

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT



STATEMENT ON SURVEILLANCE FOR MALACHITE GREEN AND LEUCOMALACHITE GREEN IN FARMED FISH

Introduction

1. The Committee has been requested by the Veterinary Medicines Directorate (VMD) of the Ministry of Agriculture, Fisheries and Food to consider the results of recent surveillance for the compounds malachite green and leucomalachite green in farmed trout. The Committee was invited to comment on the results and any possible consequences for the safety of consumers and the operators of fish farms.

Background

2. The triphenylmethane dye malachite green has been used in aquaculture throughout the world for the treatment of parasitic and fungal infections in fish and shellfish. Within the United Kingdom (UK) trout industry it has been used for many years as a general hatchery disinfectant, to treat fungal conditions on trout eggs and for the control of certain ectoparasites. Additionally, it has been found to be effective in the treatment of 'proliferative kidney disease', a systemic protozoal disease in trout. Leucomalachite green is the major metabolite of malachite green.

3. Malachite green is used as a dye for fabrics and is freely available. It is not a licensed veterinary medicine and there are no regulations on dosage or mode of use in fish farming. The Committee understands that operators apply a solution of malachite green at the water inlet of the fish tanks or transfer the fish that require treatment to a separate container. Advice has been provided to the farmed and game fish industries about the precautions that should be taken to reduce the occurrence of malachite green and its metabolites in the fish that are sold on the retail market (*vide infra*).

The 1993 review

4. In 1993 the VMD sought the advice of the Committee on the possible risk to consumers from residues of malachite green that had been detected in some samples of farmed trout purchased at retail outlets. As only low concentrations, 1 to 7.4 µg/kg, of malachite green had been detected in about 7% of the samples the Committee concluded that these residues would not adversely affect the health of consumers. Nevertheless, the Committee had reservations about a) the chemical characterisation

of the malachite green used in toxicological studies and in fish farming, b) similarities of the structure of malachite green to compounds that are carcinogenic and/or mutagenic, and c) the existence of evidence of foetotoxicity in rabbits, with there being some evidence to suggest that it may also be teratogenic. These reservations led the Committee to conclude that there were insufficient reliable toxicological data to permit a full safety assessment (Department of Health, 1994).

5. The Committee's advice was passed to the Veterinary Products Committee (VPC) in 1994. The VPC noted the Committee's views and agreed with its conclusions.

The 1995 review

6. In 1995 the Committee considered further data from 406 trout samples analysed in three surveys between July 1993 to March 1995. Sixty-seven of the samples, about 16%, contained malachite green at concentrations of 2 to 50 µg/kg. The Committee considered there were still insufficient data to reach a conclusion about the safety of this substance, and concern was expressed about the apparent increase in residue levels. Although the concentrations of residues found were considered to be unlikely to affect the health of the consumers if the substance continued to be used, the Committee recommended that toxicity data should be obtained and, in the first instance, appropriate genotoxicity studies should be carried out on malachite green and its major conversion products in fish, the carbinol derivative and leucomalachite green, (Department of Health, 1996).

Subsequent actions

7. The Committee has been informed that, in the light of the results in 1995, the British Trout Association (BTA) issued a briefing note to their members stressing the need to ensure that any trout placed on the market did not contain residues of malachite green. As part of a package of precautions, the BTA advised members that malachite green should not be administered to fry over 5 grams in weight. In support of this initiative, the VMD wrote to all registered trout farmers in the UK in July 1995 underlining its concerns about the results and repeating the BTA advice.

8. In 1996, the BTA further advised its members to limit malachite green to use only in the hatchery, on eggs and, if essential, to alevins and first feeding fry on grounds of welfare. So that due regard for consumer safety should be taken into account, the fish should only be sold on to the next stage of the production chain once they were confirmed as being free of malachite green residues. The VMD endorsed this advice by writing in August 1996 to all registered trout farmers in the UK and to over 500 fisheries producing trout for game.

Additional survey data

9. We have been informed that malachite green was detected in 15 of 208 samples tested in a survey of retail trout in 1996. In 1997 there were only two trout, out of 137 tested, which contained residues of malachite green (Table 1). Subsequently a new method of analysis, capable of determining both malachite green and leucomalachite green, was tested on the samples from 31 of the 137 trout that had been tested in 1997. These samples were randomly selected although they included the two which had contained malachite green residues. Residues of leucomalachite green were detected and quantified in seven samples. The most recently received sample which had been positive for malachite green still contained residues of malachite green and also the highest concentration of leucomalachite green (Table 2). Surveillance for residues of malachite green and leucomalachite green in trout has been undertaken in 1998 using the new analytical method. Of the twenty seven samples collected in January to August, one was found to contain both malachite green and leucomalachite green and five others were found to contain leucomalachite green alone (Table 1).

10. We have queried whether there is information about any additional sources of malachite green or leucomalachite green that might result in their accumulation in the fish but have been advised that such information is not available.

Additional toxicological data

11. We have had the opportunity of considering full reports of short-term (28 day) range-finding studies in rats and mice which have been carried out on malachite green and leucomalachite green by the National Center for Toxicological Research in the United States (US) in preparation for priority carcinogenicity testing of these compounds as part of the US National Toxicology Program (Culp *et al.*, 1998a, 1998b).

12. The results of these studies show that leucomalachite green caused a greater number of, and more severe, effects in rodents as compared to malachite green. Changes in body weight and liver to body weight ratio generally occurred at lower doses of leucomalachite green as compared to malachite green. In male rats, a dose-related increase in midzonal and centrilobular hepatocyte vacuolisation was observed in the groups fed leucomalachite green at 580 and 1160 milligrams/kilogram (mg/kg), whereas hepatocyte vacuolisation was observed in the malachite green-treated rats of either gender only in the group fed 1200 mg/kg diet. The dose levels of 1200 mg/kg of malachite green and 1160 mg/kg of leucomalachite green are approximately equimolar and were the highest doses tested.

13. Morphological evidence of cell death in the transitional epithelium of the urinary bladder was observed in all female mice fed 1160 mg/kg diet of leucomalachite green, but not in female or male mice fed 1200 mg/kg diet of malachite green.

14. There were histopathological changes in the thyroid glands of some female mice

and male rats fed the highest concentrations of leucomalachite green. However, the number of animals affected was not statistically significant. Changes in the concentrations of total thyroxine (T4) and thyroid stimulating hormone indicative of alterations of thyroid function were observed in male rats fed leucomalachite green at 1160 mg/kg diet for 4 or 21 days. Such changes were not observed in male rats fed malachite green at 1200 mg/kg diet although alterations to 3,5,3'-triiodothyronine and T4 concentrations were observed in the female rats after 21 days at this dietary concentration.

15. In a separate study, the formation of liver DNA adducts was demonstrated by ³²P-postlabelling in rats or mice fed either 100 or 600 mg/kg diet of malachite green for 28 days. Increased concentrations of adducts were observed in the livers of rats fed 96 or 580 mg/kg of leucomalachite green for the same period, but not in the livers of mice.

16. Concerns about the possible genotoxicity of malachite green and its major conversion products, i.e. leucomalachite green and the carbinol, had led to our 1995 recommendation that appropriate genotoxicity studies should be carried out on these compounds. The additional data available now have led to our seeking the views of our sister committee, the Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment, on the possible mutagenicity of malachite green and leucomalachite green. They have advised us that it would be prudent to consider both malachite green and leucomalachite green as potential *in vivo* mutagens (Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment, 1999). The full statement of the Committee on Mutagenicity is attached.

Conclusions

17. i) We *note* that recent surveillance data from the UK, together with a number of other studies, indicate that the predominant residue derived from malachite green in trout from fish farms is leucomalachite green rather than the parent substance. We *note* also that the possible presence of the carbinol derivative of malachite green has not been monitored and that there are no recent toxicological data on this compound.

ii) We *recommend* that the results of surveillance for malachite green and leucomalachite green should report separately the detected concentrations of these compounds.

iii) We *note* that leucomalachite green was not measured in surveys of farmed trout in the UK carried out between 1993 and 1996 inclusive.

iv) We are *concerned* that residues of malachite green continue to be detected in farmed trout. The toxicology data are insufficient to allow us to reach a full conclusion about the implications of these residues of malachite green and its metabolites for the health of the consumer of farmed trout. However, we *note* that, by comparison with the data provided at our review of 1995, there may have been a

decline in the proportion of fish sampled that are found to contain residues of malachite green, see Table 1. We are aware that malachite green and leucomalachite green are currently undergoing carcinogenicity tests in the United States. We *consider* that, in the light of the opinion of the Committee on Mutagenicity (that it would be prudent to assume that malachite green and leucomalachite green are potential *in vivo* mutagens), there must be some concern about the health implications for those operators of fish farms who may be exposed to high concentrations of these compounds.

May 1999

[COT Statement 1999/04]

References

Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment (1999). Statement for COT: Malachite Green and Leucomalachite Green.

Culp SJ, Mulligan TL and Beland FA (1998a). Twenty eight day range finding study in mice and rats administered malachite green and leucomalachite green in the diet (2118.03/04 -leucomalachite green). NCTR Technical Report for Experiment No. 2118.03/04, May 1998, National Center for Toxicological Research, Jefferson, Arkansas 72079.

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Department of Health (1994). 1993 Annual Report of the Committees on Toxicity Mutagenicity Carcinogenicity of Chemicals in Food, Consumer Products and the Environment, London:HMSO.

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TABLE 1: Results of recent Surveillance in the United Kingdom by the Veterinary Medicines Directorate for Residues of Malachite Green and Leucomalachite Green in Trout Muscle

Sampling period	No. of samples Analysed	No. containing residues [#]	Incidence %	Mean µg/kg	Range µg/kg	Concentration detected, µg/kg (No.)
Malachite Green						
January to December 1995*	210	35	16.7	9	2-35	2(3), 3(6), 4(2), 5(3), 6(6), 7, 8(3), 12, 13, 14, 16(4), 18, 23, 25, 35
January to December 1996	208	15	7.2	10	3-31	3(3), 4(2), 5(3), 6(2), 17(2), 21, 22, 31
January to December 1997	137	2	1.4	7	2-12	2, 12
Introduction of new analytical method						
Malachite Green						
January to August 1998	27	1	3.7	-	-	8**
Leucomalachite Green						
January to August 1998	27	6	22.2	76	4-150	4, 6, 70, 78**, 150(2)

[#] Maximum Residue Limits (MRL) not set, Limits of Quantification (LOQ) = 2 µg/kg

* Contains data from samples collected in January to March 1995 previously considered by the COT

** Same sample contained both malachite green and leucomalachite green

TABLE 2: Results of reanalysis of samples of Trout Muscle collected in 1997 for residues of Malachite Green and Leucomalachite Green using the new analytical method

Sampling period	No. of samples analysed	No. containing residues [#]	Incidence %	Mean µg/kg	Range µg/kg	Concentration detected, µg/kg
Malachite Green						
September to December 1997	31	1	3.2	-	-	5*, **
Leucomalachite Green						
September to December 1997	31	7	22.0	66	4-330	4, 5, 7, 25, 41, 48, 330**

[#] Maximum Residue Limits (MRL) not set, Limits of Quantification (LOQ) = 2 µg/kg

* When first analysed this sample had contained 12 µg/kg malachite green, see Table 1

** Same sample contained both malachite green and leucomalachite green