Opinion of the Scientific Committee on Plants regarding the evaluation of Ampelomyces Quisqualis in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market (opinion adopted by the Scientific Committee on Plants on 7 March 2001)

1. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS REGARDING THE EVALUATION OF AMPELOMYCES QUISQUALIS IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC CONCERNING THE PLACING OF PLANT PROTECTION PRODUCTS ON THE MARKET

(Opinion adopted by the Scientific Committee on Plants on 7 March 2001)

2. TERMS OF REFERENCE

The Scientific Committee on Plants (SCP) is requested to respond to the following questions in the context of the Commission's work on the implementation of Council Directive 91/414/EEC concerning the placing of plant protection products on the market.

1) Is the issue of residue levels in food and feed adequately addressed, in relation to the safety requirements of Article 5 of Council Directive 91/414/EEC?

2) Given the absence of models for assessing operator exposure for microbial pesticides - has this issue been adequately addressed in relation to Article 5 of Council Directive 91/414/EEC?

3) With regard to possible hazard to humans, should repeated dosing be part of the primary data set?

4) It is known that certain health problems can arise from working with microbial pesticides e.g. allergies developed when glasshouse workers were exposed to attenuated strains of tobacco mosaic virus (TMV). Would a post authorisation requirement to monitor the health of workers (blood testing etc.) be a prudent measure? If so, what measures would the Committee recommend?

3. BACKGROUND

The draft Commission Directive for inclusion of **Ampelomyces quisqualis** in Annex I to Directive 91/414/EEC concerning the placing of plant protection products on the market was submitted to the Committee for opinion. The Committee had been supplied with documentation comprising a monograph prepared by the Rapporteur Member State (France) based on a dossier submitted by the notifier (Ecogen), a review report prepared by the Commission and the Recommendations of the ECCO [1] Peer Review Programme.

Ampelomyces quisqualis is a fungus for use as a fungicide. Its intended use is on vine to control powdery mildew (**Uncinula necator**) as part of an integrated control programme. It may be applied up to 12 occasions/season.

4. OPINION

4.1 Question 1

"Is the issue of residue levels in food and feed adequately addressed, in relation to the safety requirements of Article 5 of Council Directive 91/414/EEC?"

Opinion of the Committee:

Based on the absence of any evidence of pathogenicity or toxin production by Ampelomyces quisqualis, the inability of the spores to survive gut transit and the lack of any adverse reaction following ingestion of pycnospores in an acute toxicity test as well as in a repeated dose 90-day oral toxicity study in rats, the SCP considers that exposure of consumers to A. quisqualis spores is not a cause for concern. The Committee is therefore of the opinion that the issue of residue levels in food and feed has been adequately addressed.

Scientific background on which the opinion is based:

4.1.1 General considerations

Unlike chemicals added to biological systems where any residues detected are the parent compound or its metabolites, living organisms introduce higher levels of organisation and this is reflected in the nature of any retained material. Material retained is more likely to consist of the organism itself, either in an active metabolic state or in some form of resting stage, non-viable cells, or fragments of the organism. In addition the added organism may produce metabolites at any stage of its production or final use which may then be further transformed by other micro-organisms and/or the host. Any assessment of safety must consider the possibility and consequences of the added organisms entering and remaining with the food chain as well as the fate of any metabolites.

4.1.2 Ampelomyces quisqualis

Ampelomyces quisqualis is a hyperparasite of the Erysiphaceae (powdery mildews). It is marketed as a water dispersible granule consisting of 58% pycnospores and 42% inert carrier. The nature of the carrier is not of toxicological or environmental concern.

4.1.3 Survival on the treated plant

Pycnospores, which are the active component of the final product, are presumed to germinate only in the presence of host mycelium and not to survive for extended periods in the absence of a suitable host.

Although no attempt have been made to measure the persistence of pycnidiospores at various stages of grape maturation, it is assumed that the number of spores surviving at harvest would be very small because **A. quisqualis** is not applied after the "veraison" phase of grape

maturation which occurs several weeks before harvest. Indeed, based on the biology of grapevine and the fungus, powdery mildew does not develop beyond the "veraison" stage.

4.1.4 Toxicity of viable spores

Two acute toxicity tests were carried out with rats by oral route. In the first experiment a single dose of 5×10^9 viable spores kg^{-1} body weight was given by gavage and the animals killed after 14 days. No effect on mortality or daily weight gain was noted and no clinical symptoms were observed other than discoloured faeces on day one. Gross necropsy also did not reveal any abnormalities.

In the second study, rats were assigned to one of four experimental groups (9 rats/sex/group), a test group (given 7.8×10^8 and 7.5×10^8 viable spores to males and females respectively), a killed test substance group (given 7.9×10^6 and 7.5×10^6 heat-inactivated spores to males and females respectively), a control group housed in the same room and a control group separately housed. Animals were killed respectively after two hours, three days and seven days after dosing. Body weight gain and animal behaviour were not affected. Viable spores of **A. quisqualis** could not be recovered from any site in the body, including the digestive tract, from any group at any time. Evidently spores lost viability within two hours of ingestion. As a consequence there was no evidence of migration to other tissues.

A repeated dose 90-day oral toxicity study in rats is also reported. Two groups of Sprague Dawley rats (10 sex/group) were administered viable spores or heat inactivated spores of **A. quisqualis**, 2x108 in 0.5 ml sterile water respectively. A similar group receiving 0.5 ml sterile water only served as control. The study was performed in compliance with an OECD protocol and under GLP conditions. No substance-related adverse effects were reported.

4.1.5 Pathogenicity

A search of the major databases performed by the notifier, failed to identify any publication relating to the infection or colonisation of humans by **A. quisqualis**.

4.1.6 Production of metabolites

A search of major databases performed by the notifier, failed to identify any reference to the production of toxins by **A. quisqualis**. The absence of significant levels of contamination and outgrowth on the mature grape also makes the **in situ** production of metabolites, toxic or otherwise, very unlikely.

4.1.7 Conclusion

The absence of any evidence of pathogenicity or toxin production by **A. quisqualis**, the inability of the spores to survive gut transit and the lack of any adverse reaction following ingestion of pycnospores in an acute toxicity test, indicate that exposure of consumers to the very low numbers of **A. quisqualis** spores which might be retained on the fruit, is not a cause for concern.

4.2 Question 2

"Given the absence of models for assessing operator exposure for microbial pesticides - has this issue been adequately addressed in relation to Article 5 of Council Directive 91/414/EEC?"

Opinion of the Committee:

In the absence of a satisfactory pulmonary toxicity study to assess the risk of operators for inhalatory exposure to spores, the Committee concludes that the risk to operators has not been adequately addressed in relation to Article 5 of Council Directive 91/414/EEC.

Scientific background on which the opinion is based:

4.2.1 General considerations

In analogy to chemical substances, intake of microbiological plant protection products (PPPs) by the operators can occur by inhalation, ingestion and skin penetration. In the case of inhalation, the size of the micro-organism-bearing particles is important to determine the site of the airways where they may deposit and the route of absorption. Assuming a behaviour similar to that of inhalable dust, particles greater than 20-30 ï • m would only be inhaled in minimal amounts, while particles from 20 to 5 ï • m would mostly deposit in the upper airways, and particles less than 5 ï • m would reach the smaller bronchioles and deeply penetrate into the lungs. The particles deposited in the nose, larynx and pharynx can successively be ingested. The penetration of the micro-organisms through intact skin is likely to occur to a very limited (if any) extent, except when the micro-organism can colonise the epidermis and become established.

Specific models to assess operator exposure to biological PPPs are not available and the experience in this area is rather limited. Since operator exposure has to be in any case assessed, the SCP is of the opinion that the methods and the models used for the chemical PPPs can be adapted to this purpose. This assumes that a microbial propagule is quantitatively comparable to a mass of chemical molecules and the quantitative contamination created by spraying suspensions of micro-organisms is analogous to that generated by chemical PPPs. Such a procedure allows the dose expected to reach the operator to be estimated and, after conversion in terms of micro-organism mass, to compare the exposure values with the inoculum concentrations used in the toxicity studies or, when available, with NOAEL [2].

Similar considerations apply to bystander exposure and to agricultural workers exposure, although the differences in adherence and skin penetration between chemical and biological PPPs may be large.

As indicated above, it has to be recognised that operator exposure assessment for micro-organisms used as PPPs is currently far from accurate and that any proposed method of assessment can only provide a general, approximated estimate of risk. Thus a sufficiently large margin of safety has to be present in order to ensure compliance with the provisions of Article 5 of the Directive 91/414/EEC. Moreover, while the quantitative assessment of exposure to micro-organisms is relevant to the assessment of the toxic and infection risk, other potential adverse effects such as allergic reactions cannot be adequately predicted and assessed with this method [this issue is addressed below in section 4.4].

4.2.2 Pulmonary Toxicity of viable spores

The Committee reviewed the study entitled "Toxicity/pathogenicity testing of **Ampelomyces quisqualis** following acute intratracheal challenge in rats" [3]. The SCP considers this study to be seriously flawed for the following reasons:

- the experimental design is inappropriate and does not allow any reasonable conclusions for the possible effects of **Ampelomyces quisqualis** due to the absence of appropriate controls;
- the absence of more than one dose level prevents the estimation of NOELs [4] or LOELs [5] ;
- study reporting is of an unacceptably poor quality and is not in compliance with GLP regulations.

This study is the only source of information submitted to the Committee on pulmonary toxicity in order to establish the absence of pathogenicity or infectivity of the spores. In the circumstances, it is not possible to establish a NOAEL for pulmonary toxicity and therefore neither is it possible to confirm the margin of safety for inhalatory exposures in operators.

4.3 Question 3

"With regard to possible hazard to humans, should repeated dosing be part of the primary data set?"

Opinion of the Committee:

The SCP is of the opinion that repeated dosing should in general be part of the primary data set, but repeated dosing can be omitted provided that adequate justification can be offered based on the biological properties of the micro-organism and the results of acute toxicity and pathogenicity studies. In the specific case of A. quisqualis the Committee is unable to comment on the necessity for repeated dosing by inhalation to assess hazard to humans due to absence of information on pulmonary toxicity.

Scientific background on which the opinion is based:

4.3.1 General considerations

Repeated dosing may not be necessary when the biological properties of the agent and the results from acute toxicity are clear enough to conclude of the non-toxicity of the agent. If on the basis of acute toxicity data or other reasons doubt persist, repeated dosing should be performed.

Repeated dosing is also not deemed necessary when the results of the acute toxicity and pathogenicity studies enable to conclude in the absence of infectivity and of toxicity due to toxins or metabolites produced by the micro-organism.

4.3.2 Conclusion regarding **Ampelomyces quisqualis**

Despite the acceptable results of the acute toxicity studies and the presence of a repeated dose oral study, the Committee is unable to comment on the necessity for repeated dosing by inhalation to assess hazard to humans due to absence of information on pulmonary toxicity

4.4 Question 4

"It is known that certain health problems can arise from working with microbial pesticides e.g. allergies developed when glasshouse workers were exposed to attenuated strains of tobacco mosaic virus (TMV). Would a post authorisation requirement to monitor the health of workers (blood testing, etc.) be a prudent measure? If so, what measures would the Committee recommend?"

Opinion of the Committee:

The possibility of occurrence of allergic reactions resulting from agricultural exposure to A. quisqualis cannot be excluded. Greater confidence on the absence of allergenic response in humans can only be provided by direct observation on subjects exposed to it in its production or use.

Therefore the SCP considers that monitoring the health of producers and users would be a prudent measure. In the case of an allergic reaction being recorded in these subjects, the causative agent should be identified and notified to the competent authority of the relevant Member State. The results of the monitoring should be made available for future re-assessment.

Scientific background on which the opinion is based:

4.4.1 Generalities on allergy

The term **allergy** denotes a condition of hyper-reactivity of the immune system of an organism which may be the cause of pathological reactions of clinical significance on repeated contact with substances (allergens) against which the organism is hypersensitive. Since food allergy is a particular phenomenon involving the gastro-intestinal immune system and the oral exposure for workers is likely to be negligible, the issue of food allergy is not addressed.

The substances acting as allergens, possess a variety of chemical structures. This fact makes it difficult to predict the allergenic potential of a substance only based on its structural or chemico-physical properties. Many substances of biological origin, in particular proteins and glycoproteins, have the potential of acting as allergens, although the (genetic) predisposition of the host plays a determinant role in the onset of the allergic reaction. Heterologous animal testing of a substance to predict the allergenic risk for man has limited value because of interspecies differences in allergic reactivity and inability of these tests to predict the inter-individual human variability in response.

4.4.2 Allergic responses to microbial aerosols

Allergic reactions induced by fungi in man may take the form of allergic interstitial pneumonitis, a syndrome caused by inhalation of organic dust and characterised by granulomatous infiltrates in the lungs and symptoms such as fever and cough. In allergic interstitial pneumonitis the hypersensitivity reaction results in a granulomatous reaction in alveolar septums, with collection of lymphocytes, plasma cells and epithelioid cells. Ordinarily eosinophils (which are common in diseases such as hay rhinitis and asthma) are not part of the cellular infiltrate. In most instances, the antigens appear to be products of

bacterial and fungal growth in stored plant products. Precipitating antibodies to the inciting antigen occur in most patients and appear to mediate the reaction. In these conditions the period of exposure required to establish the specific sensitivity is uncertain, but once sensitisation is established, inhalation exposure to the antigen commonly leads to a moderately acute illness in a matter of hours.

In the case of Farmer's lung, agricultural workers may develop chills, fever, cough, and dyspnea rapidly, a few hours after exposure to mouldy organic dusts (hay, grain, corn, tobacco, etc.). Mouldy hay is rich in thermophilic actinomycetes (**Micropolyspora faeni** and **Micromonospora vulgaris**) as well as a number of fungal species (**Aspergillus, Cladosporium, Mucor, Penicillium,** and **Humicola**). The serum of patients with Farmer's lung contains precipitating antibodies against extracts of mouldy hay dust but none to clean hay dust. Furthermore, when inhaled by aerosol, extracts of mouldy hay or cultures of the actinomycetes will produce typical attacks.

In the case of Maple bark disease, sawmill or logging workers may develop typical attacks of interstitial pneumonitis after shaving or peeling bark from maple logs that have been stored for some time after cutting. Examination of the logs reveals heavy growth of the sporulating mould **Cryptostroma corticale**. Lung biopsies may show a number of inhaled spores; these spores have not germinated or grown mycelium, but by their presence, they initiate a strong hypersensitivity reaction. Similar syndromes occur after exposure to redwood or elm sawdust and are due to sensitivity to fungi of the genus **Graphium**.

Hypersensitivity pneumonitis has also been observed after exposure to organic dust in buildings. The agents responsible for these forms have included thermophilic actinomycetes, **Pullularia**, **Cladosporium cladosporoides**, **Penicillium** species, and endotoxins.

Humidifier fever is an influenza-like illness characterised by headache, myalgia, lethargy, fever and dyspnea occurring the first day back at work after a break, usually resolving within 24 hours and not recurring despite continued exposure to the polluted environment until a further period away from work. The specific cause(s) of humidifier fever are not known, however postulated causes include allergens from protozoans, **Bacillus subtilis** and bacterial or fungal endotoxins.

4.4.3 Allergenic potential of **Ampelomyces quisqualis**

No cases of allergic reactions have been observed amongst the limited number of operators monitored for adverse effects and no case has been reported in the USA where this product has been authorised as an active substance since 1994 [6].

The allergenic potential of exposure to **A. quisqualis** following a large-scale use in agriculture is difficult to assess on a theoretical base. The possibility of occurrence of these reactions cannot be excluded, given the scarce knowledge available and the limited experience of use. A greater confidence on the absence of allergenic potential of this micro-organism in humans can only be obtained by direct observation of a significant number of subjects exposed to it in its production or use.

Given the fact that individual susceptibility is of great importance in allergic responses and allergic reactions usually concern a minority of the human population, a sufficiently large

number of subjects need to be kept under observation in order to draw valid conclusions about the absence of allergic responses.

4.4.4 Conclusion

The possibility of occurrence of allergic reactions to **A. quisqualis** cannot be excluded, given the scarce knowledge available and the limited experience of use.

Therefore the SCP considers that monitoring the health of producers and users would be a prudent measure. In the case of an allergic reaction being recorded in these subjects, it should be diagnosed as to the causative agent and notified to the competent authority of the relevant Member State. The results of the monitoring should be made available for future re-assessment.

5. DOCUMENTATION MADE AVAILABLE TO THE COMMITTEE

- 1. Terms of reference "Evaluation of **Ampelomyces quisqualis** in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market" (Doc. SCP/AMPEL/001).
- 2. Evaluation table Doc. 6864/VI/98 rev.3 (Doc. SCP/AMPEL/003-Rev.3).
- 3. Assessment of potential exposure to the Biological Pesticide AQ-100, Katinka van der Jagt, JSC International Ltd, Harrogate, UK (Doc. SCP/AMPEL/005).
- 4. RFLP Analysis of **Ampelomyces** species for quality control of AQ10 Biofungicide, Dr. W.P. Donovan, Ecogen, Langhorne, USA (Doc. SCP/AMPEL/006).
- 5. Pulmonary safety of **Ampelomyces quisqualis**, Dr. R.L. Sherwood, IIT Research Institute, Chicago, USA (Doc. SCP/AMPEL/007).
- 6. Draft review report 4205/VI/99-rev.2 (Doc. SCP/AMPEL/008).
- 7. Appendices to Evaluation of **Ampelomyces quisqualis** in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market - 14/01/2000 (Doc. SCP/AMPEL/009).
- 8. **Ampelomyces quisqualis**: extract from dossier Annex II, point 5.1.1.4, p. 72 to 80: report on the study "Toxicity/pathogenicity of **Ampelomyces quisqualis** following intratracheal challenge in rats" Barbera, P., Sherwood R.L, Thomas, P.T, 14 August 1992. submitted by DG Health and Consumer Protection, 9 November 2000 (Doc. SCP/AMPEL/010).
- 9. Evaluation of **Ampelomyces quisqualis** in the context of Council Directive 91/414/EEC concerning the placing of plant protection products on the market: comments on document SCP/AMPEL/010, submitted by Prof. Savolainen on 10 November 2000 (Doc. SCP/AMPEL/012).
- 10. Monograph prepared in the context of inclusion of **Ampelomyces quisqualis** in Annex I of Council Directive 91/414/EEC - Ministère de l'Agriculture et de la Pêche - France (Volumes 1 to 4) - October 1997.

6. ACKNOWLEDGEMENTS

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Micro-organisms WG: Prof. O'Gara (Chairman) and Committee members Dr. Delcour-Firquet, Prof. Maroni, Dr. Meyer, Prof. Silva Fernandes, Dr. Speijers, invited experts: Dr.

Alabouvette, Dr. Chesson, Prof. Defago, Prof. Gismondo, Prof. Nuti, Dr. Stead and Prof. von Wright.

Toxicology Working Group: Prof. Maroni (Chairman), and Committee Members Dr. Delcour-Firquet, Dr. Meyer, Dr. Moretto, Prof. Silva Fernandes, Dr. Speijers, Prof. Savolainen and invited expert Dr. Fait.

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[1] European Commission Co-ordination.

[2] No observed adverse effect level.

[3] Barbera P; Sherwood R.L.; and Thomas, P.T., project no LO8349, 14 August 1992 - study no 3. I I T Research Institute, Life Science Research, Chicago, USA (property of ECOGEN INC).

[4] No observed effect level.

[5] Lowest observable effect level.

[6] Source EPA http://www.epa.gov/