This is provisional English translation of an excerpt from the original full report.

Risk Assessment Report
Rafoxanide (veterinary medicines)

Food Safety Commission of Japan (FSCJ)
February 2009

Executive summary

The Food Safety Commission Japan (FSCJ) conducted a risk assessment of rafoxanide (CAS No. 22662-39-1), a parasiticide, mainly using two risk assessment reports of the European Medicines Evaluation Agency (EMEA)[1,2]. The data used for the risk assessment includes pharmacokinetic tests (rats, cows, and sheep), residue tests (cows and sheep), acute toxicity tests (mice and rats), subacute toxicity tests (rats and dogs), two-generation reproductive tests (rats), teratogenicity tests (rats and rabbits), genotoxicity tests, and pharmacological tests.

As no mutagenic activities in three *in vivo* tests and two *in vitro* tests, it was suggested that rafoxanide was not genotoxic, noting that neither chronic toxicity test nor carcinogenicity test was conducted. Thus, FSCJ decided that an acceptable daily intake (ADI) rafoxanide could be established accordingly.

The lowest no observed adverse effect level in the toxicity tests was 0.4 mg/kg body weight/day in a 13-week subacute toxicity test in dogs. Thus it was confirmed that the ADI of 0.4 µg/kg body weight/day could be established by applying a safety factor of 1,000 (i.e. species difference of 10, individual difference of 10, and an uncertainty factor of 10). The uncertainty factor of 10 above was applied because rafoxanide was considered to be neurotoxic and because neither chronic toxicity test nor carcinogenicity test was conducted.

As a result, FSCJ established the ADI for rafoxanide at 0.4 µg/kg weight/day.

[1] EMEA, COMMITTEE FOR VETERINAFY MEDICINAL PRODUCTS “RAFOXANIDE” SUMMARY REPORT (1), 1999
[2] EMEA, COMMITTEE FOR VETERINAFY MEDICINAL PRODUCTS “RAFOXANIDE” SUMMARY REPORT (2), 2001

Food safety risk assessment (extracted from Part III of the original risk assessment report)

1. ADI calculation

In the EMEA's risk assessment, an ADI was calculated by applying a safety factor of 200 (i.e. species difference of 10, individual difference of 10, and an additional factor of 2). The additional factor of 2 above was set because the optic and central nerve vacuolization observed in the tests had a serious toxic effect.

Unlike EMEA, FSCJ set an additional safety factor of 10 because rafoxanide was neurotoxic and because neither chronic toxicity test nor carcinogenicity test was conducted.Thus, FSCJ agreed that a safety factor of 1,000 (i.e. species difference of 10, individual difference of 10, and the additional factor of 10) should be applied.

The indicator of the lowest observed adverse effect level in the toxicity tests was optic and central nerve vacuolization in a 13-month subacute toxicity test in dogs. The no observed adverse effect level (NOAEL) was 0.4 mg/kg body weight/day.

Rafoxanide was devoid of mutagenic activity in three *in vitro* tests: *Salmonella*-microsomal assay, test for gene mutation in Chinese hamster ovary (CHO) cells at HPRT locus and L5178Y mouse lymphoa cells at the TK lotus. However it gave positive results *in vitro* human lymposytes chromosome aberration test at the highest concentration (250µg), reduced mitotic index of 51%. *In vitro* in a chromosome aberration test in CHO cells, rafoxanide was clastogenic in the presence of metabolic activation at toxic concentration higher or equal to 15µg/ml. Rafoxanide was not mutagenic in two *in vivo* tests: in the bone marrow micronucleus test in mice after oral administration; and in a test for unscheduled DNA synthesis (UDS) in rat hepatocytes. Since the bacterial test and in two independent *in vivo* mammalian tests were negative, it was suggested that that rafoxanide was not genotoxic, noting that neither chronic toxicity test nor carcinogenicity test was conducted.Thus, FSCJ decided to establish the ADI at 0.4 µg/kg weight/day by applying the safety factor of 1,000 to the NOAEL of 0.4 mg/kg body weight/day.

2. Conclusion

FSCJ concluded that the following value should be used as an ADI for rafoxanide:

Rafoxanide: 0.4 µg/kg body weight/day

Ministry of Health Labour Welfare will estimate the amount of human exposure to rafoxanide and elaborate new or revised maximum residue limits (MRLs) for fosfomycin in food concerned, not to exceed the ADI above. The proposed MRLs will be reviewed by the FSCJ for any advice, where necessary.