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Review of the Concentrations of Anthelmintic Substances in Animal Food Products

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ABSTRACT

This paper reviews those anthelmintic preparations which are likely to be in widespread use in Great Britain and the evidence for the depletion of these agents and their metabolites from animals used in food production. Tissue depletion studies are summarised for fenbendazole, levamisole, oxfendazole and thiabendazole. Analytical methods available for their detection in animal products are also reviewed.

INTRODUCTION

Anthelmintic agents are widely used in Great Britain in the prevention and treatment of disease in food-producing animals caused by internal worm parasites and they are an important tool in the efficient production of food (Kemp, 1975; Michel *et al.*, 1981). They are used to prevent and treat the infection of cattle and sheep by lungworms, liver fluke and gastrointestinal (GI) tract worms (tapeworm and roundworm), of pigs by lungworms and GI tract roundworms and of poultry by GI tract roundworms (Anon., 1980*a*, *b*). Michel *et al.* (1981) showed that the majority of cattle in England and Wales are probably dosed with an anthelmintic at least once per year and this is most commonly for the purpose of prophylaxis against gutworms.

This review summarises which of these agents are widely used, what concentrations of the drug or its metabolites are likely to be found in

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animal food products and what methods are available to detect such residues. Such information is essential in order to develop a programme of surveillance to determine the effects of their use on food quality.

AVAILABLE PREPARATIONS

Range of available agents

Over twenty different agents are licensed for use in farm animals in Great Britain (Anon., 1980*a*, *b*). These include copper and cobalt salts and a diverse range of organic compounds which includes organochlorine compounds (e.g. carbon tetrachloride) and nine benzimidazole derivatives. This review will cover only those anthelmintics that are in current widespread use. Five benzimidazole compounds and levamisole are most commonly used in Great Britain (Michel *et al.*, 1981). These are listed in Fig. 1. The agents are more commonly used than others because clinical field experience has shown them to be effective. The benzimidazole derivatives (Fig. 1) affect a wider range of helminths than many of the other available compounds (Coles, 1977). Amongst the most commonly used compounds, levamisole was the clear market leader for cattle treatment in England and Wales in 1978 (Michel *et al.*, 1981), but more recently fenbendazole, which is more potent than levamisole (Kemp, 1975), has become almost as widely used in cattle (Michel, personal communication).

Methods of administration

In Britain, manufacturers' recommendations often give quite detailed dosage programmes and most of these relate to prophylactic usage, which is recommended for the majority of the agents (Anon., 1980*a*, *b*). Administration may be by injection or orally via a drench, as a drinking water or feed additive or as a syringed paste (Table 1). Injection is probably most commonly used in cattle in England and Wales (Michel *et al.*, 1981). The instructions for use of these agents often recommend periods of withdrawal prior to slaughter, during which the animal should not be treated with the anthelmintic (Table 1). The lengths of these recommended withdrawal periods differ from compound to compound and in some cases, for the same compound, between different animals,

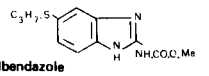
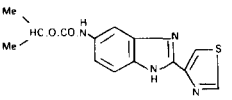
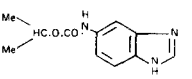
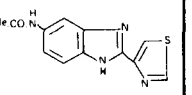
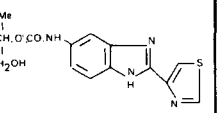
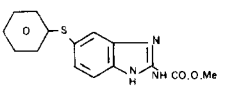
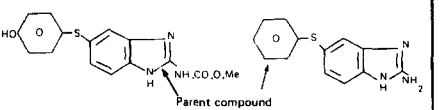
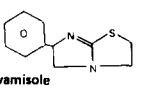
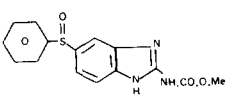
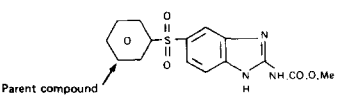
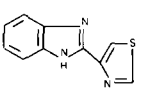
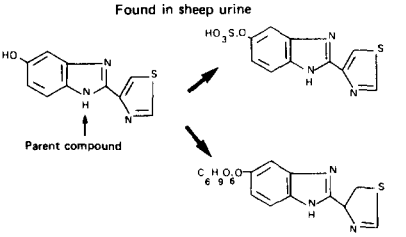
ANTHELMINTIC	METABOLITES	REFERENCE
 Albendazole	No data available	
 Cambendazole	Found in calf urine  Found in pig urine  	Van den Heuvel et al (1972)
 Fenbendazole	Found in cattle, sheep and pig urine  Parent compound	Duwel (1977)
 Levamisole	No data available	
 Oxfendazole	Found in sheep plasma  Parent compound	Marriner and Bogan (1981)
 Thiabendazole	Found in sheep urine  Parent compound	Tocco et al (1964)

Fig. 1. Anthelmintic structures and metabolites.

TABLE 1
Recommended Usage of Commonly Used Anthelmintics (Source of data: Anon., 1980*a*, *b*, 1981)

Agent	*Available formulations*	*Dosage route(s) and methods(s)*	*Food-producing animals treated (recommended withdrawal periods)*[a]	*Target parasites*
Albendazole	Liquid suspension	Oral drench	Sheep (10 days) Cattle (14 days)	GI tract roundworms; lungworms; tapeworms; adult liver flukes
Cambendazole	Pellets	Addition to feed	Pigs (14 days)	GI tract roundworms
Fenbendazole	Liquid suspension	Oral drench	Cattle, sheep and pigs (14 days)	GI tract worms; lungworms; roundworm eggs
	Powder	Addition to feed		
	Granules	Addition to feed	Cattle (14 days)	
	Paste	Oral (via syringe)	Cattle and sheep (14 days)	
	Feed block	Addition to feed		

Levamisole	Solution	Addition to drinking water	Poultry (3 days)	GI tract worms
		Oral drench	Cattle and sheep (3 or 14 days[b])	GI tract and respiratory tract worms
		Injection	Cattle and sheep (3 or 5 days[c]); pigs (5 days)	
	Granules	Addition to feed	Cattle and sheep (3 or 14 days[b]); pigs (7 days)	
Oxfendazole	Liquid suspension	Oral drench	Cattle and sheep (14 days)	GI tract roundworms; lungworms; tapeworms
Thiabendazole	Pellets	Addition to feed	Cattle (0 days)	GI roundworms (cattle, sheep, pigs)
	Paste	Oral (via syringe)	Cattle and sheep (0 or 28 days[d])	
	Powder	Addition to feed	Cattle, sheep and pigs (0 days)	
	Liquid suspension	Oral drench	Cattle and sheep (0 or 28 days[d])	
	Premix	Addition to feed	Cattle and sheep (0 days); pigs (10 days)	
	Nuts	Addition to feed	Pigs (0 days)	

[a] Between dosage and slaughter.

[b] 3 day withdrawal applies to preparations containing levamisole alone whilst 14 days withdrawal applies to preparations containing levamisole and oxyclozanide.

[c] 3 day withdrawal applies to a subcutaneously-injected preparation whilst 5 day withdrawal applies to preparations injected subcutaneously or intramuscularly.

[d] 28 day withdrawal applies to preparations also containing rafoxanide. Other withdrawal periods apply to preparations whose active ingredient is thiabendazole alone.

between different dosages routes or between different formulations (Table 1).

ANTHELMINTIC RESIDUES IN ANIMAL PRODUCTS

Although many of these agents have been used for over a decade, the literature data on residues arising from their use is far from complete. Residue data have been reported from animal trials involving the agents listed in Table 1 as follows:

Fenbendazole: Duwel (1977) has reported residues arising from the oral use of this agent in cattle, sheep and pigs. The formulations used were not described. Treatment of cattle with 5–10 mg of agent per kilogram body weight (b.w.) (the recommended oral dosage range in Britain (Anon., 1981)) led to residue levels, after 1–2 days, of 0·74–1·6 $\mu g\,ml^{-1}$ in serum and 0·3 $\mu g\,ml^{-1}$ in milk. The liver was the target organ. Residue concentrations declined in this organ from 7·9 $mg\,kg^{-1}$, two days after treatment with 10 mg agent kg^{-1} b.w., to less than 0·1 $mg\,kg^{-1}$ at fourteen days post-treatment (the recommended withdrawal period (Table 1)). Serum residue levels also declined rapidly in sheep and pigs to less than 0·5 $\mu g\,ml^{-1}$ within 24 h of treatment. The liver was also the target organ in these animals.

Levamisole: Surprisingly few residue studies have been reported in the literature for levamisole although this agent has been available for many years (Kemp, 1975) and it is probably widely used. Simkins *et al.* (1976) found milk residue concentrations of 0·50, 0·55 and 0·32 ppm of this agent 12 h after dosage with 8 $mg\,kg^{-1}$ b.w. by drench, feed addition and injection routes, respectively. Dosage at *ca.* 7·5 $mg\,kg^{-1}$ b.w. in cattle is recommended in Britain for these routes (Anon., 1981). The agent was administered as the hydrochloride in the drench, as a resinate in feed pellets and as the phosphate in an injected solution. In Britain levamisole is available only as the hydrochloride (Anon., 1980*a*, 1981). Two days after drench administration, no residues were detectable in milk (the lower limit of detection was 0·01 ppm). Marriner *et al.* (1980) found a rapid decline in sheep plasma residues from over 2 $\mu g\,ml^{-1}$ at 1 h, following subcutaneous injection of the dosage recommended in Britain (Anon., 1981) (7·5 $mg\,kg^{-1}$ b.w.) in solution, to less than 1 $\mu g\,ml^{-1}$, 6 h after treatment. There are no available literature data for levamisole

residues in pigs or poultry, although this agent is available for use in these animals (Kemp, 1975; Michel *et al.*, 1981; Table 1).

Oxfendazole: Nerenberg *et al.* (1978) found that oxfendazole residues in cattle plasma peaked at about 0·5 $\mu g\,ml^{-1}$ at 6–9 h and then declined to less than 0·1 $\mu g\,ml^{-1}$ at 48 h after oral dosage with 3 mg agent kg^{-1} b.w. (less than the recommended cattle dosage of 4·5 $mg\,kg^{-1}$ b.w. (Anon., 1981)). Details of the dosage method and formulation used were not given. Marriner & Bogan (1981) found peak plasma residue concentrations of 0·73–0·77 $\mu g\,ml^{-1}$ in sheep at 24–30 h after dosage (10 mg agent kg^{-1} b.w.), with a decrease to 0·03 $\mu g\,ml^{-1}$ after 7 days. Administration was by oral drench, the method recommended for this agent in Britain (Table 1), and a liquid suspension was used at twice the oral dosage recommended in Britain for sheep (Anon., 1981).

Thiabendazole: Residues were found by Tocco *et al.* (1965) in calf kidney (0·15 $\mu g\,g^{-1}$) and fat (0·1 $\mu g\,g^{-1}$) 34 days after oral administration of the compound in capsules at 110 mg agent kg^{-1} b.w. (the maximum recommended oral dosage in Great Britain for cattle (Anon., 1981)). No liver residues were found (the detection limit was 0·2 $\mu g\,g^{-1}$). Calf muscle, plasma, kidney and liver levels were all less than 0·08 $\mu g\,g^{-1}$, 3 and 30 days after oral dosage with 50 mg encapsulated thiabendazole kg^{-1} b.w. No thiabendazole residues were detected in fat, kidney, liver or muscle from pigs 10–30 days after oral treatment with 50 mg encapsulated agent kg^{-1} b.w. (the lower limit of detection was 0·2 ppm). Dosage was equivalent to the maximum recommended oral dose for pigs in Britain (Anon., 1981). In a separate report Tocco *et al.* (1964) described sheep blood residues of thiabendazole peaking 4 h after oral treatment with 50 $mg\,kg^{-1}$ b.w. (oral dosage in sheep in Britain is at 44–88 $mg\,kg^{-1}$ b.w.). The agent was administered in gelatin capsules. Residues in urine and faeces both reached plateaux after 24 h. The liver was the target organ with residues decreasing to 0·15 ppm, 16 days after treatment.

Other agents: There are no residue data in the literature for albendazole or cambendazole in the farm animals which are treated with these agents in Britain.

Residue data for the widely-used anthelmintic agents frequently relate to only one or two animals, even though it is known that the metabolism of many veterinary agents can vary quite markedly between different members of the same herd or flock. In practice a *minimum* of five animals

TABLE 2
Assay Methods for Anthelmintics Widely Used in Great Britain

Agent	*Assay methods*[a] *(lower limit of detection)*	*Refs.*
Albendazole	*Cattle:* tissues and body fluids,[b] NDA. *Cattle:* milk, NDA. *Sheep:* tissues and body fluids, NDA.	Coles (1977)
Cambendazole	*Pigs:* tissues and body fluids, NDA.	
Fenbendazole	*Cattle:* tissues and body fluids,[b] method not described (50 parts per 10^9). *Cattle:* milk, method not described (50 parts per 10^9). *Sheep:* tissues and body fluids, method not described (50 parts per 10^9). *Pigs:* tissues and body fluids, method not described (50 parts per 10^9).	Duwel (1977)
Levamisole	*Cattle:* tissues and body fluids,[b] polarography (100 parts per 10^9, liver, muscle, kidney, fat, blood, urine); HPLC (20–50 parts per 10^9, plasma). *Cattle:* milk, glc (10 parts per 10^9). *Sheep:* tissues and body fluids, NDA. *Pigs:* tissues and body fluids, NDA. *Poultry:* tissues/ body fluids/eggs, NDA.	Smith *et al.* (1976); Anon. (1977); Marriner *et al.* (1980)
Oxfendazole	*Cattle:* tissues and body fluids,[b] RIA (0·2 ng ml^{-1}, plasma). *Cattle:* milk, NDA. *Sheep:* tissues and body fluids, NDA.	Nerenberg *et al.* (1978)
Thiabendazole	*Cattle:* tissues, body fluids,[b] GC/MS (100 parts per 10^9, muscle, liver, kidney, fat); fluorimetry (100 parts per 10^9, liver, kidney, muscle, plasma). *Cattle:* milk, fluorimetry (100 parts per 10^9). *Sheep:* tissues and body fluids, NDA. *Pigs:* tissues and body fluids, GC/MS (100 parts per 10^9, liver); fluorescence (100 parts per 10^9 , tissues, plasma).	Tocco *et al.* (1965); Anon. (1973, 1979); Van den Heuvel *et al.* (1977)

[a] For products of food-producing animals treated with these agents in Great Britain (see Table 1).
[b] Excluding milk.
NDA, No data available.

is probably required per group to provide a basis for fair comparison between treated and control animals.

In one of the above studies (Tocco *et al.*, 1964), the net concentrations of the parent compound *and* its metabolites were measured. Whilst one or more metabolites have been identified for several of these agents (Fig. 1), very little is known about the extent to which they are metabolised in given animals, the kinetics of their metabolism or the residues of those metabolites which may exist in food products.

Progress in this area has probably been hindered by a shortage of sensitive assay methods. For example there is a need for a specific and sensitive method to detect albendazole in the body fluids and tissues of food-producing animals. Currently available analytical methods for other commonly-used agents are listed in Table 2. These have detection limits in the range 50–300 parts per 10^9 for tissues and 0·2–100 parts per 10^9 for body fluids. The radioimmunoassay analytical method of Nerenberg *et al.* (1978) is the most sensitive of those listed in Table 2. This method and those involving fluorimetry have quite rapid extraction and clean-up procedures. More extensive extraction and clean-up are required for the column chromatographic determination of these compounds in animal products (Smith *et al.*, 1976; Van den Heuvel *et al.*, 1977; Marriner *et al.*, 1980). However, gas chromatography coupled with mass spectrometry is potentially valuable to confirm or refute the results of simpler and quicker analytical methods (Van den Heuvel *et al.*, 1977). In addition to the methods listed in Table 2, there are published methods which detect benzimidazole compounds as a group (Austin *et al.*, 1976; Mourot *et al.*, 1978). These use high pressure liquid chromatography as the final resolution stage and a detection limit of 2 ng for thiabendazole is claimed (Austin *et al.*, 1976). Although these group detection methods have not been tested on benzimidazoles extracted from tissues or body fluids of treated animals, their use for the detection of residues arising from the use of anthelmintics would be favoured over the application of methods which are only capable of detecting one agent.

DISCUSSION

The generation of a body of residue data through animal trials and the development of sensitive and reproducible detection methods for anthelmintics are necessary before information can be compiled about the

extent of residues, if any, from their use. Present evidence does give some indication of the approximate residue levels to be expected for some agents in some animal products. In particular the liver is probably the target organ for benzimidazoles and the physiological turnover of these agents and levamisole in blood is probably quite rapid. At present there is little information about the extent of turnover of the administered agents in other tissues and body fluids (including milk). Little is known about the extent of turnover of their metabolites.

Despite the limited available information, surveillance of the *general animal product supply* could provide information about the extent of usage of several of these agents. This could be done by the analysis for residues of fenbendazole in liver from cattle, sheep and pigs, levamisole in cow's milk and oxfendazole in plasma from cattle and sheep. Surveillance in these cases would also give some indication of whether the relevant recommended withdrawal periods are being observed. Great caution would be required in such interpretation since it would be based on the major assumption that residue data from very limited numbers of animals in a controlled trial can be extrapolated to a much larger number of animals in the national herd.

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