Submission form for phytosanitary treatments

(Reviewed by TPPT March 2016)

Czech Republic, Central Institute for Supervising and Testing in Agriculture (UKZUZ)

Click here to find the IPPC Procedure Manual for Standard Setting on the IPP (www.ippc.int), where you can download this form.

Submission number (Secretariat Use Only):

Complete the following form, preferably in electronic format, and submit by e-mail to the IPPC Secretariat (ippc@fao.org). The call will remain open, but if you wish your submission to be considered by the TPPT in their next meeting, please send it before the 5 June 2017.

Please use one form per phytosanitary treatment. An electronic version of this form is available on the International Phytosanitary Portal (IPP) at https://www.ippc.int/en/publications/1089/. Incomplete submissions will be returned. Please save the completed submission form with the following file name: COUNTRY or RPPO NAME –Title of treatment.doc, prior to submitting to the IPPC Secretariat via e-mail. The words "Call for Phytosanitary Treatments" should be placed in the subject line of the email message.

(Text in brackets given for explanatory purposes)

Name of treatment	*Hydrogen cyanide fumigation treatment for pine wood nematode and wood boring beetles in debarked wood*

Submitted by: Central Institute for Supervising and Testing in Agriculture (UKZUZ), Czech Republic

Contact:

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Treatment description

Active ingredient	Hydrogen cyanide (HCN)
Treatment type	Chemical - fumigation
Target pest	Wood-borne life stages of *Bursaphelenchus xylophilus* Coleoptera, including *Hylotrupes bajulus* and *Anoplophora glabripennis*
Target regulated articles	Wood and wooden furniture, wood packaging material, other wooden objects
Treatment	Active substance- hydrogen cyanide (HCN) in preparation "Uragan D2" (new trade name

schedule	BLUEFUME, hereinafter BLUEFUME). BLUEFUME is a mixture (stabilized liquid hydrogen cyanide) of approx. 98 % of hydrogen cyanide with stabilizing additives. Fumigation by using of pressure cylinders. Pressure cylinders consisting of stainless steel 316L liner and composite overwrap. Cylinder is equipped with stainless steel 316L dual port valve with dip tube for liquid HCN outlet and gas port for nitrogen pressuring. Elastomeric sealing is from polychlorotrifluoroethene (PCTFE). The cylinders contain up to 27.5 kg of HCN. BLUEFUME is produced and filled into a cylinder as liquid. Due to fumigation conditions (application by spraying nozzles, temperature, pressure), the liquid evaporates (and does not condense back) and so the active substance is gas. Use in fumigation chamber Application rate: 20 g/m^3 Fumigation temperature and time: 12 °C and above; at least 24 hrs Ventilation time: at least 48 hrs Ventilation through alkali cleaning Fumigation against wood-destroying pests should not be applied when temperatures at the site of pest activity is below 12 °C. The maximum thickness of treated debarked wood should not exceed 9 cm. The fumigation may be performed only by specifically trained professional personnel. The exclusion zone is to be set based on the local conditions (it should usually range between 10-20 m) from the start of the fumigation till the end of the ventilation. The exclusion zone is set to protect bystanders and other once-in-a-lifetime exposed persons (limit 3 mg/m^3). Exclusion zone for HCN exposition duration up to 8 hours is adjusted according to real-time monitoring of exposure limit 0.6 mg/m^3. HCN from the cylinder is sucked into a special container containing an alkali cleaner and the resultant solution is disposed of as a hazardous waste in accordance with the local regulations. Under no circumstances may wooden articles be used for packaging or storing of foods, feeds or beverages be fumigated. Nor may be such articles made of wood previously fumigated.
Other relevant information	According to registration of biocidal preparation “BLUEFUME” for following usage PT08, PT14 and PT18.
References	a) File DOC III There are wood penetration data and efficacy data were submitted for evaluation of successful biocidal registration for product type- PT08. b) File DOC IV There are submitted wood penetration data, test reports and articles related to HCN wood penetration and efficacy to wood boring pests. These data were submitted for evaluation of successful biocidal registration for product type- PT08. Safety data sheets (SDS) for BLUEFUME – cylinders Validation report confirms the suitability of GC method in laboratory conditions.

The following form must be completed in accordance with ISPM 28 *Phytosanitary treatments for regulated pests*, the IPPC Strategic Framework and the *Procedure and criteria for identifying topics for inclusion in the IPPC standard setting work programme*.

Copies of all relevant supporting information and publications should be supplied with the treatment submission, preferably in PDF format, for ease of subsequent distribution.

The following form refers to the relevant sections of ISPM 28 and are numbered accordingly.

3.2 Efficacy data in support of the submission of a phytosanitary treatment

All efficacy data which were used in this form are based on laboratory tests and studies.
Two new laboratory studies were submitted: "Wood penetration ability of hydrogen cyanide and its efficacy for fumigation of *Anoplophora glabripennis*, *Hylotrupes bajulus* (Coleoptera), and *Bursaphelenchus xylophilus* (Nematoda)" (1) and "Hydrogen cyanide for treating wood against pine wood nematode (*Bursaphelenchus xylophilus*): results of a model study" (2).
Suggest to authorization of HCN fumigation for wooden transport material (i.e. pallets).

Overview of all support submitted documents is attached to this form.

3.2.1 Efficacy data under laboratory/controlled conditions (Treatments may be considered without efficacy data under laboratory/controlled conditions if sufficient efficacy data is available from the operational application of the treatment (section 3.2.2) and if no data under laboratory/controlled conditions exists this section may be left blank.)

Pest information

Identity of the pest to the appropriate level, life stage, and if a laboratory or field strain was used

Bursaphelenchus xylophilus, *Hylotrupes bajulus* and *Anoplophora glabripennis*

Conditions under which the pests are cultured, reared or grown

1. *H. bajulus*, originating from Materialprüfanstalt Brandenburg GmbH, and *A. glabripennis*, originating from the European Biological Control Laboratory, USDA, ARS.
   The experimental nematodes (*B. xylophilus*) were obtained from a CULSin vitro-laboratory culture maintained on the fungus *Botrytis cinerea* (De Bary) Whetzel.
2. *B. xylophilus* individuals were obtained from in vitro cultures on *Botrytis cinerea* fungus maintained on MEA medium. Two weeks after inoculation on *B. cinerea*, the nematodes were extracted from the fungal mycelium using Baermann's funnel extraction technique. Extracted nematodes were concentrated and transferred into fresh water to ensure their viability for subsequent experiments.

Biological traits of the pest relevant to the treatment

X

Method of natural or artificial infestation

In the above studies were used model laboratory conditions when pests were artificial infested into wooden blocks.

1. *H. bajulus* originating from Materialprüfanstalt Brandenburg GmbH
   A glabripennis originating from the European Biological Control Laboratory, USDA, ARS
   B. xylophilus were obtained from a CULSin vitro-laboratory culture maintained on the fungus *Botrytis cinerea* (De Bary) Whetzel

2. *B. xylophilus* individuals were obtained from in vitro cultures on *Botrytis cinerea* fungus maintained on MEA medium.

Determination of most resistant species/life stage (in the regulated article where appropriate)

X

Regulated article information

Type of regulated article and intended use

Wood, wooden blocks, wooden pallets

Botanical name for plant or plant product (where applicable)

1. *H. bajulus* and *A. glabripennis* were attached to spruce wooden blocks (*Picea alba* (L.) *H. Karst*.)
   Sawdust (*P. sylvestris*) infested by *H. bajulus* in vials were put into spruce blocks

2. Sawdust (*P. sylvestris*) infested by *H. bajulus* in bags were put into spruce blocks

Conditions of the plant or plant product

X

Experimental parameters

Level of confidence of laboratory tests provided by the method of statistical analysis and the data supporting that calculation

1. Statistical analysis of the data was conducted using the Kruskall-Wallis test. The analysis was performed using Statistica 10.0 software (StatSoft, Inc., Tulsa, OK, USA, 2011). The Ct product was estimated based on FAO methods.
2. Calculated cumulative Ct product values underwent basic statistical analysis (Statistica 12, StatSoft, Inc., Tulsa, OK, USA, 2013).

The results of the biological tests were evaluated in cooperation with:

Czech University of Life Sciences Prague
Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources
Kamýcká 957
Prague - Suchdol
Czech Republic - 165 00

and

Crop Research Institute (CRI)
Drnovská 507
Prague 6
Czech Republic - 161 06

Experimental facilities and equipment

All experiments were performed in a fumigation chamber localised within the Draslovka Kolín a.s.
Unique hermetical fumigation chamber (size 120x90x60cm, made of stainless steel sheets, (material 17240) with a thickness of 2 mm); gas chromatograph (Shimadzu GC-17A, RT-QPLOT, 30 m, ID 0.53 mm,GC Software Clarity DataApex, Kyoto, Japan); statistical analysis using:

1. Statistica 10.0 software (StatSoft, Inc., Tulsa, OK, USA, 2011)
2. Statistica 12.0 software (Statistica 12, StatSoft, Inc., Tulsa, OK, USA, 2013)

Experimental design

All of the experiments were performed in a hermetically sealed steel fumigation chamber with forced air circulation and temperature regulation. If there is an experimental need, the chamber allows for the continuous, non-invasive withdrawal of individual samples through an air-lock antechamber during the required time intervals. The HCN was introduced into the fumigation chamber using a syringe via a rubber septum. Inside the fumigation chamber, the HCN vapor concentration (inside and outside/ Ľ headspace/wooden spruce block) was estimated using the GC technique (Shimadzu GC-17A, RT-QPLOT, 30 m, ID 0.53 mm, GC Software Clarity DataApex). The GC method is based on comparing the detector response from the sample with an external standard with a known concentration. HCN (0.5 vol. %) in nitrogen was used as the standard (Linde Gas).

1. **HCN penetration** – 5 spruce (*Picea alba (L.)*) wooden blocks (100 x 100 x 10 mm; moisture 18.5% ± 0.44%), in saturated (i.e., after a single initial application of 20 g/m^3, small dosages were periodically applied to compensate for absorption) and non-saturated atmosphere (single initial application of 20 g/m^3). The rate of HCN penetration into the central cavity of each spruce block was measured at regular intervals over 50 h of exposure. Air samples were taken from the central cavity of the spruce block (diameter: 25 mm, depth: 60 mm) via a rubber septum, as well as from the steel fumigation chamber headspace, and were immediately analyzed using the GC technique.

   Coleoptera - Tests were performed in the fumigation chamber (described above) with HCN concentrations of 10 and 20 g/m^3 (at 24 and 40 h of exposure) at temperatures of 23-24°C and with an HCN concentration of 20 g/m^3 (at 1, 3, and 6 h of exposure) at temperatures of 20-21°C. Larvae of two species were tested: *H. bajulus*, originating from Materialprüfanstalt Brandenburg GmbH, and *A glabripennis*, originating from the European Biological Control Laboratory, USDA, ARS. Exposure was performed on small wooden blocks that were delivered from the original cultures (1 larva/1 block). The moisture content of the wooden blocks ranged from 16.5% to 21%. The thickness of the experimental wooden blocks was approximately 30 mm narrower than the top/bottom deck-boards of wooden Euro pallets. The larvae of *H. bajulus* (size 15-25 mm) were attached to 70 x 25 x 40 mm wooden blocks, and the larvae of *A. glabripennis* (size 25-40 mm) were attached to 90 x 40 x 40 mm wooden blocks. For *H. bajulus*, three repetitions (five insect-infested wooden blocks per repetition) were performed for each concentration and exposure time. For *A. glabripennis*, only two repetitions were performed for each concentration and time, due to the limits on biological material supplies and the quarantine status. After 24 h of ventilation, the wooden blocks were gently opened, and the mortality measurement was performed. A control (blank) group was tested in the fumigation chamber without HCN.

   Nematodes - The experimental nematodes (*B. xylophilus*) were obtained from a CULSin vitro-laboratory culture maintained on the fungus *Botrytis cinerea* (De Bary) Whetzel. Experiments were conducted in 50-ml polyethylene vials with modified lids. The lid modification consisted of perforating the lid and covering the opening with an 80µm mesh size of Uhelon fabric, allowing for the free access of HCN to the vial. Infested

wood sawdust (*Pinus sylvestris L.*) was used. Twenty grams of sawdust (moisture content declined during the experiment from 30 to 7%) was placed into each vial. Subsequently, individuals of *B. xylophilus* were pipetted into each vial; different numbers of nematodes (inoculum) were applied in different experiments. A magnetic stirrer was used, while pipetting, to ensure the homogeneity of the nematode inoculum. The surviving nematodes were evaluated after six exposure intervals (6, 12, 18, 24, 30, and 40 h) and two temperatures and HCN concentrations (25°C and 20 g/m^3; 20°C and 10 g/m^3). Each experiment was repeated five times. Five untreated controls were kept at the relevant temperatures outside the chamber during the period of fumigation (40 h). After fumigation, the chamber was actively ventilated to vent the HCN-containing atmosphere, and the nematodes were separated from the samples using a modified version of Baermann's method (utility model number CZ 24090 U1). The controls were evaluated in the same manner. After 12 h of extraction, the suspension of nematodes was transferred into embryonic dishes, and the numbers of nematodes in the individual samples were determined using a stereomicroscope.

2. **Nematodes -** Tests were performed in the fumigation chamber (described above) with HCN concentrations of four HCN concentrations in the gas chamber were tested (12.30, 18.21, 21.71 and 24.12 g/m^3) in exposition times 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 h. *B. xylophilus* individuals were obtained from in vitro cultures on *Botrytis cinerea* fungus maintained on MEA medium. Two weeks after inoculation on *B. cinerea*, the nematodes were extracted from the fungal mycelium using Baermann's funnel extraction technique. Extracted nematodes were concentrated and transferred into fresh water to ensure their viability for subsequent experiments. Pinewood sawdust was prepared by sawing *P. sylvestris* logs using a chainsaw. Two grams of sawdust (average moisture content 6.7 % with standard deviation 0.7) were inserted into Uhelon fabric bags with a mesh size of 18 µm to allow HCN free access to the sawdust and to restrict the possibility of spreading the nematodes from bags. 2 ml of tap water was pipetted into bags containing the sawdust to ensure that there was sufficient moisture in the bags for nematode survival. The moisture content of the sawdust was measured during sample preparation and evaluation using the data loggers. On average, moister content of sawdust containing nematodes was 32.30 % 2 h after inserting into gas chamber and 21.69 after 24 h lasting exposition. Next, 500 µl of the nematode suspension containing approximately 1200 *B. xylophilus* individuals was pipetted into each bag. A magnetic stirrer was used during inoculation to ensure inoculum homogeneity. No water drops were observed on the bottom of the bags; whole volume of 2.5 ml of water was absorbed into sawdust as is apparent from its moisture content. Next, the bags were sealed using steel wire and inserted into hollow spruce blocks (100 x 100 x 120 mm) covered by glass containing a silicon septum. The glass was fitted to the wood block using HCN impenetrable glue. Five replicates were prepared for each exposition time and HCN concentration.

Experimental conditions

Tests were performed in the fumigation chamber (described above).

1. **HCN penetration** – spruce (*Picea alba (L.)*) wooden blocks (100 x 100 x 10 mm; moisture 18.5% ± 0.44%), in saturated (i.e., after a single initial application of 20 g/m^3, small dosages were periodically applied to compensate for absorption) and non-saturated atmosphere (single initial application of 20 g/m^3)..

   Coleoptera –HCN concentrations of 10 and 20 g/m^3 (at 24 and 40 h of exposure) at temperatures of 23-24°C and with an HCN concentration of 20 g/m^3 (at 1, 3, and 6 h of exposure) at temperatures of 20-21°C.

   Nematodes - Evaluation after six exposure intervals (6, 12, 18, 24, 30, and 40 h) and two temperatures and HCN concentrations (25°C and 20 g/m^3; 20°C and 10 g/m^3).

2. **Nematodes -** HCN concentrations of four HCN concentrations in the gas chamber were tested (12.30, 18.21, 21.71 and 24.12 g/m^3) in exposition times 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 h.

Monitoring of critical parameters

Values of HCN concentration inside of the fumigation chamber were measured by GC technique.

Methodology to measure the effectiveness of the treatment

1. **HCN penetration** – Air samples were taken from the central cavity of the spruce block (diameter: 25 mm, depth: 60 mm) via a rubber septum, as well as from the steel fumigation chamber headspace, and were immediately analyzed using the GC technique.

Coleoptera – After fumigation tests were performed mortality determination. The mortality check was made after 24 h of wooden block ventilation. In case of eggs were samples observed if larvae emerged. A control (blank) group was tested in the fumigation chamber without HCN.

Nematodes - The nematodes were separated from the samples using a modified version of Baermann's method (utility model number CZ 24090 U1). The controls were evaluated in the same manner. After 12 h of extraction, the suspension of nematodes was transferred into embryonic dishes, and the numbers of nematodes in the individual samples were determined using a stereomicroscope.

2. **Nematodes** - After treatment, the glass seals were crushed, the bags with sawdust and nematodes were removed and the nematodes were extracted from the sawdust for 24 h using Baermann's extraction technique. The obtained suspensions were surveyed using the stereomicroscope, and the nematodes were quantified. An untreated control variant was prepared in the same manner, and the control samples were placed next to the gas chamber during treatment of the experimental ones. The nematode numbers were expressed as the means of surviving nematodes.

Determination of efficacy over a range of critical parameters, where appropriate
X
Methodology to measure phytotoxicity, when appropriate
X
Dosimetry system, calibration and accuracy of measurements,
Accuracy of measurement of above-mentioned gas chromatograph is ± 0,05 vol. %
3.2.2 Efficacy data using operational conditions (historical data, may in some cases substitute for the requested information below)
Pest information
Identity of the pest to the appropriate level, life stage, and if a laboratory or field strain was used
X
Conditions under which the pests are cultured, reared or grown
X
Biological traits of the pest relevant to the treatment
X
Method of natural or artificial infestation
X
Determination of most resistant species/life stage (in the regulated article where appropriate)
X
Regulated article information
Type of regulated article and intended use
X
Botanical name for plant or plant product (where applicable)
X
Conditions of the plant or plant product
X
Experimental parameters
Level of confidence of laboratory tests provided by the method of statistical analysis and the data supporting that calculation
X
Experimental facilities and equipment
X
Experimental design
X
Experimental conditions
X
Monitoring of critical parameters
X
Methodology to measure the effectiveness of the treatment
X
Determination of efficacy over a range of critical parameters, where appropriate
X
Methodology to measure phytotoxicity, when appropriate

X

Dosimetry system, calibration and accuracy of measurements

X

Factors that affect the efficacy of the treatment

X

Special procedures that affect the success of the treatment, if applicable

X

3.3 Feasibility and applicability (Information should be provided where appropriate on the following items)

Procedure for carrying out the phytosanitary treatment

Fumigation is provided by a professional pest control company licensed for professional fumigation.

Approved by the National Authority for Biocide Registration as a biocidal preparation of the type:
Type 8 – Wood preservatives
Preparations used for the conservation of wood, including timber and wood products, against wood-destroying or disfiguring products.

Cost of typical treatment facility and operational running costs if appropriate

The price is not available, however, it may be provided later following an agreement with a professional company carrying out fumigation.

Commercial relevance, including affordability

Costs of using hydrogen cyanide are lower than of using methyl bromide.

Extent to which other NPPOs have approved the treatment as a phytosanitary measure

X

Availability of expertise needed to apply the phytosanitary treatment

X

Versatility of the phytosanitary treatment

The preparation BLUEFUME (active substance hydrogen cyanide) is registered as a biocide in 13 states of EU, but is fully adaptable to the usage for phytopathological treatment.

In terms of its originality – efficiency, capacity and rate of the application process, BLUEFUME with hydrogen cyanide as the active substance has no comparable and acceptable alternative regarding the health protection (substance without CMR effects on humans), environmental protection and social-economical aspects.

BLUEFUME is in selected areas of usage a fully effective substitute for methyl bromide. HCN is not classified as substance hazardous to the ozone layer, and is a very important strategic material for the Czech Republic.

BLUEFUME is the only fully effective substitute for methyl bromide in the above mentioned usages.

The degree to which the phytosanitary treatment complements other phytosanitary measures

Due to simplicity of use and high efficacy to many species and developmental stages of various pests is usage seeming like a full-fledge substitute of methyl bromide. Moreover due to smaller structure of molecule HCN offers more possible field of application.

Summary of available information of potential undesirable side-effects

Human exposure may occur by inhalation of hydrogen cyanide vapours or absorption through the skin upon contamination of work clothing by vapours or liquid hydrogen cyanide.
The risk of exposure applies to workers in the production when exposure limits are exceeded, or in case of accident.
Workers carrying out fumigation shall use protective equipment; exposure should not occur if operating procedure is observed.
Bystanders or other persons may not be exposed to hydrogen cyanide, since it is intended only for professional use in empty secured spaces.
Due to the form in which hydrogen cyanide is supplied – pressure cylinder – accidental exposure of employees or other persons is beyond consideration.

Professional exposure
Since hydrogen cyanide is applied as gas, its main route of entry is inhalation and dermal exposure.
HCN is intended to be used for fumigation – to control insect pests and smaller rodents in closed structures and insect pests damaging various natural materials (in gas-tight application chambers).

If used professionally as intended, no adverse impacts to humans or animals, except target organisms, are expected.

Risks for employees in production, fumigation workers and bystanders
The risk of inhalation of hydrogen cyanide during production and processing is minimised by automatic monitoring of

the workplace atmosphere and signalisation of danger if permissible exposure limits are exceeded. Employees shall use specified personal protective equipment. Workers carrying out fumigation shall use protective equipment and personal detectors and are bound to strictly observe specified operating procedures.
Applicability of treatment with respect to specific regulated article/pest combinations
The results demonstrated that HCN possesses an overall good efficiency to efficacy for HCN in controlling important wood-infesting pests (B. xylophilus, H. bajulus and A. glabripennis)
Technical viability
BLUEFUME shall be stored in dry, cool, ventilated, separate storeroom. Due to the danger or accidental release of HCN, only personnel authorised to handle HCN may enter the storeroom, and only with a gasmask with suitable filter and a measuring device. Keep container tightly closed. Use explosion-proof electrical/ventilating/light/equipment. Take precautionary measures against static discharge. Shelf life At site practice storage stable. No decomposition. The shelf-life of BLUEFUME is set to 12 months.
Phytotoxicity and other effects on the quality of regulated articles, when appropriate
Due to chemical and physical properties of HCN, by this time, no case of resistance has been proven.
Consideration of the risk of the target organism having or developing resistance to the treatment
Due to toxicologist properties of HCN, by this time, no case of resistance has been proven.

Send submissions to:

E-mail: ippc@fao.org
(preferred)

Mail: IPPC Secretariat (AGPP)
Food and Agriculture Organization of the UN
Viale delle Terme di Caracalla,
00153 Rome, Italy