of productivity, fertility problems and a reduction in live-weight gain (Hope Cawdery 1984; O'Brien, Jordan and Danaher 2010). Flukicides are used to combat the disease and reduce its impact. Closantel, nitroxynil, rafoxanide and triclabendazole are amongst the few flukicides that are active against both mature and immature liver fluke. Other flukicides such as albendazole, clorsulon

†Corresponding author: Dr. Kieran Jordan, Tel: +353 25 42451; Fax: +353 25 42340;
E-mail: kieran.jordan@teagasc.ie

and oxyclozanide are active against adult liver fluke only. Triclabendazole offers an advantage over other flukicides in that it can be administered as a single treatment at the start of the dry cow period. Residues can occur in milk if recommended withdrawal periods are not observed, if cows are treated during lactation, or if cows are treated during the dry-period and residues are excreted on commencement of lactation after calving. The most suitable time to treat dairy cows is during the dry period, when no milk is being produced for human consumption. However, it is important that an adequate withdrawal period is observed and that the drug residues are eliminated when milking resumes.

The use of flukicides in animals producing food for human consumption can lead to residues of the drug entering the human food chain. For this reason, their use is controlled by the European Medicines Agency. Based on the best scientific data, maximum residue limits (MRLs) are set for flukicides. The MRL is the maximum concentration of residue resulting from the use of a veterinary drug (expressed in μg/kg on a fresh weight basis) that is recommended by the Codex Alimentarius Commission to be legally permitted or recognised as acceptable in or on a food (Codex Alimentarius Commission 2010). Allied with the MRL is a withdrawal period, which is the time required from administration of a drug to its concentration being below the MRL. During the withdrawal period, no food from that animal can enter the human food chain. Provisional MRLs in milk have been set for certain flukicides such as, clorsulon (Anon. 2012a), closantel, (Anon. 2012b), nitroxynil (Anon. 2012c) and triclabendazole (Anon. 2012d). For these flukicides, MRLs have been established in milk. However, where no MRL in milk has been established, for example rafoxanide, the use of such a flukicide in lactating animals where milk is to be used for human consumption is not permitted. Detection of such a compound in a food, at any concentration, indicates that a banned substance was used and deems that food as non-compliant.

Few studies have been undertaken on the depletion of flukicide residues from milk or on the migration of residues from milk to dairy products during product manufacture. The purpose of this paper is to review the limited number of studies that have been undertaken.

Analytical Methodology

Until recently, anthelmintic drug residue analysis focused on the analysis of benzimidazole, macrocyclic lactone and levamisole residues in milk. Methodologies were available to detect flukicide residues but these were generally applicable to single residue analysis and were not widely applied in Europe or the United States. Japanese researchers had developed more extensive analytical methods using high performance liquid chromatography coupled to electrochemical detection (HPLC-ECD). This HPLC-ECD detection system allowed the sensitive detection of flukicides, including oxyclozanide and nitroxynil, in milk to low concentrations (μg/kg levels). However, HPLC-ECD is not a widely used technique in residue analysis laboratories. In response to this deficiency, Kinsella *et al*. (2009) developed a method for simultaneous detection of 38 anthelmintic drug residues in food (including flukicides) using LC-MS/MS. Whelan *et al*. (2011a) improved this method through the application of ultra high performance liquid chromatography and improvements in sample preparation to improve sample throughput and sensitivity. This method was validated for measurement of 0.5, 1.0 and 1.5 times the

MRL in each of the 38 analytes in bovine milk. Power *et al*. (2013a,b,d) validated the method further for measurement of 0.5, 1.0 and 1.5 times the MRL for flukicide residues in bovine, ovine and caprine milk, cheese, butter and skim milk powder.

Treatment during the Dry Period

In Ireland, the dry period from the end of one lactation period to the beginning of another, is typically about 60 days. During this period, milk production has ceased and this should ideally provide an opportunity for the treatment of cows with veterinary drugs like flukicides. Some flukicide products containing the active ingredients albendazole or oxyclozanide can be used for treating dairy cows during lactating or dry cow periods, but they are not active against all stages of fluke. In 2010, all products containing the active ingredients clorsulon, closantel, nitroxynil, rafoxanide and triclabendazole were prohibited for use in lactating animals producing milk for human consumption. Subsequently, provisional MRLs of 16, 45, 20 and 10 μg/kg were established for clorsulon, closantel, nitroxynil and triclabendazole residues in milk, respectively (Anon. 2012a,b,c,d). No MRL was established for rafoxanide. There was a lack of data on the persistence of these residues following dry cow treatment. In two subsequent studies, the persistence of triclabendazole and closantel residues in milk following dry cow treatment was investigated in licensed trials. Triclabendazole (in the form of the unlicensed product Fasinex 10%) was administered to 36 cows during the dry period. At the first milking, the residue concentrations in milk of all of the 36 cows involved in the study were < 1 μg/kg, which is below the provisional MRL of 10 μg/kg (Power *et al*. 2013c). Similarly, the residue concentration in the first milk was below the MRL (45 μg/kg) when six cows were treated with closantel (Flukiver 50 mg/L) during the dry period (Power *et al*. 2013b). Residues were below the limit of detection of the method at 73 and 117 days post administration. Since these studies were undertaken, a new product containing triclabendazole as the active ingredient, Fasinex 240, has been licensed for use under strict conditions during the dry period (Anon 2012e)

Treatment during Lactation

If flukicides are administered during lactation, there is a risk of residues being transferred to the milk and products manufactured from that milk (Tables 1–3). An extensive review of benzimidazole residues (a sub-class of anthelmintics), which reported on the metabolism and persistence of residues in milk has been undertaken (Danaher *et al*. 2007). In that paper, it was reported that albendazole related drugs (netobimin, albendazole and albendazole sulphoxide) were typically below the MRL in the milk of lactating dairy cows at 48 h post-treatment. Triclabendazole residues were reported to persist in milk for at least 10 days following treatment. In recent years, the persistence of triclabendazole residues has been investigated in the milk of lactating dairy cows using more sensitive LC-MS/MS based methods. Imperiale *et al.* (2011) reported that triclabendazole residues persisted in milk up to 144 days post-treatment. The highest concentration of triclabendazole sulphone found in milk was 2400 μg/mL. Power *et al*. (2013c) investigated persistence of triclabendazole residues in milk following the administration. The highest levels of triclabendazole, triclabendazole sulphoxide, triclabendazole sulphone and keto-triclabendazole residues measured in individual milk samples were 244, 525, 1710 and 16 μg/kg, respectively. Residues of triclabendazole, triclabendazole sulphoxide,

Table 1. Transfer of flukicide residues from milk to curd, whey and cheese

	Milk (µg/kg)	Curd (µg/kg)	Whey (µg/kg)	Ripened cheese (µg/kg)	Reference
Triclabendazole[1] (summed metabolites)	918; 850	4532; 5672	219; 180	5372; 4784	Power *et al*. 2013a
Rafoxanide[1]	379; 216	1372; 880	83; 72	1944; 790	Power *et al*. 2013d
Closantel[1]	273; 236	283; 322	160; 130	339; 634	Power *et al*. 2013b
Oxyclozanide[2]	7.0	3.6	32.1 – Whey cheese	6.7	Whelan *et al*. 2010
Triclabendazole[3]	600–2000	ND	ND	1100–20000	Imperiale *et al*. 2011
Albendazole[2] (summed metabolites)	1127.9	2113.9	865.0	2273.0	Fletouris *et al*. 1998

[1]In these studies, independent duplicate experiments were undertaken and both values were shown.
[2]In these studies, only one value was given.
[3]In this study, a range of values was given.
ND = not determined.

Table 2. Migration of flukicide residues during the separation of milk.

	Milk (µg/kg)	Skim milk (µg/kg)	Cream (µg/kg)	Butter (µg/kg)	Buttermilk (µg/kg)	Reference
Triclabendazole	1082; 1001[1]	423; 508	7589; 10757	9177; 8027	1644; 1900	Power *et al*. 2013a
Rafoxanide	448; 220	32;13	3922; 2484	3764; 3688	367; 176	Power *et al*. 2013d
Closantel	306; 302	193; 144	2407; 1628	3656; ND[2]	304; 355	Power *et al*. 2013b
Oxyclozanide	ND	969.9; 95.4%	3.1; 4.6%	ND	ND	Power *et al*. 2012
Nitroxynil	ND	95; 97.5%	5.0; 2.5%	ND	ND	Power *et al*. 2012

[1]See footnote to Table 1.
[2]ND = not determined.

Table 3. Concentration of flukicide residues from skim milk to powder and stability of the residues in the powder

	Skim milk (µg/kg)	Powder (µg/kg)	Following storage (µg/kg)		Reference
			6 months	12 months	
Triclabendazole	423; 508[1]	7252; 5119	5935; 4775	1670; 1790	Power *et al.* 2013a
Rafoxanide	32; 13	328; 144	322; 130	309; 122	Power *et al.* 2013d
Closantel	193; 144	1571; 1558	ND[2]; 3084	ND; 2984	Power *et al.* 2013b

[1]See footnote to Table 1.
[2]ND = not determined.

triclabendazole sulphone and ketotriclabendazole were detectable in milk for up to 5.5, 15.5, 20 and 5 days post-treatment, respectively. Summed triclabendazole residues were less than the MRL of 10 µg/kg at 17 days post treatment (Figure 1).

There have been some studies on the persistence of nitroxynil residues in milk following administration to dairy cows (Heeschen, Tolle and Bluthgen 1972; Bluthgen, Heeschen and Nijhuis 1982; Takeshita *et al*. 1980; Takeba and Matsumoto 1992; Whelan *et al*. 2011b). The results of these studies show that nitroxynil residues can persist in milk for at least 8 weeks post-treatment. In a

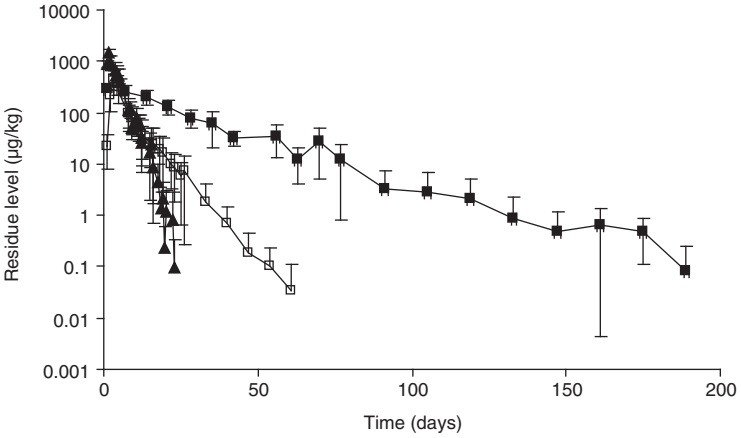


Figure 1. Withdrawal period for summed triclabendazole (▲), rafoxanide (□) and closantel (■) residues in the milk of cows (n=6) administered each analyte in three separate trials (adapted from Power et al. 2013b,c,d).

recent study, Whelan *et al*. (2011b) reported similar findings using a new sensitive UHPLC-MS/MS method, where residues were detectable in the milk of four out of six animals at 58 days post-treatment.

The persistence of oxyclozanide residues in milk has been investigated following the administration of oral doses (Bluthgen *et al*. 1982; Fujinuma, Takeba and Kamata 2006). Bluthgen *et al*. (1982) found that the highest concentration of oxyclozanide detected in milk was 130 µg/kg at 48 h post-treatment. Residues were measurable at 10 µg/kg up to 120 h post-treatment. Fujinuma *et al*. (2006) reported much lower levels of oxyclozanide in milk, typically <10 µg/kg at all time points. Whelan *et al*. (2010) investigated the persistence of oxyclozanide following administration of a combination product, which also contained the anthelmintic product levamisole. The highest concentration of oxyclozanide found in the milk of individual animals ranged between 8 and 24 µg/kg and occurred at 36 h post-treatment.

There have been few reports on the persistence of closantel and rafoxanide in milk.

Closantel residues were detectable in milk for 199 days post-treatment in one of the six cows, while the remaining five cows had no detectable residues in the milk at between 178 and 192 days (Figure 1). However, the provisional MRL of 45 µg/kg for closantel residues in milk was reached after a period of approximately 52 days post-administration (Power *et al*. 2013b). Rafoxanide residues were detectable in milk for 46 days post-treatment in one of the six animals while the remaining five cows had no detectable residue between 54 and 61 days (Figure 1; Power *et al*. 2013d).

In the case of flukicides, the excretion of residues into milk samples has been shown to vary greatly between drugs. Danaher *et al*. (2007) reported that the rate of excretion of drug residues into milk can depend on a number of factors including the physicochemical properties of the drug, the route of administration (Michels, Meuldermanns and Heykants 1987), the body condition of animal and the status of gut microflora. The withdrawal period required for residues of closantel, nitroxynil, rafoxanide and triclabendazole

to reach less than the provisional MRLs are impractical, indicating that use of these flukicides during lactation is not feasible. Rafoxanide has no MRL and the detection of any residue renders the product non-compliant. The results support the view that the dry period is the most suitable time for flukicide treatment.

Residue Transfer to Product

In general, a number of papers have reported on the persistence of anthelmintic residues in milk and the concentrations of residues in cheese produced from incurred milk. However, few papers have extended research to other important dairy products such as butter, buttermilk, cream, whey and skim milk powder. The processing of many dairy products involves the separation of fat or the elimination of water through, for example, loss of whey during cheese-making or loss of water during drying processes. Such processing creates the possibility of concentration of any residues from the starting milk either through migration with the fat, or concentration during whey separation or drying. Thus, if present in the starting milk, some dairy products have the potential to have increased concentrations of flukicide residues. This potential increase is dependent on the type of dairy product being manufactured and the drug used.

Cheese

Some studies have monitored the migration of flukicide residues to curd and whey during cheese manufacture (Table 1). Triclabendazole (Power *et al*. 2013a; Imperiale *et al*. 2011) and rafoxanide (Power *et al*. 2013d) migrated with the curd, with relatively little residue being lost in the whey. Albendazole migrated 70% with the curd and 30% with the whey (Fletouris *et al*. 1998), while the concentrations of closantel were more evenly distributed between the curd and the whey (Power *et al*. 2013b) and oxyclozanide residues were similar in the cheeses and in the starting milk (Whelan *et al*. 2010). In most cases, the ripened cheese had higher residue concentrations than the curd. The reason for this is that after separation of the curd and whey, some whey continued to be expelled from the curd leading to further concentration and therefore higher residue concentrations in the ripened cheese. Less hydrolysis of the molecule could also have contributed to this increase. In studies with nitroxynil, most of the drug residue was concentrated with the curd (Takeba and Matsumoto 1992).

Milk separation

In the case of triclabendazole, closantel and rafoxanide, the residue migrated with the fat, being detectable in concentrations that were approximately ten times higher in the cream, compared with the levels detected for each analyte in skim milk (Table 2). This is in contrast to the migration of the flukicides, nitroxynil and oxyclozanide where >90% of the residue migrated with the skim milk (Power *et al*. 2012).

Butter

Following separation, the cream may be used for butter manufacture. Similar concentrations of triclabendazole and rafoxanide were found in cream and butter, with approximately 10–20% of residue lost in the buttermilk, respectively. Closantel residues were slightly higher in the butter than in the cream, indicating concentration, again with approximately 10% of the residue lost in the buttermilk (Table 2).

Skim-milk powder

For the experiments in Table 3, a bench-top laboratory scale spray drier was used. The skim milk suspension was pneumatically atomised into a vertical drying chamber

using a two-fluid nozzle system. The inlet temperature was maintained at 185 °C and the flow-rate of the skim-milk suspension was varied by the adjustment of controls so that this flow-rate controlled the outlet air-temperature which was maintained at 90 °C ± 2 °C. Under these conditions, the residues of triclabendazole, rafoxanide and closantel concentrated in the powder and were apparently unaffected by the drying temperatures used. Thus, if there were flukicide residues in the starting milk, some of the residue will be retained in the skim milk and subsequently concentrated in the powder.

Migration Pattern of Residues

The partitioning behaviour of flukicide drug residues in dairy products is dependant on the physicochemical properties of the drugs and the dairy product. The residues of closantel, rafoxanide and triclabendazole migrated into the butter and cream, which is in good agreement with octanol-water partition coefficients (K_{ow}) shown in Table 4. The K_{ow} of these molecules would indicate that these molecules are non-polar or lipophilic in nature. In the case of closantel, nitroxynil and oxyclozanide residues were concentrated in the skim milk. This might be explained in part for oxyclozanide (medium polarity drug) and nitroxynil (polar drug) because of their lower K_{ow} of 2.3–3.1 and 5.3, respectively. The K_{ow} value for closantel of 7.0 would indicate that this molecule would concentrate in the skim milk. The pK_a value could also explain the behaviour because these three molecules are all acidic in nature. At the pH of skim milk (pH 6.7), both nitroxynil (pK_a 3.74) and closantel (pK_a 4.3) would be fully dissociated, which favours the partitioning of these molecules into the skim milk. Oxyclozanide is also a weakly acidic molecule with a pK_a of 5.4, which would be fully dissociated at pH 7.4 and approximately 75% dissociated at the pH of skim milk. Rafoxanide, which also an acid molecule with a pK_a of 6.8 would be less soluble in the skim milk. Triclabendazole sulphone (marker of triclabendazole residues) possesses both basic and acidic functional groups but does not dissociate over the range 3.0 to 9.9 (Danaher *et al.* 2007), which would indicate that its partitioning behaviour is more influenced by its K_{ow} value (3.6–5.1).

The migrational behaviour of flukicide residues during cheese production is more dynamic because of the influence of temperature, and the drop in pH from 6.2 to 5.2 during the process of syneresis. The migration patterns shown in Table 1 are similar to that predicted from the structure and chemical properties. Additionally, data is presented for albendazole residues in milk, curd, whey and cheese. Albendazole is represented by three main metabolites (all basic in nature) which are relatively polar molecules (Table 4). Albendazole related residues were found to concentrate less in the cheese as predicted by their physicochemical properties.

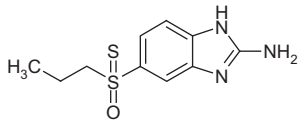
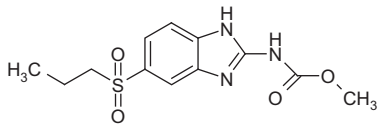
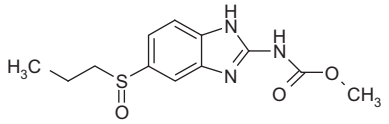
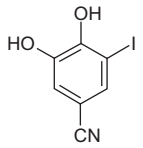
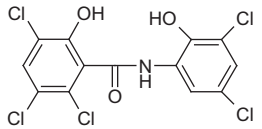
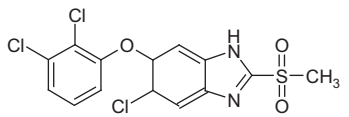
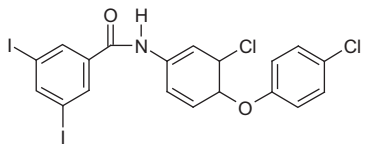
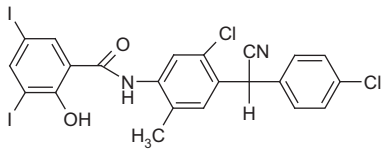
Stability of Residues in Product

If flukicide residues are present in milk, it is important to understand their stability during product manufacture, storage and freezing prior to analysis.

During manufacturing processes

Power *et al*. (2013a,b,d) showed that triclabendazole, rafoxanide and closantel were stable during pasteurisation. Similar residue concentrations were observed in milk before and after pasteurisation ($P>0.05$). These residues were also stable during the spray drying process where temperatures of 185°C were used, albeit for a short time period. In effect, none

Table 4. The chemical structures of flukicides and selected chemical properties for (A) albendazole sulphone amine, (B) albendazole sulphone, (C) albendazole sulphoxide, (D) nitroxynil, (E) oxyclozanide, (F) triclabendazole sulphone, (G) triclabendazole sulphoxide, (H) rafoxanide and (I) closantel (Danaher 2007, Anon. 2013)

	Structure	pK_a[1]	K_{ow}[2]
A		6.0 (base)	0.7–0.8
B		3.5 (base)	0.9–1.0
B		5.7 (base)	0.8–0.9
D		3.74 (acid)	2.3–3.1
E		5.4 (acid)	5.3
F		1.9 (basic)	3.6–5.1
G		6.4 (acid)	6.8
H		4.3 (acid)	7.0

[1]Acid base dissociation constant.
[2]Octanol-water partition co-efficient.

of the manufacturing processes used had any detrimental effect on the residues. Similarly, residues were stable during cooking processes (Cooper *et al*. 2011).

During storage

During storage of butter at 5 °C and cheese at 14 °C, the stability of triclabendazole, rafoxanide and closantel was studied in each of these products. In the case of butter, there was no significant difference ($P>0.05$) in the residue concentrations detected during refrigerated storage of the butter (Power *et al*. 2013a,b,d). During the ripening of cheese over a 3 week period, triclabendazole, closantel and rafoxanide residue concentrations increased significantly ($P<0.05$). This increase was probably due to the continued expulsion of whey from the cheese following separation of the curd and whey and to decreased moisture in the cheese.

During freezing

Freezing of samples prior to analysis by UHPLC-MS/MS is common practice, yet there are few studies on the effect of freezing on residue concentrations. Power *et al.* (2013a,b,c,d) studied the effect of freezing (at -20 °C) for 6 and 12 months on the stability of triclabendazole, rafoxanide and closantel residues in milk, cheese and butter. In butter, triclabendazole and rafoxanide remained stable for up to 12 months, whereas the concentration of closantel increased significantly ($P<0.05$). In cheese, there was a significant ($P<0.05$) increase in residue concentrations for all three analytes during frozen storage. If the samples dry during the freezing process, the residue concentration could be increased, or the residues may become more readily extractable. Further studies are needed to investigate the reason(s) for such changes in residue concentrations following freezing.

Significance of these Studies

These studies show that treatment of dairy cows with flukicides to combat liver fluke is best done during the dry period when there is no milk production. However, such treatment must take adequate care that residues are not in the milk after lactation resumes. Therefore, research on depletion studies for residues following treatment during the dry period is necessary. Such studies may assist the drug manufacturers in designing new more targeted veterinary drugs that have shorter depletion times. If residues were present in the milk, they were not damaged by pasteurisation or other heating processes involved in dairy product manufacture, and they migrated to product. The results also showed that undetectable concentrations of residue in milk could be concentrated during product manufacture, resulting in residue detection in the product. This is particularly important in the manufacture in infant milk formula, and infant milk formula manufacturers need to be vigilant in this regard.

Acknowledgements

This work was supported by The Dairy Levy Research Trust. Clare Power was in receipt of a Teagasc Walsh Fellowship.

References

Anonymous. 2012a. Commission implementing regulation EU No. 466/2012 of 1 June 2012 amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance clorsulon. *Official Journal of the European Communities* **L143**: 2–4.

Anonymous. 2012b. Commission implementing regulation EU No. 221/2012 of 14 March 2012 amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits

in foodstuffs of animal origin, as regards the substance closantel. *Official Journal of the European Communities* **L75**: 7–9.

Anonymous. 2012c. Commission implementing regulation EU No. 201/2012 of 8 March 2012 amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance nitroxynil. *Official Journal of the European Communities* **L71**: 37–39.

Anonymous. 2012d. Commission implementing regulation EU No. 222/2012 of 14 March 2012 amending the Annex to Regulation EU No. 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, as regards the substance triclabendazole. *Official Journal of the European Communities* **L75**: 10–11.

Anonymous. 2012e. Irish Medicines Board. 'Update on fluke treatments for use in dairy cows producing milk for human consumption' Available online: http://www.imb.ie/EN/Safety--Quality/Advisory-Warning--Recall-Notices/Veterinary-Medicines/Update-on-Fluke-treatments-for-use-in-Dairy-Cows-producing-milk-for-human-consumption~.aspx?page=1&noticetypeid=-1&year=-1 [Accessed 7 October 2013].

Anonymous. 2013. Chembase. Available online: http://en.chembase.cn/news-27.html [Accessed 7 October 2013].

Bluthgen, A., Heeschen, W. and Nijhuis, H. 1982. Gas-chromatographic determination of fasciolicide residues in milk. *Milchwissenschaft* **37:** 206–211.

Codex Alimentarius Commission. 2010. "Codex Alimentarius Commission Procedural Manual", nineteenth edition. World Health Organization, Food and Agricultural Organization of the United Nations, Rome, Italy. Available online: http://www.fao.org/docrep/012/i1400e/i1400e00.htm [Accessed 31 October 2013].

Cooper, K.M., Whelan, M., Danaher, M. and Kennedy, D.G. 2011. Stability during cooking of anthelmintic veterinary drug residues in beef. *Food Additives and Contaminants: Part A* **28***:* 155–165.

Danaher, M., De Ruyck, H., Crooks, S.R., Dowling, G. and O'Keeffe, M.J. 2007. Review of methodology for the determination of benzimidazole residues in biological matrices. *Journal of Chromatography B* **845**: 1–37.

Fujinuma, K., Takeba, K. and Kamata, K. 2006. Concentration in plasma and excretion in milk of lactating cows after oral administration of tribromsalan, oxyclozanide and bromofenofos. *Journal of the Japanese Society of Food Hygiene* **47**: 249–253.

Fletouris, D.J., Botsoglou, N.A., Psomas, I.E. and Mantis, A.I. 1998. Albendazole-related drug residues in milk and their fate during cheesemaking, ripening, and storage. *Journal of Food Protection* **61**: 1484–1488.

Heeschen, W., Tolle, A. and Bluthgen, A. 1972. Fasciolicides in milk. *Lebensmittelhygiene* **180**: 188–194.

Hope Cawdery, M.J. 1984. Review of the economic importance of fascioliasis in sheep and cattle. *Irish Veterinary News* (September): 14–22.

Imperiale, F., Ortiz, P., Cabrera, M., Farias, C., Sallovitz, J.M., Iezzi, S., Perez, J., Alvarez, L. and Lanusse, C. 2011. Residual concentrations of the flukicidal compound triclabendazole in dairy cows' milk and cheese. *Food Additives and Contaminants* **28:** 438–445.

Kinsella, B., O'Mahony, J., Malone, E., Moloney, M., Cantwell, H., Furey, A. and Danaher, M. 2009. Current trends in sample preparation for growth promoter and veterinary drug residue analysis. *Journal of Chromatography A* **1216**: 7977–8015.

Michels, M., Meuldermanns, W. and Heykants, J. 1987. The metabolism and fate of closantel (Flukiver) in sheep and cattle. *Drug Metabolism Review* **18:** 235−251.

O'Brien, B., Jordan, K. and Danaher, M. 2010. Update on the use of flukicides. *Irish Veterinary Journal* **63**: 702–704.

Power, C., Sayers, R., O'Brien, B., Bloemhoff, Y., Danaher, M., Furey, A. and Jordan, K. 2012. Partitioning of nitroxynil, oxyclosanide and levamisole residues from milk to cream, skim milk and skim milk powder. *International Journal of Diary Technology* **65**: 503–506.

Power, C., Danaher, M., Sayers, R., O'Brien, B., Clancy, C., Furey, A. and Jordan, K. 2013a. Investigation of the migration of triclabendazole residues to milk products manufactured from bovine milk, and stability therein, following lactating cow treatment. *Journal of Dairy Science* **96**: 6223–6232.

Power, C., Sayers, R., O'Brien, B., Clancy, C., Furey, A., Jordan, K. and Danaher, M. 2013b. Investigation of the persistence of closantel residues in bovine milk following Lactating-cow and dry-cow treatments and its migration into dairy products. *Journal of Agricultural and Food Chemistry* **61**: 8703–8710.

Power, C., Whelan, M., Danaher, M., Bloemhoff, Y., Sayers, R., O'Brien, B., Furey, A. and Jordan, K. 2013c. Investigation of the persistence of triclabendazole residues in bovine milk

following lactating-cow and dry-cow treatments. *Food Additives and Contaminants: Part A* **30**: 1080–1086.

Power, C., Danaher, M., Sayers, R., O'Brien, B., Whelan, M., Furey, A. and Jordan, K. 2013d. Investigation of the persistence of rafoxanide residues in bovine milk and fate during processing. *Food Additives and Contaminants* **30**: 1087–1095.

Takeba, K. and Matsumoto, M. 1992. Investigation of the residue of the fasciolicide nitroxynil in milk and dairy products. *Nippon Koshu Eisei Zasshi* **39:** 75–82.

Takeshita, Y., Kishi, T., Seki, M., Fujiyama, K., Otsuka, G. and Ahiko, K. 1980. Analysis of nitroxynil (fasciolicide) in milk and dairy-products. *Milchwissenschaft* **35:** 133–135.

Whelan, M., Chirollo, C., Furey, A., Cortesi, M.L., Anastasio, A. and Danaher, M. 2010. Investigation of the persistence of levamisole and oxyclozanide in milk and fate in cheese. *Journal of Agricultural and Food Chemistry* **58**:12204–12209.

Whelan, M., Kinsella, B., Furey, A., Moloney, M., Cantwell, H., Lehotay, S. and Danaher, M. 2011. Determination of anthelmintic drug residues in milk using ultra high performance liquid chromatography-tandem mass spectrometry with rapid polarity switching. *Journal of Chromatography* **1217**: 4612–4622.

Whelan, M., Bloemhoff, Y., Furey, A., Sayers, R. and Danaher, M. 2011. Investigation of the Persistence of nitroxynil residues in milk from lactating dairy cows by ultra performance liquid chromatography tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* **59:** 7793–7797.

Received 24 September 2013