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SCIENTIFIC OPINION

Mortality verification of pinewood nematode from high temperature treatment of shavings[1]

Scientific Opinion of the Panel on Plant Health

(Question No EFSA-Q-2009-00447)

Adopted on 16 April 2009

PANEL MEMBERS

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SUMMARY

The European Commission requested the European Food Safety Authority (EFSA) to provide a scientific opinion on the report "Mortality verification of pinewood nematode from high temperature treatment of shavings" provided by the United States Department of Agriculture (USDA).

The pinewood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle, is listed in Community plant health legislation and protective measures include a requirement that wood shavings of coniferous wood (except that of *Thuja* L.) originating in the United States (US) must be heat treated to achieve a minimum core temperature of 56 °C for at least 30 minutes.

The USDA, through the United Kingdom (UK) Authorities, requested the recognition of an alternative treatment by the European Community. The request was supported by a report of an experiment entitled "Mortality verification of pinewood nematode from high temperature treatment of shavings" and additional explanatory information. This report (and additional explanatory information) presents a treatment process whereby wood shavings are exposed to high temperatures (398 °C) for a short time (3 minutes). The report was submitted to EFSA's Scientific Panel on Plant Health for evaluation with regard to the reliability and effectiveness of the proposed treatment method, as an alternative to the current heat treatment requirement. A scientific opinion was requested to assist risk managers in considering the technical justification for derogation from the current treatment requirement for wood shavings of

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coniferous wood listed in Annex IV, Part A, Section 1, point 1.2 of the Council Directive 2000/29/EC[2].

The Panel examined the report and supporting information in detail and based its conclusions on the following:

- An analysis of the cited references, and the extent to which they support the statements made in the introduction and discussion sections of the USDA report;
- Evaluation of the materials, methods and results presented in the report and
- A statistical analysis performed by the Panel to test the hypothesis of a mortality estimate after the heat treatment lower than 100%.

No comparison is made of the effectiveness and reliability of the proposed treatment method in relation to the current treatment requirement.

The scope of this opinion is restricted to one species of *Pinus* "white pine" *(Pinus strobus)* as no wider species testing of other coniferous wood has been undertaken. Although the Panel is requested to consider the proposed treatment as an alternative to the current requirement for coniferous wood shavings, this extrapolation of the findings of the report cannot be made since no species other than white pine have been tested.

According to the Panel, the references cited do not provide adequate evidence to support the effectiveness of the high temperature treatment proposed, are in some cases incorrectly interpreted and do not sufficiently validate the premise behind the treatment as stated in the USDA report. In particular, the relationship between lethal temperature and duration of exposure was not accurately established and no conclusion can, therefore, be derived about the appropriateness of the proposed temperature/time combination.

The Panel considers that the accuracy of the results is difficult to assess due to missing data in the report for the control samples. The report claims that the treatment results in 100% mortality, but a statistical analysis is not included in the report to specify the statistical level of confidence supporting these results. The Panel performed a statistical analysis of the data provided in the USDA report and cannot exclude that the mortality estimate is lower than 99.85%.

Uncertainties relating to the experimental design are identified by the Panel. These include:

- The effect of the high temperature treatment alone, independent of the shredding process cannot be derived from this experiment.
- After the shredding process, shavings are expelled onto a mesh conveyer, which allows dust and particles to fall before the material enters the dryer. The possibility of *B. xylophilus* contamination from untreated waste material is not discussed.
- Only one temperature-time combination has been tested. Therefore the safety margins and reliability of routine operational application of the proposed high temperature treatment cannot be derived from this experiment. This is noted as particularly important in relation to the short exposure time of 3 minutes proposed.

[2] Council Directive 2000/20/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ, L.169, 10.7.2000, p. 1-112.

- A temperature of 115 °C is recorded at the end of the high temperature treatment. Thus, a continuous exposure to 398 °C for 3 minutes cannot be confirmed from the experiment described.

The Panel considers that several elements of the experiment were also not adequately described:

- The temperature of the wood shavings in the experiment is unknown. Further information on temperature measurement and placement of thermal sensors would enable more accurate evaluation of the temperature exposure over time.
- A number of factors that may influence the results of the experiment are unknown. These include: the volume of the wood shavings relative to the air volume inside the dryer, the homogeneity of the distribution of the shavings in the dryer and wood moisture levels at the start of the treatment.
- No information is provided to confirm the identity and the frequency of occurrence of the most resistant life stages of *B. xylophilus* in the treated samples. The storage conditions of the control samples are unknown, which may affect the natural mortality over time.

The Panel agrees that there is potential for development of an alternative treatment protocol based on exposure of wood shavings to high temperatures for a short duration. The Panel however, does not consider that the evidence presented in this report demonstrates the effectiveness and reliability of the proposed temperature/time regime as an alternative to the heat treatment requirement for coniferous wood shavings set out in Annex IV Part A Section I Point 1.2. of Council Directive 2000/29/EC.

Key words: ***Bursaphelenchus xylophilus*, pinewood nematode, heat treatment, wood shavings, *Pinus* sp.**

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION[3]

The current Community plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ, L.169, 10.7.2000, p. 1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Community or to be moved within the Community, the list of harmful organisms whose introduction into or spread within the EU is prohibited and the control measures to be carried out at the outer border of the Community on arrival of plants and plant products.

In addition, the Directive provides that derogations may be granted with regard to requirements referred to in Annex IV, Part A, Section I. In such cases, the derogation has to set out specific alternative requirements to prohibit the introduction and spread of harmful organisms. The phytosanitary risk must be assessed on the basis of available scientific and technical information; where this is not possible it shall be supplemented by additional enquiries or investigations.

In accordance to Annex IV, Part A, Section I point 1.2 of the Council Directive 2000/29/EC wood of conifers except that of *Thuja* L. in the form of shavings of US origin, needs to be accompanied by official statement that it has been subjected to heat treatment and a minimum core temperature of 56 °C for at least 30 minutes (56/30) has been achieved.

Pleading technical difficulties for the practical application of the 56/30 treatment to wood shavings, the USDA, through the UK Authorities requested the recognition of an alternative treatment. This consists of exposing wood shavings to high temperatures (398 °C) for a shorter time (3 min) in a continuous process (398/3). The request was supported by the description of a research experiment as well as by additional explanatory information.

The Committee discussed the information and concluded that the technical justification provided, could be a basis for a draft Decision providing derogation under an alternative treatment and certification scheme. However, a scientific evaluation is first needed to confirm whether or not such derogation could be technically justified. Therefore, the Commission, before considering further steps in this matter that could lead to the preparation of a draft Decision, decided to seek a scientific opinion from EFSA.

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion on the report ”Mortality verification of Pinewood Nematode from high temperature treatment of shavings“ provided by the USDA, with regard as to whether the proposed treatment of wood shavings of *Pinus* sp. is effective and reliable to safeguard the mortality of pinewood nematode and could be an acceptable alternative for the heat treatment requirement for coniferous wood shavings set out in Annex IV Part A Section I Point 1.2 of Council Directive 2000/29/EC.

[3] Submitted by European Commission, ref. SANCO E1/DF/svi D(2009) 510098

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002[4], to provide a scientific opinion on the report "Mortality verification of Pinewood Nematode from high temperature treatment of shavings" provided by the USDA, with regard as to whether the proposed treatment of wood shavings of *Pinus* sp. is effective and reliable to safeguard the mortality of pinewood nematode and could be an acceptable alternative for the heat treatment requirement for coniferous wood shavings set out in Annex IV Part A Section I Point 1.2 of Council Directive 2000/29/EC.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion, David Makowski, Jan Schans and Kari Tiilikkala from the Plant Health Panel and Christer Magnusson, as invited expert. It would also like to thank Olaf Mosbach-Schulz from the EFSA Assessment Methodology Unit.

[4] Regulation (EC) N°178/2002 of the European Parliament and the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ, L 31, 1.2.2002, p. 1-24.

ASSESSMENT

1. Introduction

This document presents the opinion of the Panel on Plant Health on a report provided by the USDA, of an experiment to verify the mortality of pinewood nematode from a high temperature treatment of wood shavings. The treatment method is proposed as an alternative to the current heat treatment requirement for wood shavings.

The scope of this opinion is restricted to one species of *Pinus, P. strobus* (white Pine) as no wider species testing of other coniferous wood has been undertaken. The Panel notes that the species of wood to be considered is referred to differently in the Terms of Reference of the request ("coniferous wood"), the report from USDA ("white pine") and the derogation request ("*Pinus* spp.").

1.1. Current heat treatment requirement against the pinewood nematode

Bursaphelenchus xylophilus (Steiner and Buhrer) Nickle, the pinewood nematode is listed as a harmful organism in Community plant health legislation and protective measures are taken to prevent the introduction and further spread within the European Community.

The current heat treatment requirement for coniferous wood shavings is set out in Annex IV Part A Section I Point 1.2 of Council Directive 2000/29/EC. Wood of conifers except that of *Thuja* L. in the form of shavings originating in the US, needs to be subjected to heat treatment to achieve a minimum core temperature of 56 °C for at least 30 minutes.

A minimum core temperature of 56 °C for at least 30 minutes is endorsed as one of the approved measures in the International Standards on Phytosanitary Measures, ISPM No. 15 (FAO 2007): Guidelines for regulating wood packaging material in international trade, after consideration of the effectiveness for a range of pests, including *B. xylophilus*.

1.2. Documents submitted for evaluation

The USDA report provided is entitled "Mortality verification of pinewood nematode from high temperature treatment of shavings" and describes an experiment carried out to verify nematode mortality after treatment of wood shavings at a high temperature (398 °C), for a short period of time (3 minutes). The report comprises four pages and cites four references. Two additional documents are provided; a certificate of laboratory analysis describing the pinewood nematode (*Bursaphelenchus xylophilus*) extraction procedure and a letter in which USDA responds to earlier questions raised by the European Commission.

1.3. Evaluation procedure

The Panel examined the report and supporting references in detail and bases its conclusions on:

- Evaluation of whether the cited references support the statements made in the introduction and discussion sections of the USDA report;
- An analysis of the materials, methods and results presented in the report and
- A statistical analysis performed by the Panel to test the hypothesis that the mortality rate after the heat treatment is lower than 100%.

2. Analysis of the USDA documents

2.1. General comments

Wood shavings for the purpose of the study are defined as "a wood cutting no more than 5 cm in two dimensions and less than 3 mm in thickness". The document describes a single experiment whereby sixteen pinewood nematode-infested logs (9.505 kg) are shredded into shavings of less than 3 mm thickness and subjected to a high temperature treatment. No explanation is provided to indicate whether the treatment method described is to be used for the routine production and treatment of wood shavings intended for export to the EU, or whether this represents a small-scale simulation to test the effectiveness of the high temperature treatment proposed, prior to the development of a larger-scale operation.

Four references are cited to support the technical justification for the proposed treatment. However, the four references cited are not always correctly interpreted in the introduction and discussion sections of the USDA report. In addition, the document did not include a statistical analysis of the results.

2.2. Specific comments

2.2.1. Introduction

In the introduction section, the USDA report states that the premise behind the treatment is validated by the studies of Johnson and Koch (1972), Sokhansanj and Wood (1991). The Panel agrees that the study by Johnson and Koch (1972) highlights the fact that the shavings are being treated at the limits of the product damage threshold, at which the product exhibits an exothermal reaction, i.e. charring. The study is cited to show that an exothermic reaction occurs above 380 °C (for earlywood) and 382 °C (for latewood) and thus the reasoning to support the proposal for a treatment temperature (398 °C) above the likely damage threshold is not presented. The effect of the treatment on product quality is not within the scope of the Panel evaluation.

The study by Sokhansanj and Wood (1991) dealt with the use of high temperatures and short exposure times with regard to a quarantine treatment for forage bales (alfalfa). They report on previous studies that 100% mortality of wheat midge (*Sitodiplosis mosellana*) and Hessian fly was achieved in 37 minutes at 47.5 °C, 7 minutes at 52.5 °C, and 3 minutes at 55 °C and above. However these previous experiments are not described in the paper by Sokhansanj and Wood (1991) and therefore, the mortality of the treatment using a 3 minute exposure time cannot be verified from this paper. In addition, the effectiveness of the heat treatment for wood shavings cannot be directly derived from the treatment of forage bales.

The information found in Rotili *et al*. (2001) is cited to highlight the similarity in thickness of stem portions of alfalfa forage (2.87 mm) and the wood shavings (less than 3 mm) being tested. However, this reference appears to have little relevance to the Sokhansanj paper nor the report under evaluation.

The Panel, therefore, does not agree that the papers Sokhansanj and Wood (1991) and Rotili *et al*. (2001) validate the premise behind the proposed treatment.

The study by Fleming *et al*. (2005) reports on findings from tests of microwave equipment (2.45 GHz) against pinewood nematode in red pine logs. This reference is cited to demonstrate 100% mortality of pinewood nematodes and vectoring cerambycid at 62 °C with only 2.31 minutes of exposure time. However, the Panel notes that live nematodes were found

in 2 out of 29 samples treated at 62 °C with a batch microwave system. This study thus demonstrated that nematodes can survive at 62 °C. Although no live nematodes were recorded in samples where temperatures were greater than 62 °C, the temperature range values are not given to determine the precise temperature at which 100% mortality is achieved.

In a second experiment, Fleming *et al.* (2005) assessed the effectiveness of a continuous microwave system and did not find live nematodes and vectoring cerambycid larvae in pine wood boards treated to 46 °C and 53 °C respectively, but the number of live nematodes found in the control samples was low (between 2 and 12) in this experiment. Fleming *et al.* (2005) established a function relating mortality and temperature, but its quality of fit was low (r^2=0.24). The results presented by Fleming *et al.* (2005) are thus uncertain.

The Panel thus considers that no conclusion can be derived from the literature cited about the appropriateness of the proposed temperature/duration combination.

2.2.2. Materials and methods

2.2.2.1. Experimental design

The characteristics of the experimental design are described in Figure 1.

The Panel notes that the report does not explicitly present the hypothesis tested by the experiment. It is not explained whether the experiment was set up to test the effectiveness of the heat treatment alone or to test the combination of the heat treatment and of the shredding process.

The Panel considers that several elements of the experiment were not adequately described:

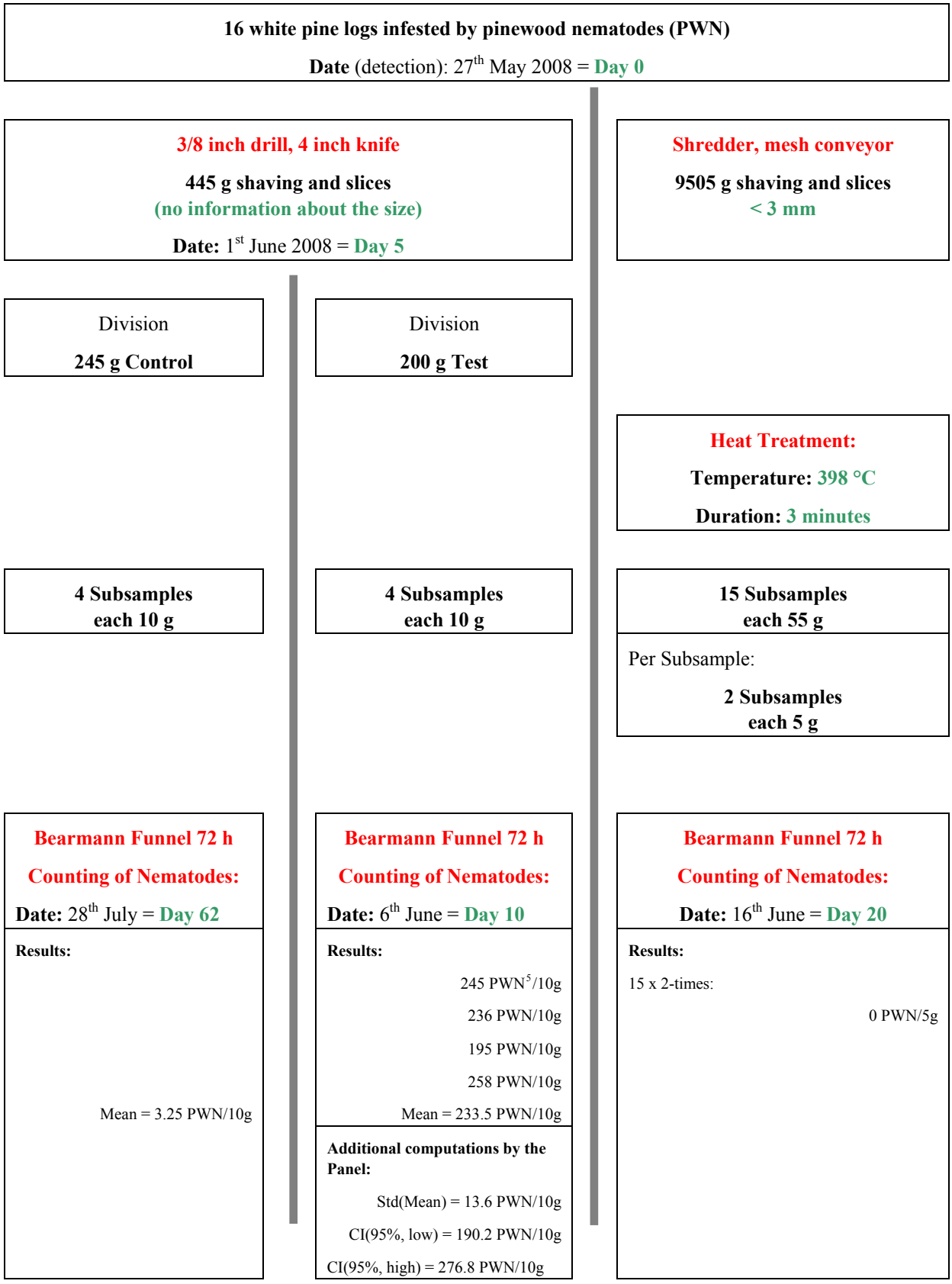
- No information about the method of temperature measurement and placement of thermal sensors is provided.
- No information on the wood moisture levels at the start of the experiment is provided although this factor is likely to affect wood temperature (Fleming *et al.*, 2005).
- No information is provided about the homogeneity of the air temperature within the dryer. In particular it is noted that the shavings enter the airflow which brings the temperature of the shavings and the ambient air to 398 °C. After proceeding through the dryer for a total of three passes, at the end of the third pass (i.e. after 3 minutes) the material is noted to have cooled to 115 °C. Thus the experiment describes a process which does not assure a continuous exposure to temperatures of 398 °C for the full three minute duration of the treatment.
- The temperature of the wood shavings is not confirmed.
- The thickness of the shavings is stated to be lower than 3 mm, but the range of variation of the shaving thickness is not presented in the report.
- No information is given about the frequency of the different life-stages of nematodes present, in particular the third dispersal juvenile stage (J_{III}), which is considered to be the most resistant to adverse conditions including desiccation and extremes of temperature (Panesar *et al.*, 1994).
- The limitations of the extraction method are not considered.

- No information is provided to confirm the identification of *B. xylophilus* and its differentiation from *B. mucronatus, B. kolymensis* and *B.fraudulentus,* and other closely related species ((EPPO, 2007); Braasch, 2008; Gu *et al* 2008).

The Panel considers that the experimental design presented in the report has several shortcomings:

- Two different procedures were used to produce shavings for the control and treated samples, and thus the effect of the heat treatment cannot be distinguished from a possible effect of the shredder in this experiment.
- The counting of nematodes was not carried out on the same day for the control and the treated material, so a direct comparison is not possible due to changes in the number of nematodes over time.
- The heat treatment was only done for only one combination of temperature and duration, which gives no information about the influence and interaction of these.

Figure 1. Experimental design



2.2.2.2. Nematode Examination

The Baermann funnel is a standard nematode extraction method relying on the active migration of living nematodes after suspension of the sample in water. The nematodes then settle to the bottom of the funnel, from which they are collected (Wingfield *et al.*, 1982). It is noted that failure to recover nematodes by this method does not guarantee freedom from infestation (Evans *et al.*, 1996).

Many modifications of this technique have been proposed to overcome limitations of the technique such as poor oxygenation (Hooper, 1986). The report does not indicate that these modifications have been implemented in order to limit the risk of underestimating the number of nematodes present and no information is provided on the incubation period and temperature at which samples were maintained. The effects of incubation temperature and time on life stages of *B. xylophilus* were investigated by Tomminen *et al.* (1991) and differences in the total number of nematodes and occurrence at each life stage were reported at different temperatures in the range between 3 °C and 40 °C. Confirmation of identity of *B. xylophilus* and the frequency of occurrence of the third dispersal juvenile stage (J_{III}) is not provided and the control and treated samples were not examined on the same day, which does not allow a direct comparison to be made.

2.2.3. Results

Test sample (measurements performed on June 6): The number of nematodes found alive in each sub-sample was provided by the USDA. The mean number of nematodes is 233.5 nematodes for 10 g. Although not computed by the author, it is possible to estimate the standard deviation and confidence intervals for the number of nematodes in the test sample. This analysis has been undertaken by the Panel (see section 3 of the opinion).

Control sample (measurements performed on July 28): Only the mean number of nematodes was provided by the author, not the values found in the sub-samples. It is thus impossible to estimate the standard deviation and confidence intervals for the control sample based on the USDA report. The mean nematode number is lower in the control sample (3.25 in 10 g) than in the test sample on June 6. This result shows that the number of nematodes decreased over time.

Treated sample (measurements performed on June 16): no nematode was found alive in the subsamples, but this result does not necessarily show that the mortality rate is 100%. No statistical analysis was performed by the author in order to test the hypothesis of a mortality level after the heat treatment lower than 100%.

2.2.4. Discussion

The discussion states that the 100% mortality rate gives physical evidence that a high temperature treatment, coupled with a minimal thickness of the material is an effective form of treatment. The Panel agrees that the results from this experiment suggest that a high temperature treatment can achieve high mortality, and the minimal thickness of the test material allows for the development of an effective treatment protocol. However, the Panel does not agree that the results presented verify the mortality rate stated, as no statistical analysis was performed.

The report states that this is further verified by the control results which indicate that over 3,000 pinewood nematodes and more than a million other nematodes would have been present

without successful treatment. However no confidence interval was presented and no reason is given to explain the presence of the high number of non-pinewood nematodes.

It is stated that the material exits the treatment at almost twice the proven mortality temperature, but the mortality temperature in relation to exposure time is not confirmed in the report, or by the references cited. The sensitivity of the treatment effect with regard to wood characteristics, storage conditions, conveyor characteristics, level of wood infestation, nematode stages (J_{III}-frequency) and variability of treatment duration and achieved temperature is not discussed. In routine operational application of the proposed treatment variations in these factors are likely to occur. In order to assess the safety margins of the treatment, it would be necessary to know the minimum time required to achieve 100% mortality at 398 °C and the minimum temperature required to achieve 100% mortality in 3 minutes exposure time. The effectiveness of routine application of the tested treatment would be further limited by the processing rate (kg wood shavings/minute, or kg wood shavings per treatment cycle) relative to the air volume inside the dryer and on the homogeneity of their distribution in the dryer, since clustering of shavings may not result in adequate exposure to the required temperature. The report describes only a single experiment using 9.505 kg shavings in one treatment cycle for this particular equipment. The discussion provides no information on the factors that would determine the maximum capacity and reliability of the equipment to be used in its routine operational application.

Reasoning to support the validity of the procedure for other species of *Pinus* is not discussed.

3. Statistical analysis

3.1. Model

To test the hypothesis that the mortality of nematodes after the treatment is lower than 100% a statistical analysis was performed by the Panel. This analysis was based on a model calculating the number of nematodes in function of time and heat treatment effect. As the number of live nematodes measured on June 6 was higher than that measured on July 28, it was assumed that the number of live nematodes decreases over time.

The model includes two components: i) a multiplicative function relating the expected number of live nematodes to time and heat treatment, and ii) a Poisson distribution describing the measurement distribution for a given value of expected number of live nematodes. This type of model is commonly used for analysing count data (McCullagh and Nelder, 1983). The first component of the model is defined by

$$E(N_t) = E(N_{10}) \cdot \exp(-\lambda \cdot (t-10)) \cdot (1-h) \quad (1)$$

with $E(N_{10})$= Expected number of live nematodes per 10 g at day 10 (June 6)

$-\lambda$ = logarithmic survival rate over time

s = $\exp(-\lambda)$ survival rate over time

h = Mortality rate of the heat treatment

$E(N_t)$ = Expected number of live nematodes per 10 g at day *t*.

The second model component is expressed as

$$y_t \sim Poisson[E(N_t)] \quad (2)$$

with y_t the number of live nematodes observed in a subsample.

E(N_{10}), λ, and s were estimated from the number of live nematodes reported by USDA at day 10 and 62 (June 6 and July 28 respectively). Equation (1) was used to compute the expected number of live nematodes at day 20 (June 16) i.e. at the date of measurement of the live nematode number after heat treatment. Equation (2) was then used to calculate the probability of measuring zero nematodes alive in 30 5g samples when the mortality rate after the heat treatment is lower than 100%.

3.2. Details of the numerical application

Estimation of N_{10}

The value of E(N_{10}) was estimated from the measurements obtained in the four 10g subsamples of the test sample: 233.5 live nematodes for 10 g (standard error=13.6). Assuming an asymptotic Normal-distribution [with t-quantile (n=3, α=97.5%) = 3.18)], the 95 % confidence interval is defined by [190.2, 276.8] live nematodes for 10 g. Under the assumption of a Poisson distribution, the 95%-confidence interval is defined by [218.84, 248.8] live nematodes for 10 g. The construction of the confidence interval is rough due to the small number of observations (4). It is not possible to choose between the Normal and Poisson distributions from such a small number. Only the first is considered further.

Estimation of the survival rate over time (λ and s)

This parameter was calculated from the estimated value of E(N10) (233.5 for 10 g) and from the mean number of live nematodes observed on day 62 (July 28) (3.25 nematodes for 10 g). The value of λ was estimated from Equation (1) as

$$\lambda = \frac{\log\left[\hat{E}(N_{10})\right] - \log\left[\hat{E}(N_{62})\right]}{62-10} = 0.082$$

From this value, the survival rate *s* over time is estimated as 92.1% after 1 day.

The confidence interval found for the estimated value of E(N_{10}) can be used to derive an approximated confidence interval for the survival rate; confidence interval at 95% = [91.8%; 92.5%]. However, this confidence interval does not take into account the uncertainty about $E(N_{62})$. Here it is not possible to take this uncertainty into account because only the mean value of the four measurements was provided in the USDA report.

Estimation of the expected number of live nematodes on June 16

The estimated value of E(N10) and of s were used to estimate E(N20) (i.e the expected number of nematodes on June 16) from Equation 1. The estimated value is equal to 102.6 nematodes for 10g with a 95%-confidence interval of [99.3; 106.8]. The estimated value for 5g is 51.3 with a confidence interval of [49.7; 53.4].

Probability of a mortality rate lower than 100%

Finally, the probability of zero nematodes observed alive on June 16 in 30 treated samples of 5g each was expressed in function of $\hat{E}(N_{20})$ and of the mortality rate *h* under the assumption of a Poisson distribution (Equation 2) as

$\alpha = \left\{Poisson\left[0\middle|\hat{E}(N_{20})*(1-h)\right]\right\}^{30} = \left\{\exp\left[-\hat{E}(N_{20})*(1-h)\right]\right\}^{30}$. The values of the mortality rate h leading to a probability α equal to 0.01, 0.05, 0.1 were computed as $h = 1 + \frac{\log(\alpha)}{\hat{E}(N_{20})*30}$. The results are h=99.7% for α=0.01, h=99.81% for α=0.05, h=99.85% for α=0.1 and $\hat{E}(N_{20})$=51.3 for 5 g. The numerical results are only marginally changed if $\hat{E}(N_{20})$ is set equal to the lower bound of the confidence interval.

3.3. Conclusion of the statistical analysis

The results show that:

- when the mortality rate is 99.85 %, there is 10 % chance to observe zero nematodes alive in 30 samples,
- when the mortality rate is 99.81 %, there is 5 % chance to observe zero nematodes alive in 30 samples,
- when the mortality rate is 99.7 %, there is 1 % chance to observe zero nematodes alive in 30 samples.

Thus, based on the assumption of a constant rate of decline in the nematode population without treatment, the Panel cannot exclude the possibility that the mortality is lower than 99.85 % and that on average in every 65 g of the original material with the given treatment one nematode may still be alive. The uncertainty about the effectiveness of the heat treatment could be reduced by using a higher number of samples or by using or by using samples with a higher density of *B. xylophilus*.

4. Uncertainties

The Panel identified several sources of uncertainties relating i) to the experimental design and methods, ii) to the results, and iii) to the reliability of the treatment, with regards to its operational application:

- After the shredding process, the shavings are expelled onto a mesh conveyer, which allows dust and particles to fall before the material enters the dryer. The possibility of *B. xylophilus* contamination from untreated waste material is not discussed.
- The temperature of the wood shavings in the experiment is unknown. The time necessary for the wood shavings to acquire the maximum temperature (398 °C) will depend on the volume of wood shavings relative to the air volume inside the dryer and on the homogeneity of the distribution of shavings in the dryer. The experiment does not provide information on these variables.
- The homogeneity of the distribution of wood shavings during the heat treatment in unknown, influencing the consistent exposure of all material to the required temperature-time regime.
- The accuracy of the results is difficult to assess due to missing data for the control samples and the effect of the heat treatment alone cannot be determined from the experiment described.

- Only one temperature and time combination has been tested. Therefore the safety margins and reliability of routine operational application of the proposed treatment cannot be derived from this experiment.
- The effects of several factors which may influence the effectiveness of the treatment are unknown:
    - shaving characteristics (moisture level, range of thickness, *Pinus* species),
    - homogeneity of the air temperature within the conveyor,
    - the frequency of the most resistant stages of the nematodes in the wood,
    - storage conditions of samples which influence the natural mortality and reduction in number of nematodes over time.

CONCLUSIONS AND RECOMMENDATIONS

The scope of this opinion is restricted to one species of *Pinus* "white pine" *(Pinus strobus)* as no wider species testing of other coniferous wood has been undertaken. Although the Panel is requested to consider the proposed treatment as an alternative to the current requirement for coniferous wood shavings, this extrapolation of the findings of the report cannot be made since no species other than white pine have been tested.

According to the Panel, the references cited do not provide adequate evidence to support the effectiveness of the high temperature treatment proposed, are in some cases incorrectly interpreted and do not sufficiently validate the premise behind the treatment as stated in the USDA report. In particular, the relationship between lethal temperature and duration of exposure was not accurately established and no conclusion can, therefore, be derived about the appropriateness of the proposed temperature/time combination.

The Panel considers that the accuracy of the results is difficult to assess due to missing data in the report for the control samples. The report claims that the treatment results in 100% mortality, but a statistical analysis is not included in the report to specify the statistical level of confidence supporting these results. The Panel performed a statistical analysis of the data provided in the USDA report and cannot exclude that the mortality estimate is lower than 99.85%.

Uncertainties relating to the experimental design are identified by the Panel. These include:

- The effect of the high temperature treatment alone, independent of the shredding process cannot be derived from this experiment.
- After the shredding process, shavings are expelled onto a mesh conveyer, which allows dust and particles to fall before the material enters the dryer. The possibility of *B. xylophilus* contamination from untreated waste material is not discussed.
- Only one temperature-time combination has been tested. Therefore the safety margins and reliability of routine operational application of the proposed high temperature treatment cannot be derived from this experiment. This is noted as particularly important in relation to the short exposure time of 3 minutes proposed.

- A temperature of 115 °C is recorded at the end of the high temperature treatment. Thus, a continuous exposure to 398 °C for 3 minutes cannot be confirmed from the experiment described.

The Panel considers that several elements of the experiment were also not adequately described:

- The temperature of the wood shavings in the experiment is unknown. Further information on temperature measurement and placement of thermal sensors would enable more accurate evaluation of the temperature exposure over time.
- A number of factors that may influence the results of the experiment are unknown. These include: the volume of the wood shavings relative to the air volume inside the dryer, the homogeneity of the distribution of the shavings in the dryer and wood moisture levels at the start of the treatment.
- No information is provided to confirm the identity and the frequency of occurrence of the most resistant life stages of *B. xylophilus* in the treated samples. The storage conditions of the control samples are unknown, which may affect the natural mortality over time.

The Panel agrees that there is potential for development of an alternative treatment protocol based on exposure of wood shavings to high temperatures for a short duration. The Panel however, does not consider that the evidence presented in this report demonstrates the effectiveness and reliability of the proposed temperature/time regime as an alternative to the heat treatment requirement for coniferous wood shavings set out in Annex IV Part A Section I Point 1.2. of Council Directive 2000/29/EC.

DOCUMENTATION PROVIDED TO EFSA

1. The Request letter from DG SANCO to EFSA Executive Director sent on 17/02/2009 ref: E1/DF/svi D(2009) 510098
2. Report "Mortality verification of Pinewood Nematode from high temperature treatment of shavings" and the results of laboratory analysis in Annex 1
3. The additional explanatory information provided to the UK authorities in Annex 2

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