

RESIDUE STUDY OF MEBENDAZOLE AND ITS METABOLITES HYDROXY-MEBENDAZOLE AND AMINO-MEBENDAZOLE IN EEL (*ANGUILLA ANGUILLA*) AFTER BATH TREATMENT

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ABSTRACT:

Mebendazole (MBZ) is extensively used in eel culture for treatment of *Pseudodactylogyrus* spp. infections. This use may lead to residues of MBZ in eel tissues. Consequently, the residue profile of MBZ in eel after treatment with the drug is of special concern. Therefore, a residue study was performed in European eels (*Anguilla anguilla*), bath-treated with MBZ at a dose of 1 mg/liter for 24 hr and kept at a water temperature of 25°C. Liver, kidney, fat, skin, and muscle tissue samples were collected at intervals during and after treatment and analyzed for MBZ and its metabolites, hydroxy-MBZ (MBZ-OH) and amino-MBZ (MBZ-NH₂), by HPLC.

Results showed that MBZ is extensively metabolized to MBZ-OH

and MBZ-NH₂. Liver and kidney were found to contain the highest levels of MBZ metabolites, and fat contained the highest levels of the parent compound. Skin contained higher residue levels for all three compounds, compared with muscle tissue. MBZ and its hydroxy metabolite were eliminated within 5 days from the edible parts (muscle and skin) of the eels, whereas MBZ-NH₂ could be detected by the 14th day after the end of the treatment period. Consequently, although MBZ and MBZ-OH constitute the residues of toxicological concern, MBZ-NH₂ should be taken as the compound of interest for estimating the withdrawal time for consumption of eel treated with MBZ.

MBZ¹ (methyl-5-benzoylbenzimidazole carbamate), a broad-spectrum anthelmintic of the benzimidazole family of veterinary drugs, is extensively used in eel culture for treatment of *Pseudodactylogyrus* spp. infections (1–3). This use may lead to residues of MBZ and its possible metabolites in eel tissues. This means that information regarding the presence of MBZ metabolites in eel tissues is of further concern.

The metabolism of MBZ has been investigated in humans (4), rats (5), sheep (6, 7), and poultry (7). In addition, *in vitro* metabolism studies have been conducted in pig, rat, and dog liver fractions (8) and in an isolated perfused gut system (9). In these studies MBZ-OH [methyl-5-(α -hydroxybenzyl)-2-benzimidazole carbamate] and MBZ-NH₂ (2-amino-5-benzoylbenzimidazole) were found to be the major metabolites. Later studies, however, have shown that NH₂-OH-MBZ is the major metabolite in humans, whereas MBZ-OH and MBZ-NH₂ are minor ones (10).

Relevant data on MBZ residues in fish are scarce. Residue studies in eels after bath treatment with 1 mg/liter of MBZ at 25°C have shown that MBZ residues can be detected for 14 days after treatment, whereas residues are absent after 21 days (11). No information, however, has been given regarding the MBZ metabolites. Recently, Hajee and Haagsma (12) developed a method for the liquid chromatographic determination of MBZ and its metabolites, MBZ-NH₂ and

MBZ-OH, in eel muscle tissue and described the presence of these metabolites in the incurred muscle samples.

The aim of the present study was to determine the residue profile of MBZ and its metabolites in eels after bath treatment with MBZ.

Materials and Methods

Test Substance. A solution of the test substance was prepared by dissolving 1000 mg of MBZ (Riedel-de-Haën, Seelze, Germany) in 5 ml formic acid and diluting to 500-ml volume with tetrahydrofurfuryl alcohol polyethylene glycol ether. For bath treatment, a dose of 1 mg/liter of water was used. Treatment aquaria were prepared by adding a 50-ml MBZ solution in each one of six aquaria, filled with 100 liters of fresh water.

Animals and Housing. One hundred wild-life European eels (*Anguilla anguilla*) of 133 \pm 20 g (mean \pm SD) were used. Fish were housed, during the habituation period and after treatment, in five aquaria (part of a recirculation system consisting of 16 aquaria). Aquaria were connected to a freshwater flow-through system. The water temperature was 25°C, and the pH was 7–7.5. Eels were allowed to acclimatize for 2 weeks. MBZ treatment took place in five aquaria each containing \sim 100 liters of aerated water. As the aquaria were disconnected from the recirculation system, the water was stagnant. Fish were not fed during both the habituation and the experimental periods.

Experimental Design. Shortly before treatment with MBZ, five eels, one from each habituation aquarium, were removed. The remaining 95 eels were transferred to the treatment aquaria with 1 mg of MBZ/liter of water, 19 eels/aquarium, and left there for 24 hr. Dose and experimental conditions were the same as those used in the therapeutic treatments in eel culture practice. At time intervals of 2, 4, 8, 12, and 24 hr after the start of the MBZ treatment, one eel was taken from each treatment aquarium. After 24-hr treatment, all aquaria were reconnected to the flow-through system so that complete refreshment of water was achieved within 1 hr. At time intervals of 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144, 192, 240, and 312 hr after cessation of treatment with MBZ, one eel was taken from each aquarium.

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¹ Abbreviations used are: MBZ, mebendazole; MBZ-OH, hydroxy-mebendazole; MBZ-NH₂, amino-mebendazole.

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Eels were anesthetized by immersing them in a bath containing 5 liters of water and 1.5 ml Hypnodil (Janssen Pharmaceutica, Beerse, Belgium). After sedation, eels were killed by freezing at -70°C for 22 min and decapitated if necessary.

During the MBZ treatment, water samples were taken from each one of the five treatment aquaria at time intervals of 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hr. At the same time intervals, water samples were also taken from an habituation aquarium that contained a MBZ concentration of 1 mg/liter, but no eels. All water samples were stored at -70°C . pH measurements were made just before sampling.

Sampling. All fish were weighed before sampling. Liver, kidney, fat, skin, and ~ 50 g of muscle tissue were sampled from each fish. Samples were weighed, frozen, and kept at -70°C until analyzed.

Muscle tissue samples were homogenized in a Moulinette homogenizer before analysis. Liver, kidney, and skin samples were cut with scissors into small pieces. Kidney and fat samples were pooled per sampling time because of the small amount of kidney and fat tissue available per fish.

Analytical Procedure. Determination of MBZ and its metabolites MBZ-OH and MBZ-NH₂ in all samples was performed using a recently developed method (12) slightly modified. Because the column, proposed in the method as described (ChromSphere B, Chrompack, Bergen op Zoom, the Netherlands), showed stability problems, it was replaced with Inertsil ODS-2 from the same supplier. With this new column, the optimal mobile phase proved to be acetonitrile:0.03 M sodium acetate buffer [(pH 5.4) = 23:77 (v/v)] at a flow rate of 0.6 ml/min.

During sample preparation, muscle, skin, liver, kidney, and fat samples were extracted with ethyl acetate. Extracts, after addition of *n*-hexane, were concentrated and cleaned up on an aminopropyl solid-phase extraction column. After elution with methanol, the reconstituted eluate was analyzed in the prescribed LC system. The UV detector was set at 289 nm. The limit of determination in muscle tissue was 0.01 $\mu\text{g/g}$ for each one of MBZ, MBZ-OH, and MBZ-NH₂.

Some recovery experiments were conducted in eel skin, liver, kidney, and fat. Recovery values were of the same magnitude as for muscle tissue. The limit of determination in skin was the same as in muscle, whereas in liver it was 0.015, 0.03, and 0.05 $\mu\text{g/g}$; in kidney 0.01, 0.025, and 0.025 $\mu\text{g/g}$; and in fat 0.02, 0.01, and 0.02 $\mu\text{g/g}$ for MBZ, MBZ-OH and MBZ-NH₂, respectively.

Pharmacokinetics. The terminal half-life of each compound in the different tissues was estimated from the corresponding mean concentration time curve with the program TopFit 2.0 (13).

Results and Discussion

The present study clearly demonstrates that MBZ given to European eels *via* the water is absorbed rapidly and metabolized into MBZ-OH and MBZ-NH₂, and this is consistent with studies in other animal species (5–7). MBZ-NH₂ proved to be the major metabolite of MBZ in the European eel.

The levels of MBZ, MBZ-OH, and MBZ-NH₂ found in each of the eel tissues examined are presented in fig. 1. In table 1, the terminal elimination half-lives of all three compounds in the analyzed tissues of eels are given.

In muscle, MBZ maximum levels were higher than those of its metabolites until 4 hr after cessation of treatment (fig. 1A). The maximum level, found (2.11 $\mu\text{g/g}$) just before the end of the treatment, rapidly decreased to the quantification limit (0.01 $\mu\text{g/g}$) within 36 hr after treatment, with a half-life of 3.2 hr (table 1). MBZ-OH residues could be detected until 12 hr posttreatment, whereas MBZ-NH₂ residues were present even 312 hr after treatment at the 0.05 $\mu\text{g/g}$ level. Estimated half-life of MBZ-NH₂ was 171 hr (table 1). The maximum muscle level found for MBZ (2.11 $\mu\text{g/g}$, 24 hr after the start of the treatment) was much higher than that mentioned by Møllergaard *et al.* (11). They detected MBZ muscle level of ~ 0.37 $\mu\text{g/g}$ 1 day after addition of 1 mg MBZ/liter of water in a large intensive eel culture system. They also reported that they could detect MBZ residues up to 14 days, whereas in our experiment the residues

could not be determined after 36 hr, although the water temperature was the same. The most probable explanation for these large differences could be the different conditions of these two experiments, such as drug formulation, treatment time, water quality, fish body weight, etc. A MBZ solution (2 mg/ml), for example, has been used in our experiment vs. a granulate formulation (100 mg/g) in theirs.

Residual levels of all three compounds in skin samples were higher, compared with those in muscle samples (fig. 1, A and B). The maximum level of the parent compound (3.82 $\mu\text{g/g}$) was obtained 24 hr after the beginning of the treatment and fell, with a half-life of 22 hr (table 1), to the quantification limit level 120 hr after cessation of treatment. MBZ-OH also reached its maximum concentration (1.38 $\mu\text{g/g}$) 12 hr after the start of the treatment and could be detected until 72-hr posttreatment, whereas the MBZ-NH₂ maximum level (2.15 $\mu\text{g/g}$) was attained 72 hr after cessation of treatment. Concentrations of the latter decreased very slowly, with a half-life of 245 hr (table 1). It is remarkable that the residue levels found in the skin were higher than those in muscle. It is known that the main route of absorption for drugs administered in the water is *via* the gills (14). However, absorption *via* the skin might not be excluded. In addition, some excretion of the administered drug as the parent compound and/or as metabolites is ascribed to the skin through the mucus of the body surface (15), whereas the most important excretory pathways in fish is the bile, the urine, and *via* the gills (14). The literature describes more examples for administered drugs, and/or metabolites persisting longer in the fish skin relative to the muscle may be due to their affinity for melanin-containing tissues (16–19). Because some consumers eat skin that is often in intimate contact with flesh during cooking, it is necessary from the safety point of view that residue studies should include samples comprising flesh and skin (20).

Liver samples showed maximum MBZ metabolite residue levels of 3.57 $\mu\text{g/g}$ for MBZ-OH 12 hr after the start of the treatment (fig. 1C) and 2.65 $\mu\text{g/g}$ for MBZ-NH₂ 4 hr posttreatment. However, 192 and 240 hr posttreatment, respectively, these levels reached their quantification limits. MBZ residue levels, in contrast to its metabolites, could be detected even 312 hr posttreatment. Kidney samples also contained appreciable levels of MBZ metabolites, but the maximum concentrations were lower than those in liver (fig. 1, C and D). MBZ-OH could be detected until 192 hr posttreatment (fig. 1D), whereas MBZ-NH₂ was present at a 1.3 $\mu\text{g/g}$ concentration even 312 hr after treatment. In contrast, the parent compound, although it reached higher levels than in the liver, it could be detected only until 48 hr posttreatment. It is remarkable that the highest MBZ-residue levels were found in the liver and kidney, consisting mainly of metabolites. The liver has a major xenobiotic-metabolizing capacity (14, 20). However, metabolizing activity has been found also in the kidneys (20). Elimination of MBZ and its hydroxy metabolite from all tissues is comparable with the exception of the skin tissue, which shows longer half-lives of MBZ and MBZ-OH than the other tissues (table 1). In comparison with the other compounds, the elimination rate of the MBZ-NH₂ is slower from all tissues.

Fat showed consistently the highest residue levels of MBZ, both during as well as after treatment. However, these levels were eliminated very rapidly, as in muscle, within 36 hr posttreatment (fig. 1E). MBZ-OH residue levels were no longer detected after 48 hr and MBZ-NH₂ residual levels were no longer detected after 192 hr. The MBZ-NH₂ level decreased with a half-life of 50 hr, comparable with the liver (table 1).

In addition, all water samples examined showed a MBZ concen-

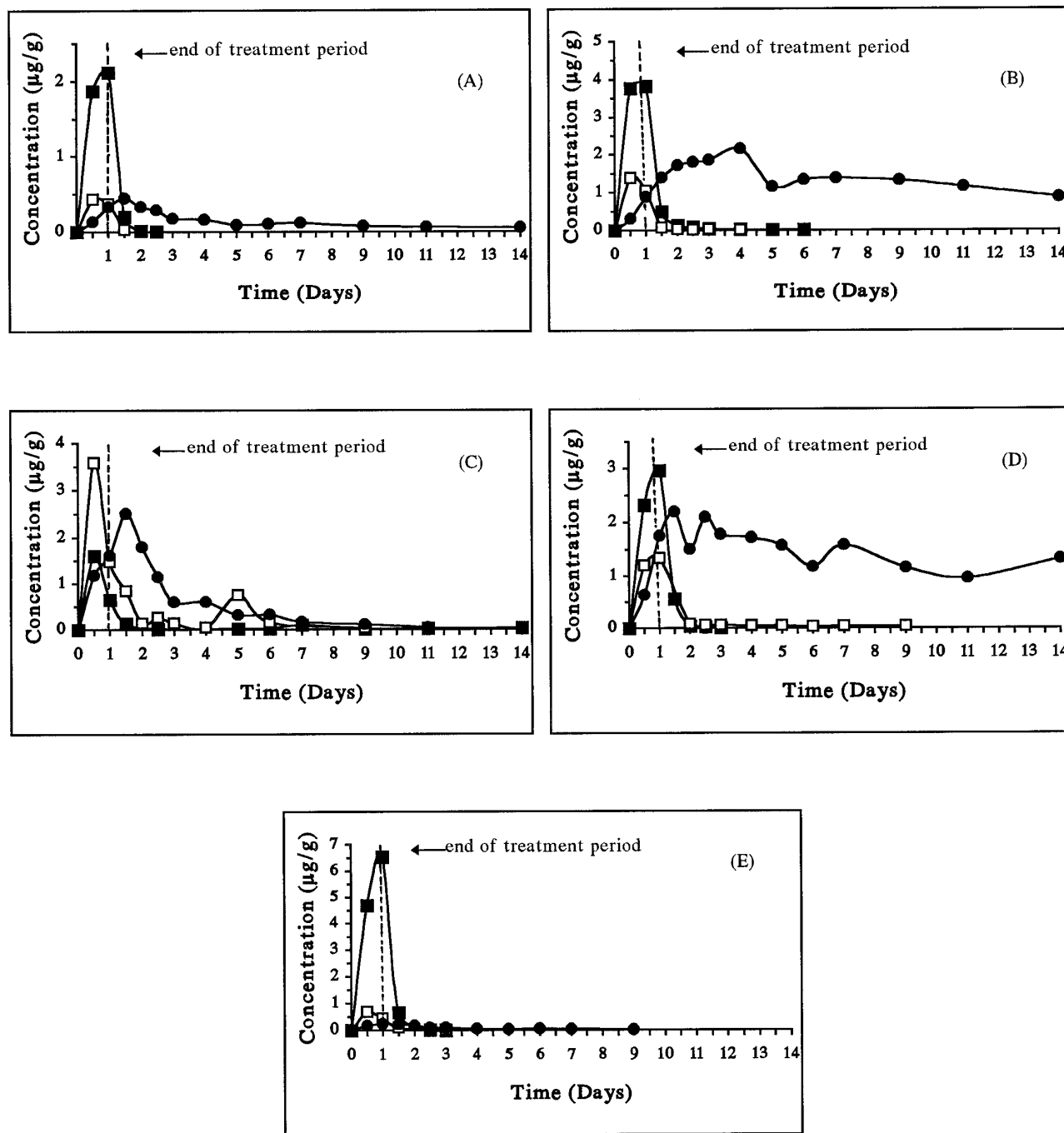


FIG. 1. Mean concentrations of MBZ (■) and its MBZ-OH (□) and MBZ-NH₂ (●) metabolites in eel muscle (A), skin (B), liver (C), kidney (D), and fat (E) after bath treatment with 1 mg MBZ/liter of water for 24 hr at 25°C.

tration of 0.5 mg/liter, but no metabolite could be detected, either in the aquaria with fish or in the aquarium without fish. Our results concerning the concentrations measured in water samples, however, are consistent with the finding of Møllergaard *et al.* (11). Lower MBZ concentrations than those added for the eel bath treatment in the water were detected in both of the experiments. A possible explanation for these lower MBZ levels is that MBZ may have been adsorbed either on organic particles present in the aquarium or on the glass surfaces of the aquarium. This is supported, in our experiment, by the lower MBZ concentrations found even in the water samples from the habituation aquarium containing the treatment MBZ dose, but no eels.

TABLE 1

Terminal elimination half-lives (hr) of MBZ and its metabolites MBZ-OH and MBZ-NH₂ in eel tissues after bath treatment with 1 mg MBZ/liter of water for 24 hr at 25°C

Tissue	MBZ	MBZ-OH	MBZ-NH ₂
Muscle	3.2	3.3	171
Skin	21.7	9.5	245
Liver	4.5	5.6	55
Kidney	6.1	1.2	348
Fat	3.6	5.2	53

It was demonstrated that MBZ and its hydroxy metabolite constitute the residues of toxicological concern, because they were demonstrated to have embryotoxic potential (21). However, it is of special concern for the residue risk assessment that the MBZ-NH₂ persists in edible parts of the eels (muscle and skin) even 14 days posttreatment. It may be concluded that it should be taken as the compound of interest for proving eel treated with MBZ and estimating the withdrawal time for consumption of eel treated with MBZ.

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