ASSURING THE SAFETY, QUALITY AND EFFICACY OF VETERINARY MEDICINES

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Your Ref: Report TR-527
Our Ref: VMD 7726
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Dear Dr Shane,

REPORT NUMBER TR-527: Malachite Green (569-64-2) and Leucomalachite Green (129-73)

I am writing to you to provide comment from the UK Government on the above draft report. I apologise for the lateness of this response, but we have sought the advice of a number of independent scientific advisory bodies in preparing this consolidated response. In addition, we have also sought comment and advice from the Food Standards Agency (FSA) and the Department of Health (DH), all of which adds to the quality of the response but unfortunately also requires a little longer to prepare.

Before providing our comments on the draft report, it might be helpful to briefly provide some background information in order to understand why the UK has such an interest in this study.

Background

The Veterinary Medicines Directorate (VMD) is an Executive Agency of the Department for Environment, Food and Rural Affairs (Defra) with the responsibilities of protecting public health, animal health, the environment and promoting animal welfare by assuring the safety, quality and efficacy of veterinary medicines in the United Kingdom

In doing so we support Defra's objectives to promote a sustainable, competitive and safe food supply chain which meets consumers' requirements and to ensure high standards of animal health and welfare. We also support the aim of the FSA to protect and improve the safety of food people eat.

The UK Government Departments and Agencies are advised by independent expert groups. The DH is advised by the Committee on Toxicity (COT) and the Committee on

Mutagenicity (COM). The VMD and the FSA are advised by the Veterinary Products Committee (VPC) and the Veterinary Residues Committee (VRC). The comments below represent a distillation of the views received.

Malachite green

Malachite green (MG) is a synthetic industrial. It was discovered many years ago, probably by accident, that MG is effective in treating parasitic and fungal infections in fish. Leucomalachite green (LMG) is a metabolite of MG. MG is not an authorised veterinary medicine. It is not listed under Annexes I, II or III of European Council Regulation 2377/90 and its use on farmed fish intended for human consumption is therefore not authorised throughout the European Union. Such use of MG is now banned in the UK.

Residue surveillance

a) farmed trout

The VMD has been analysing samples of trout for several years. The residue concentrations of MG, and in later years LMG, have been low. The significance of the residues was assessed by independent advisory committees and the independent scientific advisory committee to the UK Department of Health, the Committee on Toxicity (COT) looked at the issue for the third time in 1998, at which they reviewed residues surveillance results since 1996 and toxicological data which had become available since their last review. They also sought the advice of their sister committee, the Committee on Mutagenicity, which advised that it would be prudent to consider MG and LMG as ***in vivo*** mutagens.

The COT concluded in 1999 that the low concentrations found in a small proportion of samples of farmed trout (tested between 1996 and August 1998) would probably not adversely affect the health of consumers. However, it added that MG had not proven safe for use in fish farming. The COT expressed concern about the continued detection of residues of this substance in farmed fish.

The Food Advisory Committee (FAC) looked at the issue in October 2000 at the request of the Advisory Group on Veterinary Residues (an independent group advising the VMD, which has since been replaced by the Veterinary Residues Committee) and the FSA. The FAC expressed its concern over the continued presence of residues of both MG and LMG in a small percentage of trout and concluded that the advice issued by the British Trout Association to its members regarding the use of MG should be extended to all trout and ornamental fish farmers. It also recommended that the position should be reviewed when the NTP report is published.

b) farmed salmon

Farmed salmon was added to the UK statutory residue surveillance programme in 2001 after the FSA forwarded anecdotal evidence it had received that MG is used in salmon farming. Low residue concentrations (between 4 and 17 µg/kg) were found in 6 out of 30 samples analysed in 2001. These are in keeping with the residue concentrations found in trout in the mid 1990s and considered by the COT. In 2002, residues were found at concentrations from 2 – 35 µg/kg in 14 out of 74 samples analysed (but two of these

As you will see from the above, the UK has had an interest in the safety of malachite green and leucomalachite green for a number of years and has actively followed the progress of the NTP study from an early stage.

Comments on the draft report

The study protocol has been modified from the usual male and female groups in rats and mice and comprises only female rats and mice for malachite green chloride and male and female rats, and female mice only for leucomalachite green. The reasons for these changes are not given. The available summary is limited, but the data suggest that survival rates are acceptable between controls and treated groups and that the dose-related decrease in body weights indicates that both compounds were administered at the Maximum Tolerated Dose (MTD). It is not clear if the authors considered the potential impact of leucomalachite green content in the studies that were designed to look at the carcinogenicity of malachite green and vice-versa. The rationale for the dose selection should be given.

In the malachite green chloride component, no neoplastic effects were seen in the mice, but some non-neoplastic inflammatory effects were seen in a dose-related fashion in the urinary bladder and liver. In the female rats, there were slight increases in small numbers of neoplasms in the thyroid gland (follicular-cell adenoma or carcinoma) in the medium and high dose groups. There was also a small increase in hepatocellular adenoma and mammary gland tumours. There was, however, a marked decrease incidence in mononuclear cell leukaemia with increasing exposure.

Overall, the authors consider the evidence for the carcinogenicity of malachite green, based on the female rats, to be ***equivocal*** - this latter is defined as a marginal increase in neoplasm that may be treatment related. We would agree with this interpretation, but suggest a complete examination of the full study report and mechanistic considerations is required before any clear conclusion can be reached.

In the case of leucomalachite green, male and female rats and female mice were investigated. In the rats, dose-related decreases in bodyweight and good survival rates suggest the rat study was well designed, although there was no dose-related decrease in bodyweight in the female mice. However, in the mice there was a dose-related increase in non-neoplastic effects on the urinary bladder and an increase in hepatocellular adenoma or carcinoma. In the male rats, there was a slight increase in thyroid follicular cell adenoma or carcinoma and an increase in interstitial-cell adenoma of the testes. Concomitantly there were dose-related decreases in mononuclear-cell leukaemia and pituitary adenomas. In the female rats, non-neoplastic changes were seen in the liver and there was a slight increase (few extra tumours) in tumours in the thyroid gland, mammary gland and liver (adenomas). These increases were all marginal and not of statistical significance.

The authors considered the resulting neoplastic increases in rats to be ***equivocal*** and that in female mice demonstrating ***some evidence*** of carcinogenicity - this latter is defined as showing a chemically related increased evidence of neoplasms in which the strength of the response is less than that required for clear evidence.

Our scientific advisors considered that these conclusions seem to be an over-interpretation of the strength of evidence without further examination of the detailed evidence. In the

and pituitary tumours (a decrease) - are often related to perturbation of hormonal status in the rats and it is thus crucial to have an understanding of the potential genetic toxicity of the test substances as well as their hormone-pertubating potential. This particularly relates to the experimental induction of thyroid follicular-cell neoplasms that can be caused solely by hormonal imbalance. IARC has examined this issue in some depth (IARC Scientific Publication No.147, 1999, and IARC Monograph Series Vol. 79 - Some Thyrotrophic Agents, 2001). The consensus view is that agents that can cause thyroid follicular-cell neoplasms in rodents solely through hormonal imbalance can be identified using the following criteria:-

- there is a lack of genotoxic activity (agent and/or metabolite) based on an overall evaluation of the ***in vitro*** and ***in vivo*** data;

- the presence of hormone imbalance has been demonstrated under conditions of the carcinogenicity assay;

- the mechanism whereby the agent leads to hormone imbalance has been defined.

In the case of malachite green and leucomalachite green, the UK COM evaluated the available evidence of mutagenicity in 1999 and noted the limited number of studies available for evaluation. However, from those that were available they concluded that a positive ***Salmonella*** assay (in TA98 plus S9), positive results in a Chinese hamster lung cell assay, a negative result in a micronuclear test and a positive result in a 28-day feeding study in rats and mice using ^{32}P post-labelling assay showing DNA adducts, was sufficient evidence to suggest that malachite green may be a potential ***in vitro*** and ***in vivo*** mutagen. The data on leucomalachite green was considered too limited for an evaluation.

Thus, although the evidence is incomplete, it is not possible to conclude that the small increase in tumours, seen in the preliminary NTP reports, is based solely on hormonal imbalance. The UK advisory committees would welcome the opportunity to undertake a fuller evaluation when the full peer-reviewed report becomes available.

Conclusion

In conclusion, the evidence base for these two compounds is far from complete. Based on a quick review of the draft NTP report, it is not possible to conclude that the small numbers of tumours and, in particular, those of the thyroid, are based solely on hormonal imbalance. Our independent scientific advisors, as listed in the background above, would all welcome the opportunity of carrying out a full evaluation of the whole data set, including mechanistic considerations when the full peer-reviewed NTP report becomes available.

I hope that this is of some help to you in the forthcoming review process and once again please accept my apologies for the delay in providing this response.

Dr J F Kay