

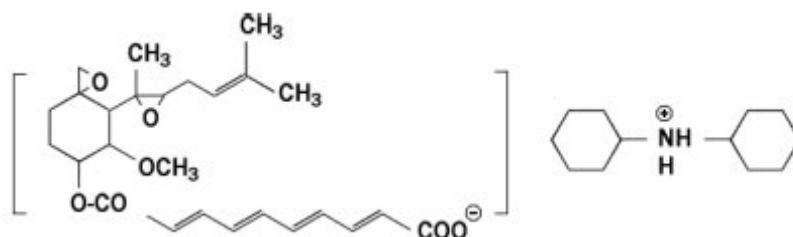
## COMMITTEE ON MUTAGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

### STATEMENT ON THE EVALUATION OF GENOTOXICITY DATA OF FUMAGILLIN DICYCLOHEXYLAMINE

#### Introduction

1. Fumagillin dicyclohexylamine (fumagillin DCHA) is an antibiotic authorised for use in honey bees for the prevention of infections caused by the *Nosema apis* parasite present in the gut of infected bees (Figure 1). Fumagillin DCHA is fed to the colony in winter over a period of several weeks in a medicated syrup as a supplementary food source to eradicate the parasites. The commercial formulation of fumagillin DCHA is a stabilised water-soluble preparation, Fumidil-B (CEVA Animal Health). Fumidil-B contains the excipient polysorbate 80, sodium phosphate (anhydrous) and sodium acid phosphate (anhydrous).

Figure 1. Structure of Fumagillin DCHA Salt



#### Background

2. Fumagillin DCHA currently has no maximum residue level (MRL) status because the Committee on Veterinary Medicinal Products (CVMP) were unable to make a recommendation when the substance was evaluated in 1999. The main reasons given were that no ADI could be established because no overall NOEL was identified for repeated dose toxicity, reproductive toxicity or teratogenicity/fetotoxicity and no conclusions could be reached on the genotoxicity or carcinogenic potential of fumagillin.

3. The veterinary medicine Marketing Authorisation Holder (MAH), CEVA Animal Health, has indicated that they may make another MRL application to address the absence of the MRL. The MAH has sought scientific advice from the CVMP and, in October 2006, the MAH stated that a 90-day repeated dose toxicity study was ongoing and that five mutagenicity tests had been performed. Reproductive toxicity studies have not been conducted.

4. Recently, reports of genotoxic effects of fumagillin DCHA in cytogenetic tests both *in-vitro* and *in-vivo* were published by the Stanimirovic group. These reports have been reviewed by VMD who have recommended that an

independent opinion should be sought on interpretation of the results, to establish if there is a potential risk to consumer safety

5. Subsequently, the MAH provided VMD with reports of six additional genotoxicity studies that it has conducted between 2004 and 2007, together with an expert report on the genotoxic potential of fumagillin and a critique of the genotoxicity studies submitted by the MAH.

### **Referral**

6. The Committee on Mutagenicity (COM) has been asked by the VMD for an opinion on the genotoxicity of fumagillin DCHA and in particular the interpretation of studies undertaken by the Stanimirovic group.

7. The COM has not been asked in this review to advise on consumer risk assessment of consumption of fumagillin, fumagillin DCHA, or its breakdown products as potential contaminants of honey.

### **Data Submitted to COM**

8. The MAH submitted studies investigating *in-vitro* chromosomal aberrations, primary DNA damage unscheduled DNA synthesis, and *in-vivo* micronucleus and primary DNA damage studies (UDS and comet).<sup>1-5</sup> The MAH also submitted a critical analysis of mutagenicity data of fumagillin DCHA salt.<sup>6</sup> The COM Secretariat retrieved additional published data on the stability of fumagillin and fumagillin DCHA, and genotoxicity studies on fumagillin DCHA and dicyclohexylamine.<sup>7-18</sup> A meeting was held between the HPA Secretariat, and the MAH and VMD on 18 December 2008 to discuss the evaluation of genotoxicity data of fumagillin DCHA. The MAH were requested to submit data on thermal and degradation studies with fumagillin DCHA, stability and genotoxicity studies with dicyclohexylamine, a summary of the genotoxicity data with fumagillin DCHA, and a critical analysis of the Nesslany studies<sup>2-6</sup> and those published by the Stanimirovic group.<sup>1,13-16</sup> The MAH submitted a detailed response to these questions<sup>19</sup> and a presentation for the COM.<sup>20</sup> Additional genotoxicity data on fumagillin acid and information on the mode of fungicidal action of fumagillin were also submitted.<sup>21-25</sup>

### **COM Consideration of Areas for Discussion**

9. The COM considered the MAH should be asked to comment on whether the effects reported by Stanimirovic were due to impurities and whether the MAH had a plausible suggestion for the *in-vivo* mutagenic effects reported by Stanimirovic. Members agreed to ask the MAH whether there were any data suggesting that the genotoxicity of fumagillin varied depending on light/dark conditions, temperature of test material dosing solutions and temperature of culture. The COM agreed to ask whether differing amounts of metabolism by epoxide hydrolase in mice and rats was one potential explanation for the results of *in-vivo* genotoxicity tests reported by these groups. Members commented that it was possible that the systemic absorption of the dicyclohexylamine salt of fumagillin was higher than the free fumagillin acid and agreed this should be raised with the MAH. The limited genotoxicity data on dicyclohexylamine was noted and Members agreed to

raise this aspect with the MAH. The COM considered the genotoxicity testing strategy used for the second tissue *in-vivo* assay and queried whether *in-vivo* rat liver comet assay was the most appropriate second tissue assay. It was noted a site of contact assay would have been preferable. Members agreed to raise this topic with the MAH.

### **Presentation by MAH**

10. The MAH provided background information on the use of fumagillin DCHA (Fumidil-B) to prevent and treat *Nosema apis* in honey bees. It was noted that the mode of action was not fully known although there were data to demonstrate irreversible inhibition of an enzyme involved in endothelial cell proliferation. A description of the submerged culture manufacture of fumagillin DCHA using cultures of *Aspergillus fumigatus* was presented. The MAH noted it was very difficult to obtain, in industrial scale, a significant better purity than approximately 90%. The impurity profile was stable from batch to batch, the main structures had been identified. Stability data for storage under three different conditions up to 12 months was available. Batches used for toxicological testing were representative of commercial batches. The EMEA had accepted data on manufacture. The MAH recommended that fumagillin DCHA was stored at 4°C to guarantee quality.

11. An overview of all the available genotoxicity data on fumagillin DCHA was presented. A comparison between the studies conducted and results obtained by Nesslany (using commercial batches of fumagillin DCHA)<sup>2-6</sup> and the studies reported by the Stanimirovic group<sup>1,13-16</sup> (using fumagillin DCHA not sourced from the MAH) was presented. For *in-vitro* studies information on purity, storage of test material, solvent and results were presented. Both research groups had found that fumagillin DCHA is an *in-vitro* clastogen using the *in-vitro* chromosomal aberration test in human lymphocytes.<sup>1-3,14</sup> The level of cytotoxicity was clearly different between the two groups but the NOEL for clastogenicity was similar. The difference could be attributed to a difference in purity and/or of a degradation product linked to preservation conditions of the test compound. The MAH showed a number of tables demonstrating slight differences in the presentation of results by the Stanimirovic group in two separate publications for essentially identical *in-vitro* chromosome aberration tests in peripheral blood lymphocytes, although this was further complicated by the reported differences in test material.

12. A comparison of the *in-vivo* genotoxicity tests undertaken by the Stanimirovic<sup>13,15-16</sup> and Nesslany<sup>4-6</sup> groups was presented. Information on purity, source and storage of the respective test materials was given. A description of the different test systems used by the Stanimirovic group (mouse bone marrow chromosome aberration and MN assays) and Nesslany group (rat bone marrow micronucleus assay) was provided. Information on the control of concentrations in dosing formulations were available for the Nesslany study. Information on the dosing solutions for both groups were available. An overview of results was presented. It was noted that plasma concentration data for the Nesslany study proved exposure of the bone marrow to the test substance.<sup>4</sup> An overview of the conduct and results for two additional studies (rat liver UDS and comet assays) undertaken by the MAH were presented.<sup>5-6</sup>

13. The MAH suggested the conclusion from the Stanimirovic group was of clastogenic effects in the mouse with seven daily oral treatments (cumulative 525 mg/kg bw) in male and female mice.<sup>13,15-16</sup> The equivalent Nesslany GLP compliant study indicated no genotoxic potential following two oral treatments of up to 1000 mg/kg bw in male and 640 mg/kg bw in female rats.<sup>4</sup> Two further *in-vivo* GLP compliant studies performed with fumagillin DCHA were negative (oral rat liver UDS (up to 250 mg/kg bw with two sampling times, and oral rat liver comet (up to 800 mg/kg bw with two sampling times)).<sup>5-6</sup>

14. The MAH briefly reported on genotoxicity studies performed with fumagillin acid. Negative results were available for *in-vitro* (bacterial and mouse lymphoma) assays. Negative results were obtained in oral MN tests in mice and rats.

15. The MAH suggested the discrepancy between the published studies from the Stanimirovic group and GLP compliant studies performed by the MAH were due to a difference of fumagillin DCHA quality and/or preservation conditions.

#### **COM Questions for MAH**

16. In answer to a question on impurity profile, the MAH reported that the profile of impurities had a relatively stable batch to batch variation, but genotoxicity data on impurities was not available.

17. In answer to a question on stability of test materials used in genotoxicity studies, the MAH suggested that differences between the Stanimirovic and Nesslany groups regarding storage of test materials was one possible explanation. The MAH noted there were no relevant genotoxicity studies of MAH derived test material conducted in light and dark conditions available.

18. Members commented that the *in-vitro* genotoxicity data from the Stanimirovic and Nesslany groups were very similar which might suggest that light/dark storage might affect cytotoxicity but not mutagenicity. One possible explanation being that fumagillin was storage sensitive but a genotoxic impurity was not storage sensitive.

#### **COM Discussion and Conclusions**

19. The COM agreed that the Stanimirovic data were limited and no definite conclusions could be reached. There were several possible explanations for the differences between the results obtained for *in-vivo* genotoxicity studies undertaken the Stanimirovic group and the MAH. These included possible differences in absorption, metabolism of the administered test material, differences in stability of the test materials including storage, and different impurity profiles between test materials used by the research groups. The COM agreed that the genotoxicity data on fumagillin acid and dicyclohexylamine tested separately did not provide sufficient information to draw conclusions on the role of these substances in the potential mutagenicity of fumagillin DCHA. The COM agreed that a repeat of the Stanimirovic study

in mice (using the same test protocol) with test material sourced by the MAH should be undertaken with appropriate measures of systemic absorption. The COM considered that a second *in-vivo* tissue evaluation should be undertaken and suggested a site of contact comet formation in the gastrointestinal tract (with an appropriate positive control substance). Negative data from appropriately conducted tests (according to the Stanimirovic protocol) using two tissues in mice would be sufficient to refute Stanimirovic data. Equivocal or positive data from such tests would confirm that fumagillin DCHA should be considered an *in-vivo* mutagen. The Committee also commented if any genotoxicity was observed with fumagillin DCHA, more genotoxicity data (*in-vitro* chromosomal aberration test in human lymphocytes) should be provided on dicyclohexylamine to evaluate its potential role. (Any study should also include fumagillin DCHA for quantitative comparison). The COM considered that the differences in statistical reporting in the Stanimirovic group publications as highlighted by the MAH were not necessarily founded.

20. Members agreed that further additional data on the influence of light/dark conditions on the genotoxicity of fumagillin DCHA were not necessary. Members agreed that the data on potential fungicidal mode of action submitted were not relevant to the potential genotoxicity mode of action of fumagillin DCHA.

21. The COM recommended the following testing strategy for fumagillin DCHA

i). A further *in-vivo* mutagenicity study using the same protocol used by Stanimirovic et al. (*Mutat Res* (2007) 628, 1-10)<sup>13</sup> to include sampling of bone marrow for MN and chromosomal aberrations.

ii) A site of contact comet assay using gastrointestinal (stomach) tissue. (The comet assay should also include an appropriate positive control substance).

iii). If any genotoxicity is observed with fumagillin DCHA, more genotoxicity data (*in-vitro* chromosomal aberration test in human lymphocytes) should be provided on dicyclohexylamine to evaluate its potential role. (Any study should also include fumagillin DCHA for quantitative comparison).

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