Risk Assessment Report on Marbofloxacin
(Veterinary Medicines)

Food Safety Commission of Japan (FSCJ)

August, 2007

Summary

A risk assessment was conducted on marbofloxacin, a new quinolone antibacterial agent effective for gram-negative bacteria and many species of gram-positive bacteria.

The assessment was conducted based on data obtained from test and experiments on animal metabolism and residual properties (in rats, dogs, swine animals and bovine animals), acute toxicity (in mice and rats), subchronic toxicity (in rats and dogs), reproductive development toxicity (2-generation reproduction in rats), teratogenicity (in rats and rabbits), and from studies on genotoxicity and microbiological effects.

Genotoxicity, effects on reproduction, and teratogenicity were not found. Although chronic toxicity/carcinogenicity studies have not been conducted, no carcinogenicity has been reported in quinolone agents in general, and marbofloxacin is considered to have no genotoxicity that can be a concern for human health. Accordingly, it is determined that it is possible to set an ADI without conducting carcinogenicity studies.

New quinolone antibacterial agents are used for human clinical treatments. Distinctive side effects include joint disturbance in immature animals and phototoxicity. A value was settled for the NOAEL for the joint effects of marbofloxacin in a study using beagle dogs. Phototoxicity is considered not strong due to its structural properties. Accordingly, intake of marbofloxacin through food is considered to have negligible phototoxicity to human health provided that it is used under proper management.

The lowest NOAEL obtained from various studies was 4 mg/kg bw/day in a 13-week subchronic toxicity study using rats or dogs. Based on this figure and the safety factor of 1,000, the toxicological acceptable daily intake (ADI) for marbofloxacin was established as 0.004 mg/kg bw/day. Microbiological ADI for marbofloxacin was set at 0.0032 mg/kg bw/day based on the findings in *in vitro* MIC_{50}.

Based on these figures, 0.0032 mg/kg bw/day was adopted as the ADI in the risk assessment on marbofloxacin. It should be noted that effects of this substance mediated by drug-resistant bacteria should be examined separately. Such effects are now in review.

Risk Assessment

[Findings Concerning Effects on Joints]

Quinolones are known to cause joint disorders, including pain and distention, to develop in immature animals. In a 13-week oral administration test of marbofloxacin using beagle dogs (3 to 4 months old), effects on joints were observed. When marbofloxacin was administered at up to 6 mg/kg bw/day for 13 weeks, no effects on joints were seen, including in histopathological examination. NOAEL for joint effects was considered to be 6 mg/kg bw/day or higher.

[Concerning Reproductive Toxicity and Teratogenicity]

Reproductive toxicity and teratogenicity of marbofloxacin were studied in 2-generation reproductive tests in rats and teratogenicity tests in rats and rabbits. In the reproductive tests using rats, inhibition of fertilizing capacity was seen in males at a high dose (500 mg/kg bw). In crossed females, the number of implantations and number of offspring were reduced and embryo mortality in utero increased. NOAEL was set at 10 mg/kg bw/day. The fertility inhibition of males reversed when the administration was temporarily terminated.

No teratogenicity was found in either rats or rabbits.

[Genotoxicity/Carcinogenicity]

In genotoxicity studies, positive results were obtained in *in vivo* Ames tests, gene conversion tests, a reverse mutation test, mitotic crossing-over test, and forward mutation test. However, positive results for Ames (TA102 strain only), gene conversion test with yeast, and forward mutation test with mammal cells (CHL V79) were also observed for other quinolone drugs. Therefore, these effects are not considered to be direct effects. Rather, they are assumed to be caused by topoisomerase II inhibition. In addition, negative results were shown in *in vivo*/*in vitro* unscheduled DNA synthesize tests in hepatic cells as well as in *in vivo* marrow micronucleus test. Based on these findings, the possibility of marbofloxacin to cause genotoxicity at a level high enough to be of concern with regard to living bodies is considered low.

While chronic toxicity/carcinogenicity tests have not been conducted, the results obtained in carcinogenicity tests in rodents for enrofloxacin and difloxacin (also types of fluoroquinolones) are negative. In a carcinogenic promotion test using levofloxacin (a formula with an extremely similar structure to marbofloxacin) in male rats, no promotion reactions were observed. In a relatively long history of clinical use in humans, no tumor development has been reported as a side effect of this compound.

In general, no carcinogenicity is recognized in new quinolone drugs, and it is unlikely for marbofloxacin to show genotoxicity of a level high enough to be of concern with regard to living bodies. Accordingly, establishment of ADI was determined possible without carcinogenicity tests.

[Phototoxicity]

Phototoxicity and photogenotoxicity of fluoroquinolones have been reported since the late 1990s. Two hypotheses have been discussed to explain the toxicity mechanism. The toxicity can be caused by a direct reaction between DNA and molecules activated by exposure to light, or by secondary damage induced by activated oxygen from light exposure and generation of free radicals. The degree of phototoxicity and photogenotoxicity of fluoroquinolones has been described in several reports. Fluoroquinolones, which have a halogen substitute group on the 6th and 8th carbons in their structures, are reported to have noticeably strong toxicity. It is also reported that phototoxicity decreases substantially when there has a methoxy group on the 8th carbon. Other reports describe weaker phototoxicity depending on the types of substitute group on the 1st carbon.

Having ring structures on the 1st and 8th carbons, marbofloxacin is structurally similar to ofloxacin, which is classified as belonging to the group of substances with weak phototoxicity/photogenotoxicity. There has been no report

of *in vivo* photogenotoxicity tests with ofloxacin or levofloxacin (optical isomer of ofloxacin). While cytotoxicity was enhanced with UV irradiation in an *in vitro* test with CHL V79 cultivated cells, and photogenotoxicity was enhanced by UV radiation[1] in a Comet assay and photo-micronucleus test, these values were relatively low when compared with other fluoroquinolones. In a test using auricle inflammation in mice after UV irradiation as an index, phototoxicity was relatively weak. In a levofloxacin test on skin erythema in human volunteers after UV irradiation as an index, no effects were observed at an irradiation level of 3 times/day (100 mg/dose). It was also reported that strong phototoxicity occurred at a rate of 1/1,800,000 in post-marketing surveillance.

Based on these findings and the fact that ofloxacin is classified as belonging to the group with weak phototoxicity/photogenotoxicity, marbofloxacin, which has an extremely similar structure to ofloxacin (both have rings on the 1st and 8th carbons), is considered to have the same phototoxicological and photogenotoxicological properties. Therefore, as long as properly managed, the residual level of marbofloxacin in ordinary foods is very minute. Accordingly, the possibility of marbofloxacin causing photogenotoxicity in living bodies through foods at a high enough level to become a health concern is negligible.

[Endpoint of Toxicological Effects]

Among the various toxicity studies reported, the index that showed effects of the test substance at the lowest dose is NOAEL shown in a 13-week subchronic toxicity test in rats and dogs (4 mg/kg bw/day).

[Endpoint of Microbiological Effects]

MIC_{50} obtained in an *in vitro* test is the only index currently available for microbiological effects. As an internationally accepted method[2], 0.260 µg/mL for MICcalc[3], 220 g for colon content, 30% for bacterial exposure fraction, and 60 kg for human body weight were adopted to calculate microbiological ADI based on the VICH formula.

$$\text{ADI (mg/kg bw/day)} = \frac{0.000260\ (\text{mg/mL}) \times 220\ (\text{g})}{0.3^{4} \times 60\ (\text{kg})} = 0.0032\ \text{mg/kg bw/day}$$

[Establishment of Acceptable Daily Intake (ADI)]

Marbofloxacin is considered to show no genotoxicity. Setting ADI, therefore, is feasible.

Among the various genotoxicity studies, the index that showed effects of the test substance at the lowest dose is NOAEL shown in a 13-week subchronic toxicity test in rats and dogs (4 mg/kg bw/day). In settling an ADI based on this figure, a safety coefficient of 1,000 should be used (10 for species differences, 10 for individual differences, 10 for the lack of chronic toxicity tests and carcinogenicity data). Accordingly, ADI based on toxicological data is 0.004 mg/kg bw/day. ADI based on the microbiological effects is 0.0032 mg/kg bw/day.

---

[1] In principle, FQ has an absorption capacity of 290 to 340 nm. The experimental irradiation in these tests was 1.25 to 37.5 kJ/m^2.

[2] VICH guidelines were adopted in March 2006 in Japan as domestic guidelines applicable to veterinary medicines.

[3] Lower limit of 90% confident limit for average MIC50 for the genus that is most relevant and has reactivity to the test substance.

[4] Estimated using data obtained in tests using rats and dogs and findings in ofloxacin administration tests using humans. Ofloxacin is a compound with a very similar structure to marbofloxacin.

The figure obtained for ADI based on microbiological data is smaller than the figure obtained for ADI based on toxicological data. This shows higher sensitivities were seen in microbiological tests. Accordingly, to establish residual standards for marbofloxacin, 0.0032 mg/kg bw/day should be used as ADI.

[Risk Assessment]

The following is the figure that should be used as the ADI in the risk assessment of marbofloxacin. It should be noted that effects of this substance mediated by drug-resistant bacteria should be examined separately. Such effects are now in review.

Marbofloxacin: 0.0032 mg/kg bw/day

The level of exposure is to be confirmed when provisional standards are reviewed based on the results of this assessment.