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SCIENTIFIC COMMITTEE ON PLANTS **SCP/PSEUDOM/002-Final**
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OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION REGARDING THE EVALUATION OF *PSEUDOMONAS CHLORORAPHIS* IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

(Opinion adopted by the Scientific Committee on Plants on 20 December 2001)

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: G1 01/342.
Telephone: direct line (+32-2)296.58.91, switchboard 299.11.11. Fax: 295.73.32.

Internet: Jean.Ferriere@cec.eu.int

A. TITLE

OPINION OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION REGARDING THE EVALUATION OF *PSEUDOMONAS CHLORORAPHIS* IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

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B. 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, is a tiered approach adequate and should repeated dosing be part of the primary (tier 1) data set?

4) Is the toxicological safety of the antibiotic metabolites of *Pseudomonas chlororaphis* adequately addressed?

5) 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?

6) The genus Pseudomonas also contains important pathogens for human e.g. *Pseudomonas aeruginosa*, which can establish in open wounds. There is one documented case where *Pseudomonas chlororaphis* was found in the wound of a soldier. Does this finding give rise to any concerns for human safety?

C. OPINION OF THE COMMITTEE

Opinion on question 1:

The Committee, noting that *Pseudomonas chlororaphis* MA342 applied to seeds does not continue to colonise the emerging plant, concludes, in the absence of sustained colonisation, the number of *P. chlororaphis* associated with the harvested grain as well as the concentration of any metabolites produced would be very low. Therefore the Committee is of the opinion that there is no cause for concern and that the issue of residues has been adequately addressed.

Opinion on question 2:

The Committee is of the opinion that operator exposure to *Pseudomonas chlororaphis* formulations has been adequately addressed (see also opinions on questions 4 and 5).

Opinion on question 3:

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 based on the biological properties of the micro-organism and the results of acute toxicity and pathogenicity studies can be offered. In the specific case of *Pseudomonas chlororaphis*, and in the light of the results of the available studies, the SCP is of the opinion that repeated dosing is not necessary to assess hazard to humans.

Opinion on question 4:

The Committee noted that the toxicological information so far available on the putative antibiotic metabolites of *Pseudomonas chlororaphis* is limited, because:

– no toxicokinetic data on DDR are available (absorption, biotransformation, distribution),
– only two studies (one *in vitro*, one *in vivo* in mice by gavage) on the genotoxicity of the metabolite DDR have been carried out, and showed aneuploidy,
– no experiments have been made to evaluate the mutagenic potential of DDR.

Although the SCP concludes that more studies would be needed for a more complete assessment of the mutagenicity potential of DDR, the potential for human exposure to DDR as well as to other possible antibiotic metabolites is so low that, even in the absence of further information, the Committee is of the opinion that no major concern exists for consumer and operator safety.

Opinion on question 5:

Although the probability of occurrence of allergic reactions on agricultural exposure to *Pseudomonas chlororaphis* is likely to be very low, the possibility of their occurrence cannot be totally excluded. Confirmation of lack of allergenic potential of this micro-organism can only be obtained by a direct systematic observation on a significant number of exposed operators. Therefore the SCP is of the opinion that a study based on the medical surveillance of workers should be conveniently carried out when introducing this agent in the field as a microbial pesticide.

Opinion on question 6:

The Committee is of the opinion that there is no cause for concern for human safety with regard to wound infection.

A. TITLE

REPORT OF THE SCIENTIFIC COMMITTEE ON PLANTS ON SPECIFIC QUESTIONS FROM THE COMMISSION REGARDING THE EVALUATION OF *PSEUDOMONAS CHLORORAPHIS* IN THE CONTEXT OF COUNCIL DIRECTIVE 91/414/EEC

B. TABLE OF CONTENTS

C. BACKGROUND

Pseudomonas chlororaphis is a naturally occurring soil bacterium that is being developed as a microbial based plant protection products (new active substance in the context of Directive 91/414/EEC[1]). A draft assessment report (monograph) has been prepared by the Rapporteur Member State (RMS, Sweden) on the basis of a dossier presented by the notifier (BioAgri AB). In order to prepare its opinion the Scientific Committee on Plants had access to the documents listed below.

[1] OJ N° L 230, 19. 8.1991, P. 1.

Pseudomonas chlororaphis is a soil bacterium, which is being developed as an inoculant to protect cereal against a wide range of soil borne fungal diseases. Its intended use is as a seed dressing for cereals.

Source documents made available to the Committee:

1. *Pseudomonas chlororaphis*: Terms of reference, submitted by DG Health and Consumer Protection, 17 December 1999 (SCP/PSEUDOM/001).

2. Acute pulmonary toxicity study, submitted by the notifier, 21 June 1999 (SCP/PSEUDOM/003).

3. Evaluation table 6865/VI/98-rev4, 13/12/99 submitted by DG Health and Consumer Protection, 17 December 1999 (SCP/PSEUDOM/004).

4. Additional information with regard to the metabolite DDR, submitted by the notifier, 01/02/2000 (SCP/PSEUDOM/005).

5. Questions from the SCP to the notifier, 28 March 2000 (SCP/PSEUDO/007).

6. End-points, submitted by DG Health and Consumer Protection, 8 June 2000 (SCP/PSEUDOM/008).

7. Draft review report 3 Nov. 1999, submitted by DG Health and Consumer Protection, 19 June 2000 (SCP/PSEUDOM/009-rev1).

8. Addendum to the monograph: evaluation of DDR, submitted by the RMS, 24 May 2000 (SCP/PSEUDOM/011).

9. Response to questions raised by the SCP, submitted by the notifier, 30 June 2000 (SCP/PSEUDOM/014).

10. Addendum to the monograph, revised Dec. 2000, submitted by the RMS, 21/12/2000 (SCP/PSEUDOM/016).

11. Appendix I CEDOMON, submitted by the RMS, 21/12/2000 (SCP/PSEUDOM/017).

12. Proposed decision with respect to the application for inclusion in Annex I to Directive 91/414/EEC, submitted by the RMS 21/12/2000 (SCP/PSEUDOM/2000).

13. Analysis of DDR in CEDOMON, submitted by the notifier, 17 April 2001 (SCP/PSEUDOM/019).

14. Response of the notifier to questions raised by the RMS, 15 Sept. 2000, submitted by the notifier, 17 April 2001 (SCP/PSEUDOM/020).

15. Response of the notifier to questions from the SCP, submitted by the notifier 17 April 2001 (SCP/PSEUDOM/021).

16. Draft Assessment Report, prepared by Sweden as rapporteur member state, April 1998 (Volumes 1 to 3).

17. Johnston JE and van der Jagt K, 1999: Assessment of potential exposure to the biological pesticide Cedomon™ Revised edition JSC International Ltd. Sept. 1999 – JSC-98-BA1, submitted by the notifier.

18. Önfelt A, Schultz N, Göstring L, 1999: Effects of Rhizoxin derivative 2,3-deepoxy-2,3-didehydrorhizoxin (DDR) on the mitotic spindle of V79 Chinese hamster cells, submitted by the notifier.

19. Abramsson-Zetterberg L, 2000: The genotoxic effect in mice of orally administered 2,3-deepoxy-2,3-didehydrorhizoxin (DDR) in a culture of *Pseudomonas chlororaphis*. Results from the *in vivo* micronucleus assay (June 2000, revised September 2000), submitted by the notifier.

D. SCIENTIFIC BACKGROUND ON WHICH THE OPINION IS BASED

I 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

The Committee, noting that *Pseudomonas chlororaphis* MA342 applied to seeds does not continue to colonise the emerging plant, concludes, in the absence of sustained colonisation, the number of *P. chlororaphis* associated with the harvested grain as well as the concentration of any metabolites produced would be very low. Therefore the Committee is of the opinion that there is no cause for concern and that the issue of residues has been adequately addressed.

Scientific background on which the opinion is based:

I.1 General statement

Unlike chemicals added to biological systems where any residues detected are the parent compound or its metabolites, microbial inoculants introduce higher levels of organisation and this is reflected in the nature of any retained material. This 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 inoculant may produce metabolites at any stage of its production or *in situ* in association with seeds on plants 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.

I.2 *Pseudomonas chlororaphis*

The Pseudomonas strain MA342, which biochemically and morphologically most closely resembles *P. chlororaphis (*syn. *P. aureofaciens),* is marketed as an oil-based product.

I.2.1 *Survival after germination*

Modifying Pseudomonas strain MA342 to express a marker gene (*gfp* green fluorescent protein) allowed patterns of colonisation of treated barley seeds to be determined pre-and immediately post-germination (Tombolini *et al*., 1999). Immediately after inoculation bacteria were found mainly under the glume (seed bract) and in grooves and cracks in the seed. After sowing and on germination, aggregates of cells continued to be seen in association with glume cells and near, but never within, the embryo.

Two experiments were carried out to study persistence of the strain on the emerging plant. The first with wheat was a field trial in which seeds were dressed with the wild-type *P. chlororaphis* or a rifampicin-resistant mutant. The second experiment, which was of shorter duration, was made with barley using only the rifampicin-resistant mutant. Barley seedlings were sampled for 27 days post-germination.

In barley, the highest concentrations of *P. chlororaphis* were found associated with the first leaf, were also detected on the second leaf, but were below detection limits thereafter. This implied that the added strain did not colonise the emerging plant and that numbers rapidly diminished after germination. This was supported by the results of the field trial in which neither the rifampicin-resistant marked derivative nor the wild-type could be re-isolated from the roots or vegetative parts of wheat at the time of plant ripening, some ten months after the seeds were first sown. The rifampicin marked inoculant strain could, however, be re-isolated from soil from around the roots in relatively low numbers (200 cfu^2 g^{-1}soil).

I.2.2 *Toxicity of the viable organism*

Since the inoculant strain *P. chlororaphis* MA342 does not persist on plants after initial colonisation, grain harvested from plants whose seeds were treated prior to sowing would be very unlikely to contain Pseudomonas in numbers greater than would be expected of any other soil saprophyte.

Even if the developing plants were to be colonised from the inoculated seeds or from the surrounding soil, the dose level would be very small and not a cause for concern given that an acute oral toxicity study with rats given a single dose of $2x10^{10}$ cfu kg^{-1} body weight failed to show evidence of toxicity (monograph Annex B p. 33). This would be equivalent to consuming approximately 2-4 kg treated seed kg^{-1} body weight. Similarly, the growth rate of broiler chicks were unaffected by the presence of either a suspension of the bacteria in water or when provided as the oil-based product (monograph annex B p. 53).

Since this strain, in common with many other soil saprophytes, fails to grow at 37^0C in culture it would be expected to be detectable only at the site of introduction and not to be invasive.

[2] Colony Forming Unit.

This is confirmed by the results from an acute inhalation test with rats (intratracheal instillation of 0.02 ml of complete formulation delivering $5x10^5$ cfu of strain MA342/rat). No viable Pseudomonas were detectable in tissues other than the lungs. In lungs, viable bacteria were detected only on the day of inhalation and not on day 4, when the next observation was made. A similar rate of clearance from the lungs was noted in mice given another strain (described as *P. aureofaciens*) although, in this case, pulmonary exposure resulted in an unexpected mortality (George *et al.*, 1999). The dose administered, however, was substantially higher ($8.3x10^6$ cfu/mouse).

I.2.3 *Pathogenicity*

A strain classified as *P. chlororaphis* has been reported to kill trout, carp and eel when inoculated (Egusa, 1992) and there has been a single report of a *P. chlororaphis* infection of chickens (Shahata *et al.*, 1988). However, such reports are rare and *P. chlororaphis* and closely related species are generally not considered pathogenic for humans or livestock and have never been associated with any clinical condition. Only *P. aeruginosa* is regularly found as an opportunistic pathogen in compromised individuals, but rarely in the healthy subjects.

I.2.4 *Production of metabolites*

Metabolites produced and exported by microbial inoculants selected for biocontrol purposes have an apparent role in the suppression of growth of target organisms, at least *in vitro*. A rhizoxin derivative (2,3-deepoxy-2,3-didehydro-rhizoxin – DDR) produced in small amounts by *P. chlororaphis* MA342 has been shown to be the major, but not the only, contributor to the antifungal properties of the strain. Transposon mutants of MA342 unable to produce this compound reduced their potential as a biocontrol agent. Strain MA342 does not produce detectable amounts of phenazines under the growth conditions employed by the notifier to grow *P. chlororaphis* (SCP/PSEUDO/017 p. 10). In this respect strain MA342 appears to differ from other strains of *P. chlororaphis/aureofaciens* where phenazine-1-carboxamide and related compounds have been shown to be the active agents (Thomashow *et al*., 1990; Chin-A-Woeng *et al*, 2000; Ligon *et al.*, 2000, Seveno *et al*, 2001).

DDR is a macrolide antibiotic which binds to tubulin and is a potent inhibitor of mitotic spindle formation and hence mitosis. The lowest active concentration for the anti-mitotic effects of this compound was 2.5×10^{-11} M in an *in vitro* study (Önfeld *et al.*, 1999).

Detectable amounts of DDR are produced during the exponential growth phase of strain MA342 (from 6.55 to 28 μg/ml for small-scale fermentations and from 4.0 to 60.0 μg/ml for commercial scale fermentations). The formulation can contain up to 12% of the bacterial suspension giving approximately 1-7 μg/ml ($2\text{-}11 \times 10^{-6}$ M) DDR in the final product assuming that no breakdown occurs. However, DDR does degrade during aqueous storage (into unknown products) and on the basis of an experimentally determined half-life and the time taken from fermentation to formulation, DDR in the newly formulated product would be present only in very low quantities. Based on measured DDR levels on freshly treated seeds (SCP/PSEUDOM/014 p. 3), the notifier also envisages further degradation of any residual DDR during storage of the product but experimental data to support this supposition are not available.

Since the formulation itself contains only trace amounts of DDR carried over from the fermentation medium, it has to be assumed that, for the product to be effective, DDR is synthesised *in situ* on the seeds before and immediately after germination when the applied Pseudomonas are metabolically active. However, since the producer strain does not persist on the plant and as DDR is rapidly degraded to unknown compounds (half-life <25 hours under aqueous conditions), and metabolised by soil and other organisms, it is highly unlikely that any DDR produced would be detectable at harvest. Analysis of samples of cereals produced from treated seed confirmed this expectation.

Although the notifier suggests likely points of cleavage in the DDR molecule under acidic or alkaline conditions or in the presence of enzymes, no experimental data are presented to confirm the nature of the breakdown products produced during formulation, storage or use. Although a possible presence of DDR breakdown products of unknown structure cannot be completely excluded, amounts of metabolic residues produced *in situ* are expected to be negligible due to the absence of colonisation on the harvested grain.

The nature of other possible antifungal metabolites produced by MA342 also is not known but, on the basis of data from closely related strains, probably include siderophores (Hohlneicher *et al*., 1995) and, possibly, a variety of other compounds (e.g. Paulitz *et al*., 2000).

I.3 Conclusions

Given that the *P. chlororaphis* and related species are widely distributed soil-inhabiting saprophytes (OECD, 1997), food crops are probably regularly recolonised with low numbers of organisms of this type. There is no indication that the specific strain introduced via dressed seeds adds in any significant way to the natural level of the resident Pseudomonas population.

The concentration of any metabolite residues would be expected to reflect the degree of colonisation with *P. chlororaphis*. Since the harvested grain is not colonised, amounts of metabolic residues produced *in situ* are expected to be negligible. Analysis has confirmed this situation for the specific metabolite DDR.

P. chlororaphis is generally considered non-pathogenic to humans and livestock, and the specific strain MA342 has been shown to be not acutely toxic when given as a single large dose to rats. Consequently, it could be expected that low levels of contamination with the organism (viable or non-culturable) do not pose a hazard.

II. 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?

<u>Opinion of the Committee</u>

The Committee is of the opinion that operator exposure to *Pseudomonas chlororaphis* formulations has been adequately addressed (see also opinions on questions 4 and 5).

Scientific background on which the opinion is based:

The notifier provided an estimate of operator exposure to *Pseudomonas chlororaphis* for the following exposure scenarios: loading the formulated product into the seed-dressing equipment, bagging the treated seed, sewing, stamping and weighing the bags containing the treated seed and cleaning the equipment (Johnston and van der Jagt, 1999).

The Pesticide Handlers Exposure Database (PHED) was used to estimate exposure during loading, assuming an inhalation rate of 29 L/min (moderate activity). Worst case scenarios (maximum seed-dressing capacity and application rate) gave an inhalation exposure of 1.3 (1.3×10^4 cfu), 0.069 (6.9×10^2 cfu) or 0.32 (3.2×10^3 cfu) µg/kg bw for closed-pour-large capacity, closed-pour-small capacity seed treatment facility, and open-pour-grower setting, respectively.

Since no model was available for bagging and handling the bags, exposure was estimated with the assumption that the total dust in the workplace was equal to the maximum permissible level for organic dust (5 mg/m^3). In this worst case scenario, the estimated exposure was 9.6 (9.6×10^4 cfu) µg/kg bw.

Dermal exposure was not quantified or estimated because skin absorption of *Pseudomonas chlororaphis* does not occur, since intact skin represents a significant barrier to bacteria.

Exposure to the metabolite DDR can be extrapolated from that calculated for the bacterium. Maximum DDR concentration in the formulated product is 0.36 µg/mg of *Pseudomonas chlororaphis* (formulation contains minimum 20 mg/ml of *Pseudomonas chlororaphis*, maximum DDR concentration 7.2 µg/ml, therefore 7.2 µg/20 mg= 0.36 µg/mg) then maximum DDR inhalation exposure, as derived from the above reported data, would be 0.000468, 0.00002484, 0.0001152 or 0.003456 µg/kg bw for closed-pour-large capacity, closed-pour-small capacity seed treatment facility, open-pour-grower setting, and bagging and handling the bags respectively (SCP/PSEUDOM/020; SCP/PSEUDOM/021). In view of the very low expected amounts of inhaled DDR (in the nanograms range) and the episodic exposure frequency of the operators, any toxicological adverse effect can hardly be expected from such exposure.

Dermal exposure cannot be estimated with available data. However, due to the fact that the commercial formulations of *P. chlororaphis* contain only limited concentrations of DDR (maximum 7.2 µg/ml) and skin penetration rate of large molecules is usually rather low, and given that skin protection with gloves would be recommended for handling any biological plant protection products, no toxicologically significant DDR absorption is expected to occur through the operator skin.

It is therefore concluded that operator exposure to *Pseudomonas chlororaphis* has been adequately addressed and does not raise safety concerns.

III. Question 3

With regard to possible hazard to humans, is a tiered approach adequate and should repeated dosing be part of the primary (tier 1) 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 based on the biological properties of the micro-organism and the results of acute toxicity and pathogenicity studies can be offered. In the specific case of *Pseudomonas chlororaphis*, and in the light of the results of the available studies, the SCP is of the opinion that repeated dosing is not necessary to assess hazard to humans.

Scientific background on which the opinion is based:

III.1 General considerations

In general, it is advisable to perform repeated dose studies in order to get a proper insight into the ability of micro-organisms to cause adverse effect in mammals. However, repeated dosing may not be necessary when the biological properties of the agent and the results from acute toxicity are clear enough to conclude on the non-toxicity of the agent. If on the basis of acute toxicity data or other reasons, doubt remains, 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 the absence of infectivity.

III.2 *Pseudomonas chlororaphis*

The results of the acute toxicity studies are clear enough to conclude that *P. chlororaphis* does not induce toxicity or pathogenicity. Moreover, *P. chlororaphis* is not able to grow at temperatures higher than 33.5°C and will therefore not be invasive in mammals (see Scientific background of question 1). Therefore repeated dosing is not considered to be necessary.

III.3 Conclusions

Based on results of the acute toxicity studies, it is concluded that in the case of *P. chlororaphis*, repeated dosing is not necessary to evaluate hazard to humans.

IV. Question 4

Is the toxicological safety of the antibiotic metabolites of *Pseudomonas chlororaphis* adequately addressed?

Opinion of the Committee:

The Committee noted that the toxicological information so far available on the putative antibiotic metabolites of *Pseudomonas chlororaphis* is limited, because:

- **no toxicokinetic data on DDR are available (absorption, biotransformation, distribution),**
- **only two studies (one *in vitro*, one *in vivo* in mice by gavage) on the genotoxicity of the metabolite DDR have been carried out, and showed aneuploidy,**
- **no experiments have been made to evaluate the mutagenic potential of DDR.**

Although the SCP concludes that more studies would be needed for a more complete assessment of the mutagenicity potential of DDR, the potential for human exposure to DDR as well as to other possible antibiotic metabolites is so low that, even in the absence of further information, the Committee is of the opinion that no major concern exists for consumer and operator safety.

Scientific background on which the opinion is based.

For the production of metabolites, see I.2.4.

IV.1 *In vitro* study

Few data are available concerning the toxicity of DDR. The genotoxicity of this macrolide has been tested *in vitro* on V79 Chinese Hamster cells (Önfelt *et al*, 1999). V79 Chinese Hamsters cells were grown in the presence of increasing amounts of DDR dissolved in acetone because DDR has limited solubility in complete medium (nominal concentrations 2.5 $x10^{-9}$ to 2.5 x 10^{-5}M) for 30 minutes. Chromosomal arrangements in mitotic cells were examined after staining of chromosomes with Giemsa, and depolymerisation of spindle microtubules was verified by immune fluorescent staining. DDR causes inhibition of the mitotic spindle and abnormal chromosomal arrangements at low concentrations. The lowest nominal concentration showing a significant increase of c-mitotic cells compared to controls was found to be $2.5x10^{-11}$M. DDR is an aneuploidy inducing agent. In this experimental system, DDR was found to be 100-fold more active than colcemid. DDR caused a decrease in cell survival at > 10^{-8}M.

IV.2 *In vivo* study

Male mice of the strain NMRI were given the bacterial suspension with different concentrations of DDR dissolved in ethanol, by oral gavage (0.2, 2.05 and 18.6 mg/kg bw - Abramsson-Zetterberg L, 2000). Negative controls received the same volume of fresh tryptone-soya broth with 20% ethanol. A group of mice (positive control) was administered by oral gavage the same volume of colchicine dissolved in PBS (13 mg/kg bw). Thirty-eight and 62 h after the start peripheral blood was drawn. The highest concentration of DDR used (18.6 mg/kg bw) significantly increased the frequency of micronucleated polychromatic erythrocytes (fMPCE). This increase was transient (only seen 38h after treatment). A statistically non-significant trend to increase was also observed with lower dose of DDR (2.05 mg/kg bw). In this experiment, DDR seemed to be less toxic than colchicine, but the experiments are not comparable. In fact, DDR is given in the presence of bacteria whereas colchicine is given alone in buffer solution.

IV.3 Conclusions

DDR is the only *Pseudomonas chlororaphis* metabolite tested as it appears to be the key metabolite involved in biocontrol of fungal phytopathogen. In experiment, DDR induced aneuploidy. In an *in vivo* test on mice by gavage, a NOEL for a transient micronucleated polychromatic erythrocyte increase was observed to be at 0.2 mg/kg bw.

The SCP concludes that more studies would be needed for a more complete assessment of the mutagenicity potential of DDR. However, the potential for human exposure to

DDR as well as to other possible antibiotic metabolites is so low[3] that, even in the absence of further information, the Committee is of the opinion that no major concern exists for consumer and operator safety.

V. Question 5

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:

Although the probability of occurrence of allergic reactions on agricultural exposure to *Pseudomonas chlororaphis* is likely to be very low, the possibility of their occurrence cannot be totally excluded. Confirmation of lack of allergenic potential of this micro-organism can only be obtained by a direct systematic observation on a significant number of exposed operators. Therefore the SCP is of the opinion that a study based on workers' medical surveillance should be conveniently carried out when introducing this agent in the field as a microbial pesticide.

Scientific background on which the opinion is based.

For generalities on allergy and allergic response to microbial aerosols the reader is referred to the opinion of the Scientific Committee on Plants on *Paecilomyces fumoroseus* adopted on 30 November 2000[4] (sections 4.4.1 and 4.4.2).

V.1 Allergy to bacteria

The nature and extent of allergies induced by Gram positive and Gram negative bacteria are difficult to define.

Results obtained in a study where subjects were submitted to intradermal tests with five different peptidoglycan preparations (PG) from *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Steptococcus pyogenes* showed that the subjects with dual and late reactions to staphylococcal strains PGs displayed significantly higher PG antibody titers than the subjects with negative reactions (von Mayenburg *et al.*, 1982).

Some studies suggest a potential role of bacterial respiratory tract infections in the development of bronchospasm (asthma) and progression of chronic obstructive pulmonary disease (COPD) (Cazzola *et al.*, 1991**)**.

Bronchial obstruction after inhalation of *Haemophylus influenzae* has been reported. An exhaustive search of the major databases identified only one publication relating to *Pseudomonas aeruginosa* allergy in cystic fibrosis (Skov *et al*, 1980). No data are reported about *P. aeruginosa* allergy in healthy subjects.

[3] See opinions on questions 1 and 2 in this report.
[4] http://europa.eu.int/comm/food/fs/sc/scp/out80_ppp_en.html

V.2 Allergenic potential of *Pseudomonas chlororaphis*

No cases of allergic reactions have been observed amongst the limited number of operators monitored for adverse effects.

The allergenic potential of exposure to *P. chlororaphis* 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. As with any other inhalable biological material, 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.

V.3 Conclusions

The possibility of occurrence of allergic reactions to *P. chlororaphis* 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, the causative agent should be determined and the competent authority of the relevant Member State notified. The results of the monitoring should be made available for future re-assessment.

VI. Question 6

The genus Pseudomonas also contains important pathogens for human e.g. *Pseudomonas aeruginosa* which can establish in open wounds. There is one documented case where *Pseudomonas chlororaphis* was found in the wound of a soldier. Does this finding give rise to any concerns for human safety?

<u>Opinion of the Committee:</u>

The Committee is of the opinion that there is no cause for concern for human safety with regard to wound infection.

<u>Scientific background on which the opinion is based:</u>

No information was found from searches made in several databases on the alleged case of an open wound infection by *P. chlororaphis* in humans. In the absence of clinical details on such a quoted case, its importance in the context of the requested opinion is negligible. In addition, the fact that no replication of *P. chlororaphis* occurs at temperature >33.5 °C makes the invasion of an open wound by *P. chlororaphis* highly unlikely.

Among Pseudomonas, only *P. aeruginosa* is regularly found as an opportunistic pathogen in compromised individuals, and rarely in healthy subjects. Furthermore, *P. chlororaphis* belongs to a DNA homology group different from *P. aeruginosa* and, since there are no plasmids in the bacteria in the technical product, exchange of DNA is highly unlikely.

In conclusion, the Committee is of the opinion that there is no cause for concern for human safety with regard to wound infection.

E. REFERENCES

1. Cazzola M, Matera M.G and Rossi F.: Bronchial hyperresponsiveness and bacterial respiratory infections, in Clinical Therapeutics 13 (1): 157-171, 1991 Jan-Feb.

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Toxicology Working Group: Prof. Maroni (Chairman) and Committee Members: Dr. Delcour-Firquet, Prof. Leszkowicz, Dr. Meyer, Dr Moretto, Prof. O'Gara, Prof. Petzinger, Prof. Savolainen, Prof. Silva Fernandes, Dr. Speijers, and invited experts Dr. Chesson, Prof. Galli, Dr. Gismondo, Dr. McGregor.

Micro-organism plant protection products Working Group: 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.