A REVIEW OF FLUBENDAZOLE AND ITS POTENTIAL
AS A MACROFILARIACIDE

A report submitted to Dr Gary Weil (PI DOLF) - a study supported by the Bill & Melinda Gates Foundation

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Goal: This report covers data on flubendazole reviewed in preparation for preparing a full drug development proposal to the B&M Gates Foundation for the use of the drug for filariasis
1. INTRODUCTION

Flubendazole is a member of the benzimidazole class of heterocyclic aromatic organic compounds, a class that has been used extensively in a wide range of species. This class includes a range of active anthelminthic compounds that represent arguably the most important collection of anti-worm agents available today.

This report is the result of considering current literature, and of discussions with many experts in this field of work including those at pharmaceutical companies who are, or were, involved with the commercial distribution of this anthelminthic agent. The document does not attempt to review all the literature available but rather to present the main components that are relevant to the idea that this agent may have a major role as a macrofilaricide for improving the mass drug administration activities against river blindness (onchocerciasis) and lymphatic filariasis.
It should be noted that some information on this compound was not available due to unavoidable circumstances (e.g. data/information had been destroyed, data not released for consideration by scientists, etc.). However, it is believed that the information that was not available would not have affected the overall conclusions made in this present report and the consequent second B&MG grant submission.

2. BASIC DATA

Chemical Structure:

There are two major metabolites for flubendazole, namely the hydrolysed form (FLU-NH2) and the reduced form (FLU-OH).

![Chemical Structure Diagram]

*Figure 1. Structures of flubendazole (FUZ) and its metabolites, hydrolysed flubendazole (FUZ-NH2) and reduced flubendazole (FUZ-OH).*

The three forms, parent and 2 metabolites are active in individuals that are treated with this drug and therefore all three compounds must be considered in the test procedures used to determine the safety of flubendazole if it to be used for filariasis.
Two polymorphs of flubendazole exist - flubendazole and flubendazole polymorph B (2011-03-22. CAS : 31430-15-6) with Form B being variably described as being active or inactive.

Flubendazole, as with the benzimidazole group, is a specific inhibitor of microtubule assembly, acting by binding to the heterodimeric subunit, the tubulin molecule. The preferential binding of the benzimidazoles for parasite tubulin is some 100-400 fold greater than that to host tubulin; this fact is an important key to their safe use anthelminthic agents.

**Names used for Flubendazole**

- Flubendazole
- Fluvermal
- Tricyclo[5.2.1.0]decan-8-one
- Tricyclo[5.2.1.0(2,6)] Decan-8-One
- tricyclo(5.2.1.02,6)decan - 8 - one
- Flubendazol
- Tricyclo[5.2.1.02,6]decan-8-one
- Flubenzimin
Some minor differences exist in the commercial description of flubendazole.
<table>
<thead>
<tr>
<th>Substance Name</th>
<th>Flubendazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Flubendazolum [INN-Latin],</td>
<td></td>
</tr>
<tr>
<td>* (5-(4-Fluorobenzoyl)-1H-benzimidazole-2-yl)carbamic acid methyl ester,</td>
<td></td>
</tr>
<tr>
<td>* Methyl 5-(p-fluorobenzoyl)-2-benzimidazolocarbamate,</td>
<td></td>
</tr>
<tr>
<td>* Methyl N-(5-(p-fluorobenzoyl)-2-benzimidazolyl)carbamate,</td>
<td></td>
</tr>
<tr>
<td>* 2-Benzimidazolocarbamic acid, 5- (p-fluorobenzoyl)-, methyl ester,</td>
<td></td>
</tr>
<tr>
<td>* AIDS-084892, * AIDS08492,</td>
<td></td>
</tr>
<tr>
<td>* Carbamic acid, (5-(4-fluorobenzoyl)-1H-benzimidazol-2-yl)-, methyl ester,</td>
<td></td>
</tr>
<tr>
<td>Synonyms</td>
<td>* Carbamic acid, [5- (4-fluorobenzoyl)-1H-benzimidazol-2-yl]-, methyl ester,</td>
</tr>
<tr>
<td>* CCRIS 4480, * EINECS 250-624-4,</td>
<td></td>
</tr>
<tr>
<td>* Flubendazol [INN-Spanish],</td>
<td></td>
</tr>
<tr>
<td>* Flubendazole [USAN:BAN INN],</td>
<td></td>
</tr>
<tr>
<td>* Flubenol, * Flumoxal, * Flumoxane, * Fluvermal,</td>
<td></td>
</tr>
<tr>
<td>* N-[5-(4-Fluorophenyl)carbonyl]benzimidazol-2-yl)methyloxymethanamide,</td>
<td></td>
</tr>
<tr>
<td>* NSC 313680, * NSC313680,</td>
<td></td>
</tr>
<tr>
<td>* R 17899, * R 17,889,</td>
<td></td>
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<tr>
<td>Chemical Name</td>
<td>methyl N-[5-(4-fluorobenzoyl)]-3H-benzoimidazol-2-yl]carbamate</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>Chemical Structure</td>
<td><img src="image" alt="Chemical Structure" /></td>
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</tbody>
</table>
Flubendazole is a synthetic anthelmintic belonging to the benzimidazole carbamates which acts by inhibiting the microtubular assembly in absorptive cells of nematodes. It acts by binding to tubulin, the dimeric subunit protein of the microtubules. It inhibits microtubular assembly in absorptive cells: i.e. of intestinal cells of nematodes. This is shown by disappearance of cytoplasmic microtubules, accumulation of secretory granules in the cytoplasm due to a block in their transport, leading to an impaired coating of the cellular membrane and a decreased digestion and absorption of nutrients. Irreversible lytic degeneration of the cell, due to the accumulation of secretory substances (hydrolytic and proteolytic enzymes), results in the death of the parasite.

Flubendazole is a broad-spectrum anthelmintic agent effective against endoparasites such as gastro-intestinal ascarids, hookworms, whipworms found in dogs, and active a range of gastro-intestinal parasites in pigs and poultry such as roundworms, and tapeworms, Ascaris suum (large roundworm), gapeworms, Haemonchus contortus (red stomach worm), Oesophagostomum dentatum (nodular worm), Trichuris suis (whip worm), Strongyloides ransomi (adult), Metastrongylus apri (lungworm). Flubendazole is ovicidal.

3. USE IN NON-HUMAN SPECIES

Flubendazole is a very commonly used compound in the veterinary world and is freely available for aquatic, avian and mammalian species. There are very few validated descriptions of adverse reactions to its use and the few described are associated with very high doses or the use of pro-drugs (which have considerably different pharmacology to flubendazole itself or the two metabolites, reduced flubendazole and hydrolyzed flubendazole).
Use with Aquatic Species

In fish it is used for controlling a number of organisms, including hydra, intestinal parasites (Heximata, gill flukes and Camallanus) possible by adsorption through the fish’s skin. Regularly used dose levels are 0.5 g of 10% flubendazole in 20 gallons (75 liters) of water. Overall it is reported to be safe at comparatively high levels in its use with fish and the like. No delayed expression of toxicity were observed for 21 d after a 96-h exposure to flubendazole was noted, probably reflecting to the relatively high elimination constants for the chemical.

Use in Birds

Flubendazole is commonly used in the management of avian species. For example commercially available flubendazole-based products are efficacious against three species of helminth parasites of chickens: Ascaridia galli, Heterakis gallinarum and Capillaria spp; flubendazole achieves an overall efficacy of 99.4% for the three parasite species without any adverse side effects.

**Flubenvet (2.5%)** as a poultry dewormer is used as a medicated feed supplement commonly used to deworm chickens, turkeys and geese. Flubenvet is active against mature and immature nematodes of the respiratory and gastrointestinal tract. There are 3 major worms that usually affect chickens - these are roundworms, gapeworms and tapeworms. Flubenvet contains the active ingredient Flubendazole which has no adverse effect on egg laying or hatching. It is the only licenced in feed wormer for chickens currently available in the UK. It is very effective and can prevent a large number of problems and long term damage to your birds. You do not need to withdraw eggs for consumption when it is given at the correct dose and it is simple to administer in food.

The PK/PD aspects of flubendazole have been well studied in birds with acceptable results such as the following example: After oral administration of the
veterinary medicine Flubenol 5% the concentrations of the flubendazole-derived residues were determined by a liquid chromatographic-mass spectrometric method. The highest residue concentrations were obtained for the reduced metabolite. With the therapeutic dose, the maximum mean residue concentrations obtained for this compound in thigh muscle, breast muscle and liver were 312, 288 and 1043 ug/kg, respectively. The values for flubendazole, the parent molecule, were 114, 108 and 108 ug/kg, respectively. The residues of the hydrolysed metabolite were negligible in the sampled muscle tissues. After 24 h of depletion, the sum of the residues of parent and metabolites in muscle tissue still exceeded 50 ug/kg. After 8 d of depletion, flubendazole-derived residues at low concentrations could still be measured in both muscle tissues and liver. Thus flubendazole in this species has a wide tissue distribution, and consequently can reach parasites in different tissues.

Use in Domestic Species

Flubendazole has been used extensively in poultry and swine but lesser so on ruminants. the pharmacology in the latter species has been carried out and the data available for studies that would need to be carried out with bovine onchocerciasis in the development of the drug for use in filariasis. No major differences appear to be likely with ruminants compared to mono-gastric animals. Little is published concerning this drug’s use in small domestic animals (e.g. dogs) but oral reports stated that it was safe and effective.

4. USE IN HUMANS

Flubendazole, as originally formulated, is used to treat intestinal nematodes in humans. Flubendazole is registered and sold in Europe (EMEA) as Fluvermal (Johnson and Johnson, Sante Bea). Flubendazole is reported as an ingredient of Fluvermal in the following countries:

- Algeria
- Benin
The excipients used in Fluvermal are Talc (E553b), Saccharose, Amidon de pomme de terre, Amidon, Sodium laurylsulfate (E487), Magnésium stéarate (E572), Cellulose microcristalline (E460).

A 100mg dose of Fluvermal is most commonly proscribed for treating pinworms (Enterobius vermiculus)). This is followed by a second dose of 100mg 15-21 days later to ensure reinfection is avoided, as flubendazole does not kill pinworm eggs. 100mg taken 3 times a day for 3 days is effective against larger nematodes, but only marginally effective against tapeworms. Fluvermal is available over the counter for human use in Europe under this brand name, and in 100mg tablets or as a 20mg/ml oral solution.
FLUVERMAL INSERT DATA

The form of flubendazole available for human use

Flubendazole

FLUVERMAL, tablets, 100 mg
FLUVERMAL, oral suspension, 20 mg/ml
FLUVERMAL, tablets + oral suspension, 100 mg + 20 mg/ml

Contraindications
Fluvermal is contraindicated in persons with a known hypersensitivity to the drug or its components.

Special warnings and special precautions for use
Use in infants < 1 year: as well-documented experience in children below 1 year of age is scarce, Fluvermal should only be given to very young children if their worm infection interferes significantly with their nutritional status and physical development.
The tablets contain lactose. Patients with rare hereditary diseases of galactose intolerance, Lapp’s lactose deficiency or malabsorption of glucose-galactose should not use this medicinal product.
The oral suspension contains parabenes that can cause allergic reaction (possibly delayed).

Interaction with other medicaments and other forms of interaction
None known.

Pregnancy and lactation
Fluvermal has shown embryotoxic and teratogenic activity in one study in rats.
No such findings have been reported in teratology studies in the rabbit, mice or other studies in rats.
Experience in humans has not shown any increase in the risk of malformations.
Nonetheless, it is better to avoid using the product in pregnant women or in those liable to become pregnant.
It is not known whether flubendazole is excreted in human milk. Therefore caution should be exercised when Fluvermal is administered to nursing women.

Effects on ability to drive and use machines
Fluvermal does not affect the mental alertness or driving ability.

Undesirable effects
Transient abdominal pain and diarrhoea have only rarely been reported, in cases of massive infestation and expulsion of worms.

Hypersensitivity reactions such as exanthema, rash, urticaria and angioedema have rarely been observed.

**Overdose**

*Symptoms*

In the event of accidental overdosage, abdominal cramps, nausea, vomiting and diarrhea may occur. Although the maximum recommended treatment duration of Fluvermal is limited to three days there have been rare reports of liver function disturbances, hepatitis and blood dyscrasias described in patients who were treated for hydatid disease with massive doses for prolonged periods of time.

*Treatment*

There is no specific antidote. Within the first hour after ingestion, gastric lavage may be performed. Activated charcoal may be given if considered appropriate.

As described below flubendazole was tested along with other benzimidazoles in humans for its effect on *Onchocerca volvulus* but as the preparation induced SAE’s at the injections site it was not pursued further at that time (1981) - although it appeared to be very effective against this filarial pathogen.

5. SAFETY ISSUES

There are known issues with all this group of anthelminthics in terms of safety. The basic reason appears to be related to the effect of these agents in disrupting the tubulin of host cells and consequently the functions related to these structures. There are considerable differences in effects between species which may relate primarily to the pharmacodynamics in these species. However, it has been suggested that the vehicles that the primary compound is delivered in may also be important.
The use of this property of binding tubulin in maintaining the health of the host (patient) is considered a potential advantage when used at higher levels. Combined with other cancer inhibiting drugs it is being proposed as as a treatment for certain types of neoplasia. In a study with leukemia and myeloma it was found that flubendazole induced cell death in leukemia and myeloma cell lines and primary patient samples at nano-molar concentrations. Moreover, it delayed tumor growth in leukemia and myeloma xenografts without evidence of toxicity. Mechanistically, flubendazole inhibited tubulin polymerization by binding tubulin at a site distinct from vinblastine. In addition, cells resistant to vinblastine because of overexpression of P-glycoprotein remained fully sensitive to flubendazole, indicating that flubendazole can overcome some forms of vinblastine resistance. Thus it was suggested by these authors that flubendazole is a novel microtubule inhibitor that displays pre-clinical activity in leukemia and myeloma.

An EMEA report (EMEA/CVMP/33128/2006-FINAL July 2006) states that flubendazole has a low acute oral and subcutaneous toxicity. The acute oral LD50 values were greater than 5000 mg/kg bw in mice, rats and guinea pigs. Acute subcutaneous LD50 values were greater than 5000 mg/kg bw in the rat and the mouse and 4679 and 4834 mg/kg bw in male and female guinea pigs, respectively. Any toxicity occurring appears to depend on route and on formulation.

FOOD RESIDUES

Flubendazole is one of the safest of drugs used in food animals in terms of the food residues. Residues of veterinary medicinal products, as defined by the European Union, are "pharmacologically active substances (whether active principles, excipients or degradation products) and their metabolites which remain in foodstuffs obtained from animals to which the veterinary medicinal product in question has been administered". The MRL is the maximum concentration of residue following administration of a veterinary medicine which is legally permitted or acceptable in food under the laws of the EU.
This is particularly important in the poultry industry where benzimidazoles are veterinary drugs widely used for prevention and treatment of parasitic infections. Metabolites of benzimidazoles have been reported in several matrices including eggs. However, to date flubendazole is the only anthelminthic that has an established MRL of 400 µg kg⁻¹ in egg. A value of 50 µg kg⁻¹ was established for all other anthelmintics. Thus flubendazole appears to be in terms of residues safer than most other anthelminthic agents.

6. POTENTIAL OF FLUBENDAZOLE AS A MACROFILARICIDE

A safe, field usable chemotherapeutic agent that will rapidly kill adult filarial worms is urgently needed in tropical medicine. Ivermectin, distributed as Mectizan® by Merck & Co.Inc. has had an enormous impact on two major human filarial infections of developing countries, onchocerciasis and lymphatic filariasis. However, this agent works primary against the microfilarial stage and lacks the ability to rapidly kill the adult parasites. Since the adult worms can survive for many years producing offspring, it has been necessary for control programs to continue drug distribution for more than a decade, i.e., until the adult worms eventually die; a labor-intensive and expensive proposition. Other agents used in filarial control programs, such as diethylcarbamazine and albendazole, may be more effective macrofilaricides than ivermectin, but for various reasons are not suitable, or are unable, to fill the role of a being rapidly-acting macrofilaricide. Thus, a drug, administered once, or at least in multiple doses over a very short period, that safely kills adult filarial worms would be a major contributor to the current efforts to rid the world of filarial infections and the diseases they cause. A field useful agent has typically been required to be administered in an oral dosage form, but a truly safe agent administered by another route, including parenteral approaches, could be acceptable and may even be advantageous.
Given the challenges of discovery and development of agents for human use, a drug as described above is arguably most likely, at least at present, to come from the benzimidazole group of anthelmintics. Although several benzimidazoles are currently employed in human chemotherapy, there are other potential candidate macrofilaricidal agents in other drug classes. However, time is of the essence in finding a new drug for use in ongoing filarial control programs, the first priority is to consider the benzimidazoles as the most likely source of a macrofilaricide. This group has provided many important effective agents for both veterinary and human medicine over the past 50 years, beginning with thiabendazole and now most prominently including albendazole and mebendazole for human parasites and a whole range of agents in veterinary medicine. Benzimidazoles work by interfering with the equilibrium among tubulin subunits, tubulin and microtubules. Not surprisingly, benzimidazoles can affect host tubulin as well as that of the parasites, are typically positive in mammalian cell cytotoxicity assays and cause chromosomal non-disjunction during mitosis. However, the benzimidazole anthelmintics show a differential preference for binding to nematode tubulin compared to mammalian tubulin, an important factor for development of a drug against nematodes in mammals. Benzimidazoles are also anti-fungal agents as well as anthelmintics, a fact that may be important in filarial conditions such as elephantiasis that involve secondary infections often involving fungi; albendazole is one of the two drugs used in the global lymphatic filariasis elimination program.

The most appealing benzimidazole with regard to filarial parasites is flubendazole as it is highly active against filariae in a number of hosts. It has the typical
benzimidazole structure with an added fluorine as the major structural difference from other benzimidazoles. It is a very efficacious macrofilaricide in a variety of experimental animals, with perhaps its most dramatic and relevant action being its ability to completely eliminate adult *Dirofilaria immitis* from dogs after a single injection.

Flubendazole was developed by Janssen in the mid 1970’s and is currently licensed in Europe for the use as an anthelmintic in humans for intestinal nematodes (at 5 mg /kg for 3 days). Flubendazole is a potent and efficacious anthelmintic for gastrointestinal nematode infections in swine, poultry and companion animals, as well as against lungworms in swine. It is usually given over 3 days at ~5 mg/kg, but is probably also efficacious even as a single dose at this same rate. In a number of experimental filarial rodent models, flubendazole was found to have essentially 100% efficacy as a macrofilaricide at reasonable doses and schedules. A trial in human onchocerciasis was also carried in Mexico in the early 1980’s with promising results. However, wider testing in humans was restricted at that time by problems associated with the route of administration and the relatively unsophisticated carrier agent used at that time, some 39 years ago. In addition, the introduction of ivermectin at about this time lessened the urgency to replace diethylcarbamazine for onchocerciasis control with a new macrofilaricide.

As noted, flubendazole is highly efficacious in various experimental filariasis models, including the feline *Brugia pahangi* model, a host in which it occurs naturally. Efficacy varies with parasite species, location in the host, and host species (Table 1). It should be noted that flubendazole is highly efficacious and potent as a macrofilaricide in these models only when given parenterally (in keeping with its very low oral bioavailability in standard formulations). Given parenterally, flubendazole is arguably the best macrofilaricide tested in animal models. Importantly, no adverse reactions were reported in any of these animal studies. An important observation, relevant to current
problems faced by the global control and elimination programs for human lymphatic filariasis and onchocerciasis, is that in cats and jirds infected with *Brugia spp.*, flubendazole is active against adult worms but poorly active against the microfilarial stage. The significance of this observation lies in the fact that a major problem for filarial control programmes using ivermectin is that individuals co-infected with high levels of circulating *Loa loa* microfilariae may suffer severe adverse events. Over 124 people have died in the past ten years, usually with signs and symptoms of central nervous system pathology related to microfilarial death. An agent that will kill adult filariae but not microfilariae may be a breakthrough for this important practical problem, which currently limits ivermectin distribution programs in many Africa countries.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>LED&lt;sub&gt;10&lt;/sub&gt; × 5 (mg/kg)</th>
<th>LED&lt;sub&gt;50&lt;/sub&gt; × 1 (mg/kg)</th>
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<tbody>
<tr>
<td><em>Jird</em></td>
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<td>ND</td>
<td>100</td>
<td>[7]</td>
</tr>
</tbody>
</table>

*Adult parasites in the peritoneal cavity.*
*Adult parasites in the lymphatics.*
*I3 larvae.*
*Not titrated, only dose reported.*
*Multimammate rat.*
*Microfilariae transplanted into the skin.*

Most of the efficacies reported at these doses in these studies were 100%. Efficacy determinations are dependent on the time of necropsy; efficacy is higher (i.e., number of worms observed) in jirds necropsied 8 weeks post-treatment compared with 6 weeks post-treatment. (McCall PERL COMM.)

LED<sub>50</sub>, lowest dose that was at least 50% effective.
Following the encouraging findings in rodent models, a study was carried out in Mexico in the early 1980’s in which several potential macrofilaricides, including flubendazole, were tested in humans infected with *Onchocerca volvulus*. This study was terminated early due to problems associated with reactions at the intra-muscular injection site where the flubendazole in its oil-based carrier was administered. Nevertheless, efficacy data on adult *O. volvulus* worms in surgically removed nodules from these patients suggested that flubendazole is a potent macrofilaricide. At 3 weeks after initiation of treatment (750 mg once per week for 5 weeks), significant degeneration of the adult worms was detected; at 5 weeks (Table 2), there was very effective destruction of the adult worms compared with the other anti-filarial agents.

<table>
<thead>
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<th>Status of parasites</th>
<th>2 months post Rx</th>
<th>3 months post Rx</th>
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<tbody>
<tr>
<td></td>
<td>DEC*</td>
<td>FLUB*</td>
</tr>
<tr>
<td>Degenerated adults</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Intact adult worm</td>
<td>44</td>
<td>11</td>
</tr>
<tr>
<td>Females with empty uteri</td>
<td>6</td>
<td>1</td>
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<tr>
<td>Females with only oocytes</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Reduction in dermal microfilariae*</td>
<td>Yes</td>
<td>No</td>
</tr>
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</table>

*DEC (100 mg) was administered twice daily for 14 days and 750 mg FLUB was injected intramuscularly once a week for five doses.
*There was no significant ocular or skin pathology related to microfilarial death in those receiving FLUB. The only significant post-FLUB treatment reactions were associated with inflammation at the injection site. Dermal microfilarial loads stayed at pretreatment levels in the FLUB-treated individuals for approximately 6 months.
DEG: Diethylcarbamazine; FLUB: Flubendazole; post RX: After last treatment with flubendazole.
*Data taken from [6,10].
As mentioned above, flubendazole is currently registered for human use in Europe for treatment of gut-residing nematodes, an action that does not require efficient uptake into the host’s circulation. A challenge for ensuring its suitability for filariasis will be to develop a new formulation that will produce blood and tissue levels sufficient to destroy tissue-resident parasites, such as the filariae. Efficacy against filariae was not observed following oral dosing of flubendazole in any of the early animal studies, but it should be noted that none of these studies used any of the new formulation methods now common in the pharmaceutical industry. Encouraging results come from Lanusse’s group, which showed that the tissue-residing stage of the cestode, *Echinococcus granulosis*, can be killed by an orally administered flubendazole formulated with the now commonly-used excipient hydroxypropyl-β-cyclodextrin. Newer formulations such as this could greatly enhance the likelihood of developing flubendazole as a suitable macrofilaricidal for human filariasis for oral dosing. A hydroxypropyl-β-cyclodextrin formulation might indeed be suitable given the increased degree of bioavailability it provides; for example, it markedly enhances the bioavailability of albendazole, mebendazole and flubendazole. This material is approachable and is a gold-standard reagent for enhancing bioavailability of lipophilic drugs, and can be used in both liquid and solid dosage forms.

As the target infective adult filariae are complex and biochemically resourceful (many nematodes have the ability to switch biochemical pathways when stressed), it is likely that a relatively long duration of exposure to the drug will be needed. This may involve the need for dosing on multiple (e.g., 3-5) days to maintain lethal levels of the
agent for the needed period of time. For many nematodes, acute exposure to benzimidazoles has few noticeable effects, even at very high concentrations; this is true for flubendazole in various adult filariids. As the drug acts by disrupting the tubulin-microtubule equilibrium in cells, leading to cessation of nutrient transport and eventual cell death, these effects take time to become evident. \textit{In vitro} experiments have shown that flubendazole concentrations as low as 100 ng/ml (incubated for 32 hours) disrupt tissue structure in parasitic nematodes in the same clade as filariae.

In addition to pharmacodynamic challenges, there are other hurdles to developing a safe and effective formulation of a drug for the treatment of complicated infections such as lymphatic filariasis and onchocerciasis. A primary concern with the benzimidazoles is safety. As these drugs interfere with microtubules, they have the potential of interfering with host cells, especially during cell division. Thus, the use of drugs such as albendazole is generally contra-indicated for pregnant women; this is likely to apply with a new flubendazole formulation that provides for systemic exposure. However, it should be noted that albendazole has been used very successfully in mass drug programs across the world since 1999, and that inadvertent treatment studies in pregnant women have not detected adverse effects on the unborn. Nevertheless, a major hurdle for a formulation that produces enhanced bioavailable flubendazole will need to be carefully evaluated for embryotoxicity. It may turn out that flubendazole is only useful for filarial infections in males and females outside childbearing ages. However, such a product still would be a most useful advance for control programs.
What will it take to determine if flubendazole is an important answer to the needs of filarial control and elimination programs? Scientifically, it will initially require the determination of the blood and tissue levels needed for macrofilaricidal efficacy; closely related is the need to determine the levels that induce toxicity. Both issues are central to moving forward with development of flubendazole. Based on recent experimental data from animal models, it is highly likely that current modern formulation techniques, including micronization, hydroxypropyl-β-cyclodextrin complexing or another new approach, will be able to provide the blood levels needed to kill adult worms. The testing of newly developed formulations for efficacy against filariae itself poses some challenge. Filarial infections are generally host specific and thus each filariae-host model is to some degree unique in form and properties. Flubendazole in a new formulation should be evaluated in a range of filarial models to encompass all the variations and characteristics of these infections, and to make predictions about the pharmacokinetic parameters likely to be required for efficacy in human infections; this would allow formulations to be evaluated on the basis of pharmacokinetic data rather than efficacy per se, which requires extended periods of time post-treatment. A combination of many disciplines and institutions will be needed, including, as with the pioneering onchocerciasis-ivermectin control program, “public-private partnerships” between the pharmaceutical industry, non-governmental organizations and academic scientists. Drug companies have the expertise needed to develop new formulations and are central to the final production phase needed; field-based expertise (Ministries of Health, NGDO’s, academics) are essential for developing a practical field-based mass drug administration intervention and will be important partners in any successful effort.

The benefit of developing a safe and practical agent that needs distribution only once, or perhaps twice, is substantial when compared to what is currently in place, i.e., annual distribution for 8-12 years in filarial control and elimination programs; a highly
effective macrofilaricide would still be important even if a 3-5 daily course of treatment is needed. Financial savings as well as significant savings in terms of health personnel time commitment would be realized.

Flubendazole has great potential as a macrofilaricide. Its reformulation using modern pharmaceutical platforms should be expedited to enable efficacy testing as soon as possible. Although flubendazole faces, as does any new anthelmintic, important challenges with regard to safety and formulation, the potential benefits that could result relatively quickly from a safe, usable formulation of flubendazole make this a top priority for the filarial world today.
FLUBENDAZOLE REFERENCE LIST

This is a list of references that have been considered in this Review Document. Although extensive it does not purport to be a complete list of all references that mention flubendazole.


Bunnag, D., Harinasuta, T., Viravan, C., Jarupakorn, V., Chindanond, D., and Desakorn, V. (1980) Clinical trial of flubendazole on hookworm, Trichuris trichiura and


Maki, J., Kondo, A., and Yanagisawa, T. (1983) Effects of alcoholic extract from Ma-Klua (Diospyros mollis) on adults and larvae of the dwarf tapeworm, Hymenolepis nana in mice and on the infectivity of the eggs, Parasitology 87 (Pt 1), 103-111.


by Echinococcus granulosus. Preliminary results (author's transl)], *Nouv Presse Med* 10, 3121-3124.


Mackenzie, C. D., Geary, T. G., Gerlach, J. A. Possible pathogenic pathways in the adverse clinical events seen following ivermectin administration to onchocerciasis patients. Filaria J. 2 (supp 1) S5 (2003).


Gyapong, J. O., Chinbuah, M. A., Gyapong, M. Inadvertent exposure of pregnant women to ivermectin and albendazole during mass drug administration for lymphatic


8. APPENDICES

8.1

**FLUVERMAL INSERT**

Flubendazole

FLUVERMAL, tablets, 100 mg
FLUVERMAL, oral suspension, 20 mg/ml
FLUVERMAL, tablets + oral suspension, 100 mg + 20 mg/ml

**Contraindications**

Fluvermal is contraindicated in persons with a known hypersensitivity to the drug or its components.

**Special warnings and special precautions for use**

Use in infants < 1 year: as well-documented experience in children below 1 year of age is scarce, Fluvermal should only be given to very young children if their worm infection interferes significantly with their nutritional status and physical development.

The tablets contain lactose. Patients with rare hereditary diseases of galactose intolerance, Lapp’s lactose deficiency or malabsorption of glucose-galactose should not use this medicinal product.

The oral suspension contains parabenes that can cause allergic reaction (possibly delayed).

**Interaction with other medicaments and other forms of interaction**

None known.

**Pregnancy and lactation**

Fluvermal has shown embryotoxic and teratogenic activity in one study in rats. No such findings have been reported in teratology studies in the rabbit, mice or other studies in rats.

Experience in humans has not shown any increase in the risk of malformations. Nonetheless, it is better to avoid using the product in pregnant women or in those liable to become pregnant.

It is not known whether flubendazole is excreted in human milk. Therefore caution should be exercised when Fluvermal is administered to nursing women.
Effects on ability to drive and use machines
Fluvermal does not affect the mental alertness or driving ability.

Undesirable effects
Transient abdominal pain and diarrhoea have only rarely been reported, in cases of massive infestation and expulsion of worms.

Hypersensitivity reactions such as exanthema, rash, urticaria and angioedema have rarely been observed.

Overdose
Symptoms
In the event of accidental overdosage, abdominal cramps, nausea, vomiting and diarrhea may occur. Although the maximum recommended treatment duration of Fluvermal is limited to three days there have been rare reports of liver function disturbances, hepatitis and blood dyscrasias described in patients who were treated for hydatid disease with massive doses for prolonged periods of time.

Treatment
There is no specific antidote. Within the first hour after ingestion, gastric lavage may be performed. Activated charcoal may be given if considered appropriate.
1. Flubendazole is a benzimidazole derivative which is administered for therapeutic applications in pigs, chickens, turkeys, and other birds. The preparations available include tablets, pastes, pellets, and powders. In some countries, it is also available as an on-label treatment for domestic animals.

2. Flubendazole was shown to be poorly absorbed from the gastro-intestinal tract with most of the administered dose recovered unchanged in the feces. It was of low acute toxicity.

Daily oral doses of up to 40 mg/kg bw per day of flubendazole were given to dogs for 2 weeks. Some slight histological changes, which were difficult to interpret, were seen in both the male and female genital tracts. The changes in females were considered to be within age-range normal limits and not related to treatment. It was also found that the changes in males (prostatic hyperplasia) were probably not treatment-related. However, as a precautionary measure, it was decided to increase the dose level from 40 mg/kg bw per day to 50 mg/kg bw per day for the subsequent study. The plasma level of flubendazole in the rat, equivalent to 1.17-3.47 mg/kg bw per day.

Flubendazole was tested for monitoperapy in a wide range of in vitro and in vivo assays, all of which gave negative results. In cytotoxicity studies, no significant changes were found. The test was repeated with the same results. In general, no increase in tumor incidence was observed and there were no other treatment-related effects.

The reproductive toxicity of flubendazole was investigated in a range of species. No effect on fertility was observed in rats or dogs. In ovariocystic disease, no effect on fertility was observed at very high dose levels. There was evidence of teratogenicity in the rabbit. The results of a published study, in which the agent was used, were not consistent with the observed effects. In the rat, no significant effect on fertility was observed with 50 mg/kg bw per day of flubendazole used in a commercial formulation of 60 mg/kg bw per day.

3. Flubendazole had no significant anticoagulant activity.

4. It was noted that the EC50 of 0.013 mg/kg bw per day for applying a safety factor of 200 to the NOEL of 5.5 mg/kg bw per day in the 5-month dog study.

The safety factor of 200 was used to take into account the fact that the doses were administered 1 day per week.

It was agreed that the EC50 evaluation was not significantly different from the CVMP Working Group on Safety of Medicines, of the data and it was therefore agreed that the EC50 should be adopted.

5. Carboxylic hydroxylation and benzene reduction were the main biotransformation pathways in pigs and the (4H-2,1-benzothiazine-2-yl) methylene benzene metabolite was the major urinary component in pig kidney. However, there was no validated analytical method for the routine determination of this metabolite in tissues and it was therefore agreed that the flubendazole parent compound should be considered the marker molecule.

The following provisional MRLs were established:

- Chickens: 1 to 5 mg/kg
- Poultry: edible tissues (muscle, liver, kidney) 0.05 mg/kg
- Pig tissue (muscle, liver, kidney) 0.1 mg/kg
- It was calculated that the limits set at these values were considerably lower than those consumed by animals but consistent with consumption of residues of flubendazole in tissues of animals which did not exceed these levels would not exceed the ADI proposed above.

6. An analytical method was available for the determination of the parent flubendazole using HPLC with UV detection at 312 nm. The limit of detection of the method was 0.01 mg/kg for all tissues. Validation data for this analytical method were provided only for plasma/tissues.

7. The following data are required before 31 December 1994:

- Further information on the relationship between extraneous flubendazole-related residues and total extraneous & bound residues in treated tissues.
- Further information on whether ADI is the appropriate route for pig and other species if it is not, a suitable complementary method for determination of this metabolite should be determined and fully validated.
- Further information on the relative proportions of parent flubendazole and metabolites in edible tissues in the chicken after treatment with 40 mg flubendazole in the feed (the higher recommended dose).
- Data for residues in individual amounts and for analytical method validation for all pharmaceuticals, metabolites, degradation and distribution studies performed for swine and poultry.
- Data on quantitative determination between pharmaceutical, absorption, depletion and distribution of flubendazole in plasma and safety data allowing NOEL to be set for poultry.
- The sensitivity of the analytical method for the determination of residues should be improved and the methods should be validated for food-producing species (other than poultry).
8.3

### FLUBENDAZOLE REVIEW

#### flubendazole

<table>
<thead>
<tr>
<th>Indications</th>
<th>Special Precautions</th>
<th>Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage</td>
<td>Adverse Drug Reactions</td>
<td>MIMS Class</td>
</tr>
<tr>
<td>Contraindications</td>
<td>Drug Interactions</td>
<td>ATC Classification</td>
</tr>
</tbody>
</table>

#### Related Information

#### See related flubendazole information

<table>
<thead>
<tr>
<th>Indications</th>
<th>Listed in Dosage.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage</td>
<td>PO Enterobiasis 100 mg as a single dose. Repeat 2-3 wk later if needed. Ascariasis; Hookworm infections; Trichuriasis 100 mg twice daily for 3 days. Click to view flubendazole Dosage by Indications</td>
</tr>
<tr>
<td>Contraindications</td>
<td>Pregnancy.</td>
</tr>
<tr>
<td>Adverse Drug Reactions</td>
<td>GI disturbances; headache, dizziness; allergic reactions; raised liver enzyme values; alopecia; bone marrow suppression.</td>
</tr>
<tr>
<td>Drug Interactions</td>
<td>Enzyme inducers eg phenytoin or carbamazepine; and inhibitors eg cilomide.</td>
</tr>
<tr>
<td>Mechanism of Action</td>
<td>For details of the mechanism of action, pharmacology and pharmacokinetics and toxicology ... click to view flubendazole</td>
</tr>
<tr>
<td>MIMS Class</td>
<td>Antihelmintics</td>
</tr>
<tr>
<td>ATC Classification</td>
<td>P02CA05 - Flubendazole ; Belongs to the class of benzimidazole derivative agents. Used as antihelmental.</td>
</tr>
</tbody>
</table>
FLUBENDAZOLE REVIEW

8.4

Brand name: FLUBENDAZOLE Brandet
Formulas: Tablets
Categories: Gastrointestinal System Drugs
Code: 7-20
Composition: Flubendazole 100mg

Detailed Information:
Composition:
Each tablet contains Flubendazole 100mg

Properties:
Flubendazole, a benzimidazole carboxamide anthelmintic, is an analogue of mebendazole. It is active against most nematodes and other worms. Flubendazole acts by inhibition or destruction of cytoplasmic microtubules in the worm's intestinal or absorptive cells, leading to death of the worm within several days.

Indication:
Flubendazole is used in the treatment of single or mixed infections caused by Enteroobia, ascariasis, hookworm, and trichuriasis.

Contraindications:
- Hypersensitivity to the drug
- Pregnancy and lactation

Side effects:
Flubendazole is well tolerated but may cause transient abdominal pain, diarrhea, headache, allergic reactions, and raised liver enzyme values with the high doses.

Precautions:
- Patients receiving high doses should be supervised closely with blood counts and liver function being monitored. No special diet or laxatives.

Dosage and administration:
For the treatment of enterobiasis:
- Adults and children: 100mg as a single dose repeated if necessary after 2-3 weeks.
- For ascariasis, hookworm, and trichuriasis: 100mg twice daily for 3 days.
8.5

Veterinary Drug Residues in Food

Updated up to the 34th Session of the Codex Alimentarius Commission (2011)

**VETERINARY DRUG DETAILS**

Flubendazole

- Functional Class
  - Anthelmintic agent

- Search JECFA
  - Click the above link to access the relevant JECFA residue monograph(s)

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>MRL</th>
<th>Year of Adoption</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig</td>
<td>Muscle</td>
<td>10 μg/kg</td>
<td>1995</td>
<td></td>
</tr>
<tr>
<td>Pig</td>
<td>Liver</td>
<td>10 μg/kg</td>
<td>1995</td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>Liver</td>
<td>500 μg/kg</td>
<td>1995</td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>Muscle</td>
<td>200 μg/kg</td>
<td>1995</td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>Eggs</td>
<td>400 μg/kg</td>
<td>1995</td>
<td></td>
</tr>
</tbody>
</table>

printer-friendly version
8.6

FLUBENDAZOLE REVIEW

COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

FLUBENDAZOLE (extrapolation to poultry)

SUMMARY REPORT (4)

1. Flubendazole is a benzimidazole anthelmintic. It is the fluoro-analogue of mebendazole and has many similar properties. It is administered orally to pigs, chickens and game birds.

Flubendazole is currently entered into Annex I of Council Regulation (EEC) No. 2377/90 for turkeys, chicken, game birds and porcine species as follows:

<table>
<thead>
<tr>
<th>Pharmacologically active substance(s)</th>
<th>Marker residue</th>
<th>Animal species</th>
<th>MRLs</th>
<th>Target tissues</th>
<th>Other provisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flubendazole</td>
<td>Sum of flubendazole and (2-amino-1H-benzimidazole-5-yl) (4-fluorophenyl)-methanone</td>
<td>Chicken, turkey, game birds and porcine</td>
<td>50 µg/kg, 50 µg/kg, 400 µg/kg, 300 µg/kg</td>
<td>Muscle, Skin + fat, Liver, Kidney</td>
<td></td>
</tr>
<tr>
<td>Flubendazole</td>
<td>Chicken</td>
<td>400 µg/kg</td>
<td></td>
<td>Eggs</td>
<td></td>
</tr>
</tbody>
</table>

2. A request was submitted to the EMEA for the extrapolation of the existing entry in Annex I of Council Regulation (EEC) No. 2377/90 for turkeys, chicken and game birds. The scientific justification for this extension was assessed taking into account the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL). Based on the approach explained in this guideline the CVMP considered whether the extrapolation to poultry would be possible.

3. In setting the ADI in the original assessment of flubendazole, the data summarised in the paragraphs below were considered:

4. Flubendazole had low oral bioavailability in the rat, dog and the target species. In rats, the half-life for plasma elimination was around 6 hours. In all species, more than 50% of the administered dose was excreted in the faeces as unchanged flubendazole. The absorbed portion of the drug was rapidly metabolised so that concentrations of the parent drug in the blood and urine were very low. The urine contained a mixture of metabolites. The main metabolic pathways were the same in all the species studied and involved reduction of the ketone functional group and hydrolysis of the carboxylic moiety.

5. Flubendazole was of low acute oral and subcutaneous toxicity. The acute oral LD50 values were greater than 5000 mg/kg bw in mice, rats and guinea pigs. Acute subcutaneous LD50 values were greater than 5000 mg/kg bw in the rat and the mouse and 4679 and 4834 mg/kg bw in male and female guinea pigs, respectively. The substance was more toxic when administered intraperitoneally with an acute intraperitoneal LD50 of 528 and 434 mg/kg bw in male and female rats, respectively.
8.7
8.8 WHO REPORT 832 VETERINARY DRUG RESIDUES
EVALUATION OF CERTAIN VETERINARY DRUG RESIDUES IN FOOD

Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives

World Health Organization
Geneva 1993
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Mr D. Schutz, Office of Toxic Substances, Environmental Protection Agency, Washington, DC, USA (WHO Consultant)

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Dr K. Woodward, Veterinary Medicines Directorate, Ministry of Agriculture, Fisheries and Food, New Haw, Addlestone, Surrey, England (WHO Temporary Adviser)
closantel, with the remaining residue consisting of 3- and 5-monoiodo-
closantel. No evidence for the existence of other metabolic pathways
was reported. In cattle liver, approximately 10% of the radioactivity
was unchanged closantel, and 40–77% was accounted for by 3-mono-
iodoclosantel. In faeces, 6% of the radioactivity was identified as a sulfate
conjugate of a closantel derivative.

Residue depletion of closantel from plasma parallels that from the edible
tissues. Within a given species, there is a reasonably constant tissue:plasma
ratio which is independent of time. The tissue:plasma ratios in liver and in
fat are, respectively, approximately four times and 12 times as large in
cattle as in sheep. The tissue:plasma ratios for muscle and kidney in sheep
and cattle are comparable.

**Maximum Residue Limits**

Taking into account this information and the specific residue data
discussed on pages 8–10, the Committee recommended amended MRLs
for closantel in sheep (Table 2) and new MRLs for cattle (Table 3). The
recommendations for cattle are based on the studies in which an oral dose
of closantel of 10 mg per kg of body weight and an intramuscular dose of
2.5 mg per kg of body weight were given; from these the Committee
calculated that the theoretical maximum daily intake of closantel residues
at 42 days withdrawal time would be below the ADI of 1.8 mg for a 60-kg
person. It should be noted that the manufacturer recommends an oral dose
of 5 mg per kg of body weight for cattle. Residues from such an oral dose
will be lower than those given in Table 3.

3.1.2 **Flubendazole**

Flubendazole had not been previously reviewed by the Committee. The
compound is used as an anthelmintic in pigs and poultry. It belongs to the
group of benzimidazole carbamates.

**Toxicological data**

A substantial database was available for assessment, including data on
kinetics and metabolism, acute toxicity, short-term and long-term toxicity,
reproductive and developmental toxicity, and genotoxicity.

The absorption, metabolism, and excretion of flubendazole have been
studied using radiolabelled drug. Flubendazole is poorly absorbed and is
metabolized in a qualitatively similar way in all species studied. More than
50% of the ingested drug is eliminated unchanged in the faeces. The
absorbed drug is rapidly metabolized, so that levels of parent drug in the
blood and urine are extremely low. The main site of metabolism is the liver,
and major metabolic pathways are carbamate hydrolysis and ketone
reduction. It seems probable that flubendazole undergoes enterohepatic
circulation.

Single oral doses of flubendazole were slightly toxic to experimental
animals, the median lethal dose (LD₅₀) being greater than 5000 mg per kg
of body weight in mice, rats, and guinea-pigs.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue (mg/kg parent drug)</th>
<th>Estimated daily intake (mg clopamide equivalents)</th>
<th>Theoretical maximum daily intake (mg clopamide equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oral dose&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Intramuscular dose&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Oral dose&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle</td>
<td>&lt; 0.4</td>
<td>1.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Liver</td>
<td>0.8</td>
<td>0.7</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>(1.14)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(1.17)&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.7</td>
<td>1.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat</td>
<td>0.7</td>
<td>0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>0.31</td>
</tr>
</tbody>
</table>

<sup>a</sup> Based on concentrations at 28 days withdrawal time. For the original discussion of residue data for sheep, see Annex 1, reference 97.

<sup>b</sup> Calculated from the observed residue levels.

<sup>c</sup> Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat.

<sup>d</sup> 10 mg/kg of body weight.

<sup>e</sup> 5 mg/kg of body weight.

<sup>f</sup> Estimate of total residues; after oral administration, clopamide accounted for 70% of the total residues in liver.

<sup>g</sup> Estimate of total residues; after intramuscular administration, clopamide accounted for 60% of the total residues in liver.
### Table 3
Recommended MRLs for closantel in cattle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue (mg/kg parent drug)</th>
<th>Estimated daily intake&lt;sup&gt;a, b&lt;/sup&gt; (mg closantel equivalents)</th>
<th>Theoretical maximum daily intake&lt;sup&gt;a&lt;/sup&gt; (mg closantel equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oral dose&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Intramuscular dose&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Oral dose&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.19</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>0.16</td>
<td>0.56</td>
<td>0.16</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.83</td>
<td>1.39</td>
<td>0.05</td>
</tr>
<tr>
<td>Fat</td>
<td>0.7</td>
<td>2.36</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>0.32</td>
<td>0.91</td>
<td>1.70</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated from the observed residue levels.

<sup>b</sup> Based on a daily intake of 0.5 kg of meat made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat.

<sup>c</sup> 10 mg/kg of body weight, 28 days withdrawal time.

<sup>d</sup> 2.5 mg/kg of body weight, 42 days withdrawal time.

<sup>e</sup> Estimate of total residues; closantel accounted for 10% of the total residues in liver.

<sup>f</sup> Estimate of total residues; closantel accounted for 90% of the total residues in kidney.

<sup>g</sup> Estimate of total residues; closantel accounted for 70% of the total residues in fat.
Flubendazole was given orally in gelatin capsules to dogs at doses of 2.5, 10, or 40 mg per kg of body weight per day, 6 days a week for 3 months. Some atrophic changes and congestion of the epididymis were observed in the male genital tract at doses of 10 and 40 mg per kg of body weight per day, and atrophic changes occurred in the female genital tract at all doses. The changes in the female genital tract were considered to be within normal limits for dogs of the age of those used in the study. On histological examination of male sex organs, changes in the testes could not be clearly associated with flubendazole treatment. The findings in male dogs may not be compound-related, but because of the lack of conclusive evidence as to the cause of these changes, the Committee concluded that the no-observed-effect level (NOEL) was 2.5 mg per kg of body weight per day.

Carcinogenicity studies were performed in mice and rats at doses up to 30 and 20 mg per kg of body weight per day, respectively; no treatment-related effects were observed. There was no treatment-related increase in any type of neoplasm. The Committee was of the opinion that flubendazole had no carcinogenic potential at the highest doses administered in these studies.

The results from a range of in vitro and in vivo genotoxicity tests were all negative.

The Committee considered data from reproduction, embryotoxicity, and teratogenicity studies. Studies in mice, rabbits, and pigs were negative. Flubendazole was extensively studied in segmented reproduction studies in rats performed as required for human drug regulation purposes and accepted by the Committee in lieu of a multigeneration reproduction study. In several rat developmental studies, doses of up to 40 and 160 mg per kg of body weight per day, given on gestation days 6-15, did not produce any embryotoxic or teratogenic effects. In a rat teratogenicity study published in 1987, using material extracted from a commercial preparation, gross skeletal and internal fetal malformations were recorded at doses of 40 and 160 mg per kg of body weight per day. The NOEL in this study was 10 mg per kg of body weight per day.

An ADI of 0-12 μg per kg of body weight was established for flubendazole, based on the NOEL of 2.5 mg per kg of body weight per day in the 3-month study in dogs and a safety factor of 200. This safety factor was used by the Committee to take account of the fact that the doses were administered only 6 days per week in this study; the precise consequences of which could not be assessed.

The Committee noted that the ADI also provided a safety margin corresponding to a factor of about 1000 with respect to the NOEL of 10 mg per kg of body weight per day derived from the rat teratogenicity study. Furthermore, the Committee considered that further carcinogenicity studies would not be required, since the highest dose used in the negative studies that it had evaluated exceeded the ADI by a factor of approximately 2000.
Residue data
The Committee considered data on the metabolism of flubendazole and the depletion of flubendazole residues from the edible tissues of pigs, and on the depletion of flubendazole residues from the edible tissues and eggs of laying hens.

When pigs or poultry are treated with flubendazole, the tissue with the highest residue concentration and slowest depletion rate is the liver. The major metabolite in pig liver is (2-amino-1\(H\)-benzimidazol-5-yl)-4-fluorophenylmethanone, which is found at a much higher concentration than parent flubendazole. Residue concentrations are higher and more persistent in egg yolk than in egg white.

Pigs. A residue-depletion study was conducted using 18 feeder pigs given a dose of 1.5 mg per kg of body weight of \([^{14}C]\)flubendazole daily for 5 days (Table 4). Total residue concentrations were highest in liver throughout the 30-day withdrawal period.

Three male pigs received flubendazole at 30 mg per kg of body weight in the feed for 5 consecutive days. Flubendazole levels measured by high-performance liquid chromatography (HPLC) were less than 0.01 mg/kg (the sensitivity limit of the method) in plasma, liver, kidney, muscle, and fat at withdrawal times of 16, 30, and 54 hours.

A similar residue study was conducted using single oral doses of 5 mg per kg of body weight in three groups of five male pigs. Tissues and plasma were analysed using a radioimmunoassay with quantification limits of 1 \(\mu\)g/kg in plasma and 5 \(\mu\)g/kg in tissues. Animals were slaughtered in groups of five at 24, 72, and 168 hours after dosing. At 24 hours withdrawal time, the tissues contained 7-12 \(\mu\)g/kg of parent flubendazole. All residues were below the detection limit at 72 hours.

In another study, seven sows were treated with 30 mg per kg of body weight flubendazole in the diet for 10 consecutive days. The sows were

<table>
<thead>
<tr>
<th>Withdrawal time</th>
<th>Muscle</th>
<th>Liver</th>
<th>Kidney</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 hours</td>
<td>262</td>
<td>3865</td>
<td>2678</td>
<td>212</td>
</tr>
<tr>
<td>5 days</td>
<td>35.5</td>
<td>1863</td>
<td>435</td>
<td>50.1</td>
</tr>
<tr>
<td>10 days</td>
<td>10.5</td>
<td>529</td>
<td>78.2</td>
<td>16.3</td>
</tr>
<tr>
<td>16 days</td>
<td>8.66</td>
<td>433</td>
<td>76.6</td>
<td>15.6</td>
</tr>
<tr>
<td>23 days</td>
<td>8.67</td>
<td>194</td>
<td>49.9</td>
<td>13.5</td>
</tr>
<tr>
<td>30 days</td>
<td>2.51</td>
<td>106</td>
<td>22.5</td>
<td>3.38</td>
</tr>
</tbody>
</table>

* For each withdrawal time, values are means for three pigs.
Table 5

<table>
<thead>
<tr>
<th>Withdrawal time (days)</th>
<th>Plasma (mg/l)</th>
<th>Muscle (mg/kg)</th>
<th>Liver (mg/kg)</th>
<th>Kidney (mg/kg)</th>
<th>Fat (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.007</td>
<td>≤ 0.01</td>
<td>0.210</td>
<td>0.080</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.005</td>
<td>≤ 0.01</td>
<td>0.146</td>
<td>0.054</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>4</td>
<td>0.002</td>
<td>≤ 0.01</td>
<td>0.069</td>
<td>0.010</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>7</td>
<td>0.001</td>
<td>≤ 0.01</td>
<td>0.073</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>11</td>
<td>≤ 0.001</td>
<td>≤ 0.01</td>
<td>0.080</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
<tr>
<td>14</td>
<td>≤ 0.001</td>
<td>≤ 0.01</td>
<td>0.016</td>
<td>≤ 0.01</td>
<td>≤ 0.01</td>
</tr>
</tbody>
</table>

* For liver and kidney at 1 day withdrawal time, values are means for three animals; all other values are means for four animals.

slaughtered 7 days after the last treatment with flubendazole. Mean levels of flubendazole measured by HPLC were 59, 67, 13, and 33 µg/kg for liver, kidney, muscle, and fat, respectively.

**Poultry**. A total of 28 laying hens received [14C]flubendazole at a dose equivalent to 30 mg per kg of body weight in food for 6 consecutive days. At all withdrawal times tested from 1 to 14 days after treatment, the concentration of radioactive equivalents of flubendazole in blood and plasma was less than 0.01 µg/ml, which suggests that absorption was poor. After total radioactivity levels reached a steady state in 5–6 days, eggs contained an average of 0.12 mg of flubendazole equivalents per kg. Radioactivity in the yolks (0.34 mg/kg) was much higher than in the egg white (0.02 mg/kg). The highest observed levels of radioactivity in individual tissues, calculated in terms of flubendazole equivalents, were 0.21 mg/kg in liver and 0.08 mg/kg in kidney 24 hours after the last dose. Table 5 shows residues in plasma and tissue for various withdrawal times.

When chickens were treated with flubendazole at 60 mg per kg of body weight for 7 days, residues were detectable in egg yolk for 11 days after treatment ended. Residue levels were higher in yolk than in white. Eggs and tissues were analysed by an HPLC method sensitive to 0.01 mg/kg. Of the tissues, liver had the greatest amount of residue at zero withdrawal time, although flubendazole could not be detected in any tissue by 6 and 7 days withdrawal time. The residue data are summarized in Table 6.

**Methods of analysis for residues in tissues**

For the studies described on pages 14–15, plasma and tissue levels of flubendazole in pigs were measured by radioimmunoassay or by an HPLC method with ultraviolet detection at 313 or 254 nm, which is sensitive to 0.01 or 0.02 mg/kg, respectively. Another HPLC method has been developed for flubendazole, with ultraviolet detection at 254 nm, that gives excellent separation between flubendazole and the major metabolite.
resulting from carbamate hydrolysis. However, the method described applies to the analysis of pure substances and does not include extraction procedures for tissues.

An HPLC method that has detection limits of 20-50 μg/kg has been developed for simultaneously determining eight benzimidazoles in tissue. This method might be suitable for measuring flubendazole and the major metabolite found in pig tissue, (2-amino-1H-benzimidazol-5-yl)-4-fluorophenylmethanone. Typical recoveries from spiked samples (0.1 mg/kg) were above 70% for flubendazole in liver, kidney, and muscle.

**Maximum Residue Limits**

In reaching its decision on MRLs, the Committee took into account the following points:

- An ADI of 0-12 μg per kg of body weight was established. This would result in a maximum ADI of 720 μg for a 60-kg person.
- The marker residue is the parent drug for all tissues and for eggs.
- The total daily intake of flubendazole-related residues in food would be about 620 μg (see Table 7), if assumed to be accounted for by pig tissue and eggs at zero withdrawal time, and calculated on the basis of the data presented in Table 4 and the study in chickens treated with a dose of 30 mg per kg of body weight.

**Eggs.** The daily intake of flubendazole-related residues will probably remain below the ADI even when flubendazole is given at 60 mg per kg of body weight, although this dose produces a much higher concentration of residues in eggs. The argument that increased doses of flubendazole will not increase residue levels because of the drug's low systemic availability appears not to be valid for eggs: the levels of parent flubendazole in egg yolk found in the study with 60 mg per kg of body weight are double the residue levels of all flubendazole-related residues found in the study with 30 mg per kg of body weight.

An MRL for whole egg of 400 μg/kg of parent flubendazole is recommended.
Estimated total daily intake of flubendazole-related residues in food

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Observed residue (mg/kg parent drug equivalents)</th>
<th>Estimated daily intake (µg parent drug equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pig tissue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>0.262&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79</td>
</tr>
<tr>
<td>Liver</td>
<td>3.865&lt;sup&gt;b&lt;/sup&gt;</td>
<td>386</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.678&lt;sup&gt;b&lt;/sup&gt;</td>
<td>134</td>
</tr>
<tr>
<td>Fat</td>
<td>0.212&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
</tr>
<tr>
<td><strong>Eggs</strong></td>
<td>0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>622</td>
</tr>
</tbody>
</table>

<sup>a</sup> Calculated from the observed residue levels. Based on a daily intake of 0.5 kg of meat (made up of 0.3 kg of muscle, 0.1 kg of liver, 0.05 kg of kidney, and 0.05 kg of fat) and 0.1 kg of eggs.

<sup>b</sup> Based on concentrations at 6 hours withdrawal time in a study in which pigs received an oral dose of [14C]flubendazole at 1.5 mg per kg of body weight daily for 5 days.

<sup>c</sup> Average concentration detected in eggs on day 6 of a study in which laying hens received [14C]flubendazole in the diet at 30 mg per kg of body weight for 7 days.

Poultry. As no withdrawal period is required for poultry, parent flubendazole is an adequate marker residue. MRLs of 500 and 200 µg/kg are recommended for parent flubendazole in poultry liver and muscle, respectively.

Pigs. Although edible tissues from pigs require no withdrawal period from a human food safety perspective, a withdrawal period based on good practice in the use of veterinary drugs has been applied.

Parent flubendazole is the only analyte available as the marker residue for pig liver. Methods are available for determining flubendazole, and the residue data indicate that misuse can be detected by monitoring for parent flubendazole in pig tissue.

An MRL of 10 µg/kg is recommended for the parent compound in pig liver and muscle.

3.1.3 Ivermectin

Ivermectin (a mixture of ≥ 80% 22,23-dihydroavermectin B<sub>1a</sub> (H<sub>2</sub>B<sub>1a</sub>) and ≤ 20% 22,23-dihydroavermectin B<sub>1b</sub> (H<sub>2</sub>B<sub>1b</sub>)) had previously been evaluated at the thirty-sixth meeting of the Committee (Annex I, reference 9I), when an ADI of 0–0.2 µg per kg of body weight was established, based on a NOEL of 0.1 mg per kg of body weight per day for maternal toxicity in the CF<sub>1</sub> mouse and a safety factor of 500.

Toxicological data

The Committee reappraised the developmental toxicity of ivermectin and