

**Experimental Application Of The Balsam Fir Sawfly
Nucleopolyhedrovirus Against Its Natural Host,
The Balsam Fir Sawfly**

May 2004

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Experimental Application of the Balsam Fir Sawfly Nucleopolyhedrovirus Against Its Natural Host, the Balsam Fir Sawfly

Nature of Proposed Pesticide Application

The Province of Newfoundland and Labrador continues to face serious and widespread infestations of balsam fir sawfly (*Neodiprion abietis* – Hymenoptera: Diprionidae). These infestations are threatening substantial investments in silviculture and consequently the long-term wood supply for the forest industry. For a fifth year, the Canadian Forest Service (CFS), in co-operation with the Newfoundland and Labrador Department of Natural Resources (NLDNR) and Forest Protection Limited (FPL), is proposing to carry out an experimental research application of a highly species-specific microbial biological control agent (balsam fir sawfly nucleopolyhedrovirus – NeabNPV) on selected silviculturally treated forest stands forecast to receive moderate to severe the balsam fir sawfly defoliation in 2004 and at the leading edge of the infestation. Applications of this biological control agent will be made using fixed-wing aircraft and/or helicopters.

Description of Balsam Fir Sawfly Problem

Insect population levels

The balsam fir sawfly is native to and has been an occasional pest on balsam fir in Newfoundland. Recently, it has become more important as a pest of young and semi-mature balsam fir (*Abies balsamea*), particularly in pre-commercially thinned stands (PCTs). The population overwinters in the egg stage in fir needles and larvae usually hatch in late-June to mid-July depending on the weather. Larvae feed on previous-year and older foliage for a number of weeks before pupating. Adult sawflies emerge in August, mate and eggs are laid in the needles of the current year. Populations have been regulated by natural pathogens, parasites and predators. Outbreaks have normally been of short duration (3 - 4 years) and were terminated by natural factors, predominantly NeabNPV. Although localized damage was often severe, tree mortality was limited. Defoliation, however, can cause significant growth loss to affected trees without tree death. Research at CFS has shown that, after defoliation has ceased, there may be a 13- to- 18-year period of reduced growth before the trees recover to pre-infestation growth rates (Piene et al. 2001).

The current infestation in western Newfoundland was detected in 1991 near Bottom Brook, east of Stephenville. By 1994, approximately 1,216 hectares (ha) of defoliation were recorded. In 1995, high population levels were observed. Moderate and severe defoliation was mapped on 12,600 ha, with some 10 percent mortality occurring in young fir stands. The situation in 1996 saw the infestation continue to expand with defoliation on 19,700 ha, including 15,400 ha in the moderate and severe categories. In 1997, the infestation expanded to the northeast and southeast into larger areas of valuable balsam fir (PCT) stands. A total of 53,000 ha were defoliated in 1997 with 30,300 ha in the moderate and severe categories. Pockets of defoliation were also detected on the Burin Peninsula and in Bay d'Espoir. The moderate and severe defoliation in 1998 totaled approximately 24,400 ha with 16,500 ha occurring in western Newfoundland, 5,800 ha in Bay d'Espoir and 2,100 ha on the Burin Peninsula. In 1999, moderate and severe defoliation occurred on 18,400 ha with 12,400 ha in western Newfoundland, 3,300 ha in Bay d'Espoir and 2,800 ha on the Burin Peninsula. In 2000, approximately 22,000 ha in western Newfoundland and 19,000 ha in the Bay d'Espoir

were defoliated. Moderate to severe defoliation was recorded on 38,000 ha in western Newfoundland and 9,000 ha in the Bay d'Espoir in 2001.

In 2002-2003, moderate to severe defoliation reached 60,000 ha in western and southern Newfoundland. The western area extended from south of Grand Lake, north to Old Man's Pond and from Stag Lake-Cook's Brook across the Humber Arm near Gillams and east to Steady Brook-Corner Brook Lake. This area is of particular concern because a significant proportion is PCT. These PCTs have been established, at an average cost of \$1,000+/ha (a total amount in excess of \$10 million). These are critical to maintaining an adequate wood supply for the forest industry.

The impact of balsam fir sawfly infestations, if left unchecked, will result in substantial loss of this investment. The failure to adequately protect the investment in silviculture, and the potential loss of future harvestable stands, would be significant to the social and economic well-being of the people, particularly on the west and south-west coasts of the Island. This is true both in terms of direct employment and in spin-off economics.

Apart from NeabNPV, there does not appear to be any other significant natural factor influencing balsam fir sawfly populations. With prolonged, severe defoliation, affected trees will be stressed, lose growth and be subject to mortality from secondary insects and diseases. It is estimated that, since the balsam fir sawfly outbreak began, the Province has lost in excess of 2 m³ of growth per hectare per year, a loss in excess of 120,000 m³ of incremental growth.

Control Options

A pest management program is being developed against the balsam fir sawfly in Newfoundland to protect valuable young stands and silviculturally treated areas of balsam fir. The purpose of the program is to reduce balsam fir sawfly population levels in treated areas to minimize the loss of foliage, tree growth and to prevent tree mortality due to secondary infestations in trees weakened by balsam fir sawfly attack. Unfortunately, control options for balsam fir sawfly are limited. Experimental programs have been carried out by CFS and its collaborators in 1998, 1999, 2000, 2001, 2002 and 2003 in Newfoundland and in other jurisdictions to develop biological control options for a number of sawflies, including the balsam fir sawfly, yellowheaded spruce sawfly (*Pikonema alaskensis*), pine false webworm (*Acantholyda erythrocephala*) and introduced pine sawfly (*Diprion similis*). Progress has been made and work is continuing.

Dylox

The organophosphate insecticide, Dylox 420 (trichlorfon) is no longer considered as an option. It was used in 1998 under an emergency registration from the Pest Management Regulatory Agency (PMRA) of Health Canada. Based on experimental trials conducted in the same year, it was determined that lower dosages than those recommended could be effective. NLDNR requested registration of Dylox and, for 1999 only, PMRA granted a temporary registration for Dylox for use against balsam fir sawfly. There were a number of conditions related to buffer zones (no spray zones), dose parameters and monitoring requirements attached to the temporary registration.

Dylox is fully registered for use against the yellowheaded spruce sawfly but is not being pursued further for balsam fir sawfly. This is due to public resistance to its use and issues surrounding buffer zones. In 1998, buffer zones for Dylox around water bodies were established at 100 m at the federal level and 200 m provincially. This restricted control measures to approximately 3,100 ha. In 1999, buffer zones were established at 200 m both federally and provincially. This resulted in the further exclusion of significant areas of infested stands from the protection program.

Bacillus thuringiensis

The most common biological insecticide to be applied aerially in forests against the spruce budworm and hemlock looper is *Bacillus thuringiensis* var. *kurstaki* (B.t.k.). B.t.k. was developed as a control product for, certain pest insects belonging to the order Lepidoptera (butterflies and moths). For B.t.k. to be effective, it must be ingested by an appropriate host insect. A protein crystal within the wall of the bacterial spore must first be digested by specific proteases within the alkaline midgut of the host insect. The B.t.k. toxin must bind to specific receptors on the midgut epithelial cells to work. Sawflies belong to the order Hymenoptera (includes bees, ants and wasps) and their larvae are not susceptible to B.t.k.

Bacillus thuringiensis var. *israelensis* (B.t.i.) is registered for use in the control of mosquito and blackfly (order Diptera) larvae. Its mode of operation is the same as that of B.t.k. but proteins digested from the larger crystal bind specifically to receptors on the cells of the midgut of larval mosquitoes and blackflies not those of lepidopteran larvae. In 1999, B.t.i. was tested experimentally against balsam fir sawfly on a small area. B.t.i. was found not to be effective.

Neem

Neem (azadirachtin) is a botanical insecticide extracted from the neem tree (*Azadirachta indica*) native to India and parts of Africa. Certis (a manufacturer of one neem product) applied for and received temporary registration from PMRA for Neemix 4.5 for use against several sawfly species including balsam fir sawfly. Neem has a number of properties that affect target pests. Depending on the amounts applied, these include insecticidal, insect growth regulatory and anti-feedant activities. Neem is registered for use in many countries including the USA where it is registered for indoor and outdoor use. It may be applied aerially and/or from the ground to horticultural-ornamental plants, trees, shrubs and agricultural crops. An operational program using Neemix 4.5 was carried out by NLDNR on about 1,500 ha in the Bay d'Espoir in 2001. In 2002, Neemix 4.5 was applied to just over 6,000 ha near Corner Brook. Neemix 4.5 was not available for use in 2003 and will not be available in 2004 because the temporary registration has expired.

Balsam fir sawfly nucleopolyhedrovirus

Nucleopolyhedroviruses (NPVs) are a large group of viruses with covalently closed, double-stranded DNA genomes of 88-153 kilobases. NPVs are found only in arthropods, primarily insects. NPVs have a high degree of host specificity affecting a single insect species or only ones that are closely related. NPVs are not related to any known human, veterinary or plant pathogenic viruses. Specificity and safety tests of NPVs have shown that there are no toxicological or other deleterious effects on mammals, birds, amphibians, aquatic

microorganisms and beneficial and other nontarget insects. Population crashes due to NPV epidemics occur in many species of sawflies. NPVs are transmitted through ingestion by a suitable host larva. Viral polyhedral inclusion bodies (PIBs) dissolve in the midgut, releasing the virions to infect midgut epithelial cells. Sawfly NPVs only infect the midgut epithelium so that, following a single replicative cycle, infected cells containing PIBs are sloughed off into the frass and out of the body where they can infect other host insects. Death normally occurs within 1 to 2 weeks but, during that time, the host is producing infective units of the disease. Sawfly NPVs are highly host specific and it has been necessary to develop a different virus for each host species. Attempts to use NPVs to suppress sawfly populations have usually met with success.

Progress 1997-2003

Field trials

In 1999, NeabNPV was applied to 1 ha of balsam fir forest in order to field amplify the virus. From this 1-ha application, enough NeabNPV was obtained to treat 1,800 ha of forest at an application rate of 1×10^9 PIBs/ha. On July 22-23, 2000, three blocks, each 50 ha in area, between Pinchgut Lake and Big Gull Pond near Corner Brook, Newfoundland, were treated aerially with NeabNPV at 3×10^9 PIBs/ha. (In all trials, a 20% aqueous solution of molasses was used as the carrier for the virus and the mixture was applied at a rate of 2.5 L/ha using Cessna 188 AgTrucks equipped with Micronair AU4000 atomizers). Aerial field trails (1×10^9 PIBs/ha) were conducted on July 21-22, 2001, east and north of Stag Lake near Corner Brook and north of St. Alban's, Bay D'Espoir, on July 24, 2001. The three blocks near Stag Lake totaled 2200 ha and the block in the Bay D'Espoir was 600 ha. On July 21-23, 2002, a total of approximately 5000 ha was treated (1×10^9 PIBs/ha) in three blocks to the south, east and north of Corner Brook. In 2003, NeabNPV was applied to areas totalling approximately 5,000 ha. The locations of these application plots were around Old Man's Pond, north of Deer Lake across from Pasadena and to the south west of Pasadena. In all trials in all years, there was good deposit on the targeted areas resulting in higher levels of NeabNPV infection in larval populations in the spray blocks compared to the control blocks. Additionally, it was generally found that the number of balsam fir sawfly pupae and eggs was lower in the spray blocks compared to the control blocks in the year of the spray. In the year immediately following NeabNPV applications, it was found that the number of eggs per shoot, the percentage of successful egg hatch and the resultant number of larvae per shoot were lower in the spray blocks than in the controls. As a result, defoliation in the control blocks was much greater than in any of the spray blocks, which had little defoliation. An objective of the 2002 field trial was to determine if application of NeabNPV against first-instar balsam fir sawfly would kill the insects in time to give some foliage protection in the year of application. There was some foliage protection but it was limited.

In the NeabNPV field trials to date, we have found that i) NeabNPV is easy and cheap to produce, ii) our formulation allows for smooth flow from the aircraft and good deposit on the foliage, iii) a single application at $1-3 \times 10^9$ PIBs/ha against first- and second-instar larvae results in increased levels of NeabNPV infection in larval populations within 15 days and iv) application in one year can affect the population of balsam fir sawfly larvae in the next year resulting in significantly decreased defoliation.

Proposed Field Trials for 2004

The balsam fir sawfly infestation was apparent in Corner Brook and on the north side of the Humber Arm (Summerside – Hughes Brook) in 2001. In 2002, the infestation had spread further towards the northeast and the balsam fir sawfly infestation near Old Man's Pond was sprayed with NeabNPV. On the other side of the Humber River, defoliation could be seen up the valley past Steady Brook towards Little Rapids. In 2003, applications were made around Old Man's Pond, between Old Man's Pond and Deer Lake and to the south-west of Pasadena.

In 2004, NeabNPV will be applied against first- and second-instar balsam fir sawfly larvae at a rate of 1×10^9 PIBs/ha. A 20% aqueous solution of molasses will be used as the carrier for the virus and the mixture will be applied at a rate of 2.5 L/ha using fixed-wing spray aircraft (Cessna 188 AgTrucks or Air Tractor AT-802s) equipped with Micronair AU4000 atomizers. Experimental treatment blocks (figures 1-4) will have three transect lines, perpendicular to the line of aircraft flight, with 10 groups of three sampling trees spaced evenly along the length of the transect. Similar but untreated control blocks will also be established. In the treatment and control blocks, pre-spray samples will be collected shortly after 100% egg hatch has been reached and once a week beginning one week after the spray and for three subsequent weeks. Additionally, these blocks will be monitored in 2005 and 2006 to determine the impact of NeabNPV in years following NeabNPV application. Sprays will be monitored for product deposition and meteorological conditions using standard techniques. Defoliation estimates will also be made in August or September.

Separate spray blocks (approximately 20 ha each) will be established for NeabNPV production in areas where there is high balsam fir sawfly larval densities. These blocks will be sprayed at a rate of 5×10^9 PIBs/ha when the larval index is peak second instar. Infected larvae will be collected and NeabNPV purified using established methods.

Additionally, disks will be cut from balsam fir trees in the spray and control blocks from 2000 through 2003 for stem analysis. This will be done to determine what effect, if any, the experimental applications of NeabNPV had on tree growth and wood production.

CFS and its cooperators utilize appropriate current equipment and technology. CFS complies with existing regulatory guidelines. Parameters of the spray blocks will be determined on the ground using hand-held GPS personal navigators. Block coordinates will be transferred computers on board the aircraft. These aircraft are equipped with the latest navigational and spray equipment including mapping (GIS) and positioning (GPS) systems, automatic on/off spray-boom controls, variable speed-pressure-flow monitors and controllers, real-time, on-board meteorological sensors and radar altimeters. At the time of the spray application, this equipment and related application software ensure the highest level of accuracy of application currently available. CFS supervisors will assess the favourability of weather parameters before and during spray application. To ensure environmental safety, spray bases will have available appropriate, current and approved safety and emergency equipment, materials and methods.

Worker Safety

CFS has well-established safety guidelines for workers involved in insect control. Personnel handling the NeabNPV formulation (mixer/loader) will wear the required safety equipment as indicated on the experimental label during mixing and loading onto the aircraft. In addition, approved safety precautions and established rules and guidelines will be adhered to concerning personal hygiene of all mixer/loader personnel working with NeabNPV formulations as indicated on the experimental label. Hospital and emergency telephone numbers will be posted in a conspicuous place to be used in the event of accident. Applicable contingency measures will be available to personnel in the event of an accident.

Public Health Considerations

To minimize the risk of exposure of people to insecticide spray, "no-spray" buffer zones will be left around known places of permanent human habitation and around areas such as cabin developments, parks, camps and day use areas. In 2004, spraying near habitation will be subject to terms and conditions of the Operator's Licence from the Department of Environment & Conservation in consultation with the appropriate Health and Community Services personnel. Cabins will be adequately buffered in relation to the product being applied. In addition, a 1.6 km buffer zone is left around identifiable intakes to known community water supplies. If, during the course of a spray mission, unauthorized personnel are detected in or near a treatment area, the aerial supervisor will instruct the spray aircraft pilot to provide extra buffers or to terminate the mission, as circumstances dictate.

Environmental Safety

In terms of environmental safety, all stipulations in the licence issued by the provincial Department of Environment & Conservation will be followed. These include the reporting of any incidents, such as spills, to the appropriate authorities. In connection with this, CFS and NLDNR have contingency plans that are reviewed and approved annually prior to receiving of an Operator's Licence. These plans outline procedures for spill reporting, emergency first aid for exposure, insecticide spill only, aircraft crash in bush, aircraft accident on or near the airport, jettisoned aircraft load, drum decontamination and disposal and other general regulations and instructions as necessary.

Public Notification

As part of the program, the public and media in the vicinity of the proposed treatment areas will be notified, prior to commencement of the program, through advertisements and/or news releases and through appropriate direct contact if required. Information included will be the product being used, general areas of spray blocks, timing of application, contact numbers, etc. Access roads to the general areas will be posted with signs indicating treatment, product, dates, and phone numbers for more information. A phone-in information line will be set up and the general public can call to find out the status of areas receiving treatment.

Regional offices of the NLDNR and the Department of Environment and Conservation, as applicable, will be provided with maps showing spray blocks. These maps will be made available for viewing by the general public during regular office hours. District offices of the NLDNR will be made aware of spray blocks in their area and are provided with applicable detailed maps so they can inform the public on specific local blocks when requested. Also,

Dr. Christopher Lucarotti (CFS-AFC), who is in charge of the efforts to get NeabNPV registered for operational use against the balsam fir sawfly, will be present in Newfoundland during the spray period. He may be called upon at other times to assist in answering questions and concerns from the public.

Potential Spray Conflicts

There are always potential conflicts with insect control programs; for example, proximity to habitation, water supply areas, recreational areas (fishing, camping, berry picking) and potential impacts on wildlife. However, in approving a product at the federal registration level, and in granting a licence at the provincial level, mitigating measures are identified which eliminate or significantly reduce the potential for conflicts. These mitigating measures are outlined on the product label as approved by the PMRA and in terms of any buffer zones as stipulated in the Operator's Licence. In addition, the proponent is also required to post signs and advise the public about the program to lessen accidental exposure.

Integrated Pest Management Approach

In 1997, a cooperative research agreement involving the CFS, NLDNR, Corner Brook Pulp and Paper Ltd. and Abitibi-Consolidated Inc. was initiated to investigate the ecology of the balsam fir sawfly. The prevalence of natural control factors such as viruses, fungi and parasites and their effect on balsam fir sawfly populations are being investigated. The impact of the balsam fir sawfly on and differences observed between thinned and unthinned stands is also being investigated. In 1998, additional financial resources were obtained through a Natural Sciences and Engineering Research Council (NSERC) – CFS – Industry grant which is administered through the University of New Brunswick. This funding continued through 2001. Funding for 2000-2003 was also obtained from the CFS Biotechnology Strategy, by CFS researchers, to study the functional genomics of NeabNPV. Additional funds have been obtained from the NSERC BioControl Network and CFS Enhanced Pest Management for the period 2001-2006. These cooperative research programs, in identifying natural factors that influence balsam fir sawfly populations, will hopefully lead to an integrated pest management strategy against this pest.

In November 2000, CFS research staff had a registration pre-submission consultation with officials from PMRA. The purpose of the consultation was to determine the requirements that would have to be met in order to get a registration for the operational use of NeabNPV. A great deal of progress has been made since then including: i) four years of field efficacy trials, ii) five years of field work by three graduate students studying balsam fir sawfly ecology, iii) the NeabNPV genome has been fully sequenced, iv) bioassays against non-target insects and *Daphnia magna* (freshwater invertebrate crustacean) and v) mammalian toxicology – pathogenicity tests have been carried out. NeabNPV did not have any detectable effect on any of the insect species (other than sawflies), *Daphnia* or on the mammalian animals and cell lines tested. It is hoped that an application for NeabNPV registration will be submitted to PMRA in May 2004.

Registration Approval Process

Any pest control product manufacturer who wishes to sell a pesticide in Canada must first register that product under the Pest Control Products Act. To receive registration, the

manufacturer must follow the registration process administered by PMRA. Registration involves the submission of an application by the manufacturer. The company must first carry out extensive studies on the product. The application must be supported by a very thorough data package documenting the effects of the pesticide on users, bystanders and the environment. A scientific evaluation of the product is then performed by PMRA. The scientific evaluation may take years, as the evaluation may require long- and short-term human health effects, residues in food, ground water contamination, effects on wildlife and environmental fate. A registration will be granted only if the safety of the pesticide and its merit and value for the proposed use are found to be acceptable. If problems with the product are identified, registration will not be granted. All products are subject to reevaluation, with provision for suspension or cancellation.

Once the Federal Government approves a registration, the provincial governments become more involved. Each province has legislation dealing specifically with pesticide use in that province. In Newfoundland and Labrador pesticide use is regulated under the *Pesticides Control Act*. This legislation requires all organizations and companies using pesticides to apply for and receive a Pesticide Operator Licence. This licence regulates aspects of an operation not covered by federal legislation and requirements. As with federal regulations, the Pesticide Operator Licence is designed to minimize risk to human health and the environment. Aspects of a pesticide operation, such as buffer zones, spill response, public information and notification programs, monitoring requirements, weather conditions, are all specified in the licence as they relate to a particular spray program. The federal registration system, combined with the provincial licensing and regulatory system, ensures that any pesticide that is used in Canada has passed a comprehensive environment/health evaluation.

Provincial legislation also requires individuals to be trained in the safe use of pesticides. Only individuals that successfully pass the provincial pesticide applicator exam (administered by the Department of Environment and Conservation - Pesticides Control Section) are granted an applicator license and authorized to handle pesticides. Compliance and enforcement activities are also carried out by the Pesticides Control Section.

As with all commercial pesticide operations, the 2004 experimental NeabNPV program will be regulated by the Pesticides Control Section of the Newfoundland and Labrador Department of Environment and Conservation.

Attachments

- (1) Product Label (draft).
- (2) Product Material Safety Data Sheet (draft).
- (3) Maps of Proposed NeabNPV Application Sites.
- (4) Copy of Operators License Applicable to Forest Insecticide Use (Terms and Conditions) from the Newfoundland and Labrador Department of Environment and Conservation.
- (5) PMRA Research Permit.

DRAFT LABEL

ABIETIVIRUS

Flowable Biological Insecticide

RESTRICTED For Use In Forestry

**READ THE LABEL BEFORE USING
KEEP OUT OF REACH OF CHILDREN**

GUARANTEE: *Neodiprion abietis* Nucleopolyhedrovirus, NeabNPV (Newfoundland strain):
4 x 10⁹ polyhedrin inclusion bodies (PIBs) per milliliter.

REGISTRATION NO: 35-RP-04 PEST CONTROL PRODUCTS ACT

Net Contents: 40 mL (1.6 x 10¹¹ PIBs)

Natural Resources Canada
Canadian Forest Service – Atlantic Forestry Centre
P. O. Box 4000, 1350 Regent Street
Fredericton, New Brunswick, E3B 5P7

NOTICE TO USER: This control product is to be used only in accordance with the directions on this label. It is an offence under the *Pest Control Products Act* to use a control product act under unsafe conditions.

NATURE OF RESTRICTION: This product is to be used only in the manner authorized. Consult provincial pesticide regulatory authorities about use permits which may be required.

RESTRICTED USE: For use against balsam fir sawfly larvae (*Neodiprion abietis*) in forests.

DIRECTIONS: Mix with a 20% aqueous solution of molasses at a rate of 1 mL Abietiv to 10L molasses solution. Aerial application. Apply 1-5 billion (10⁹) PIBs/hectare of Abietivirus in 2.5 L 20% aqueous solution of molasses/hectare. Apply when larvae are young (preferably first and second instar) and are actively feeding. To be effective, larvae must ingest foliage with deposits of Abietivirus. Uniform spray deposit coverage of the foliage is essential for optimum control. Recommended droplet size is 100 µm.

PRECAUTIONS

KEEP OUT OF REACH OF CHILDREN

CAUTION – EYE IRRITANT

PRECAUTIONS: KEEP OUT OF REACH OF CHILDREN. Avoid contact with skin, eyes or clothing. Wear a long-sleeved shirt, long pants, water-proof gloves and eye goggles when handling, mixing/loading or applying the product and during all clean-up/repair activities.

Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

FIRST AID

IF SWALLOWED - Rinse mouth and throat with copious amounts of water. Do not induce vomiting.

IF ON SKIN/CLOTHING – Take off contaminated clothing. Wash skin with plenty of soap and water.

IF INHALED – Move to fresh air.

IF IN EYES – Hold eye open and rinse slowly and gently with water. Remove contact lenses, if present, then continue rinsing eye.

GENERAL – Seek medical attention immediately if irritation or signs of toxicity occur and persist or are severe. Take container or experimental label with you when seeking medical attention.

STORAGE: Store in the refrigerator at 4°C. Store container upright and keep tightly closed when not in use. Shake vigorously to resuspend contents immediately prior to addition to molasses solution.

DISPOSAL: Do not reuse container. Follow provincial instructions for any required cleaning of the container prior to its disposal. Make empty container unsuitable for use and dispose in accordance with provincial requirements. For information on the disposal of unused, unwanted product and the cleanup of spills, contact the provincial regulatory agency or the manufacturer.

ABIETIVIRUS

Insecticide biologique à pulvériser

USAGE RESTREINT pour la Foresterie

**LIRE L'ÉTIQUETTE AVANT L'UTILISATION
GARDER HORS DE LA PORTÉE DES ENFANTS**

GARANTIE: *Neodiprion abietis Nucleopolyhedrovirus*, NeabNPV (souche Terre-Neuve):
4 x 10⁹ corps d'inclusion polyhedriques (CIPs) par millilitre.

**NUMÉRO D'HOMOLOGATION 35-RP-04,
LOI SUR LES PRODUITS ANTIPARASITAIRES**

Contenu net: 40 mL (1.6 x 10¹¹ CIPs)

Ressources naturelles Canada
Service canadien des forêts– Centre de foresterie de l'atlantique
C.P. 4000, 1350 rue Regent
Frédéricton, Nouveau-Brunswick, E3B 5P7

AVIS À L'UTILISATEUR: Ce produit antiparasitaire doit être employé strictement selon le mode d'emploi qui figure sur la présente étiquette. L'emploi d'un tel produit dans des conditions dangereuses constitue une infraction à la *Loi sur les produits antiparasitaires*.

NATURE DE LA RESTRICTION: Ce produit doit être employé strictement selon le mode d'emploi autorisé. S'informer auprès des autorités provinciales de régulation des produits antiparasitaires pour vérifier si un permis d'utilisation est requis.

USAGE RESTREINT: Pour l'utilisation en forêt contre les larves du diprion du sapin (*Neodiprion abietis*).

MODE D'EMPLOI: Mélanger à une solution aqueuse de mélasse à 20% au taux de 1mL d'Abiativ pour 10L de solution de mélasse. Arrosages aériennes. Pulvériser 1-5 milliards (10⁹) de CIPs/hectare d'Abietivirus dans 2.5L de solution de mélasse à 20%/hectare. Appliquer lorsque les larves sont jeunes (préférentiellement aux premier et second stades de développement) et se nourrissent activement. Pour qu'Abietivirus soit efficace, les larves doivent ingérer des aiguilles avec des dépôts. Une couverture uniforme du feuillage par les dépôts de pulvérisation est essentielle pour un contrôle optimal. La grosseur recommandée des gouttelettes est de 100 µm.

PRÉCAUTIONS

GARDER HORS DE LA PORTÉE DES ENFANTS

ATTENTION-IRRITANT POUR LES YEUX

PRÉCAUTIONS: GARDER HORS DE LA PORTÉE DES ENFANTS. Éviter le contact avec la peau, les yeux ou les vêtements. Porter des vêtements longs, des gants imperméables et des

lunettes de protection pour manipuler, mélanger/charger ou appliquer le produit et pendant les opérations de nettoyage et de réparations. Bien se laver à l'eau et au savon après avoir utilisé le produit. Retirer les vêtements contaminés et les laver avant de les porter à nouveau.

PREMIERS SOINS

SI INGÉRÉ – Rincer la bouche et la gorge avec beaucoup d'eau. Ne pas faire vomir.

SUR LA PEAU OU LES VÊTEMENTS – Enlever les vêtements contaminés. Laver la peau à l'eau et au savon.

SI INHALÉ – Se déplacer à l'air frais.

DANS LES YEUX – Rincer lentement et doucement avec de l'eau en gardant l'oeil ouvert.

EN TOUT LES CAS - Obtenir de l'aide médicale immédiatement si des irritations ou des effets toxiques se manifestent et persistent ou sont sévères. Apporter le contenant ou l'étiquette au moment d'obtenir de l'aide médicale.

ENTREPOSAGE: Conserver au réfrigérateur à 4°C. Garder le contenant bien fermé et en position verticale entre les utilisations. Brasser vigoureusement pour ressuspendre le contenu immédiatement avant de l'ajouter à la solution de mélasse.

ÉLIMINATION: Ne pas réutiliser le contenant. Se conformer à la réglementation provinciale pour le nettoyage requis du contenant avant la mise au rebut. Rendre le contenant inutilisable et l'éliminer conformément à la réglementation provinciale. Pour avoir des informations sur l'élimination de tout produit inutilisé et dont on veut se départir et pour le nettoyage d'un déversement accidentel, contacter l'agence de réglementation provinciale ou le fabricant.

NeabNPV Product Profile and Proposed Use Patterns

- a) *Neodiprion abietis Nucleopolyhedrovirus* NeabNPV Baculoviridae
- b) NeabNPV is a baculovirus within the genus *Nucleopolyhedrovirus* (NPV). NPVs are a large group of viruses with covalently closed, double-stranded DNA genomes of 88-153 kilobases (kb) (NeabNPV genome is approximately 95 kb). In NeabNPV, numerous singly enveloped virions are occluded within polyhedral inclusion bodies (PIBs) that are 0.5–1.0 µm in diameter. Polyhedrin is a 29-kiloDalton protein. Baculoviruses are restricted to arthropods, mostly insects. NPVs have a high degree of host specificity affecting a single insect species or ones that are closely related. Sawfly NPVs are especially host specific and those described to date only seem to infect and replicate in the midgut epithelial cells of a single host species (Wallace and Cunningham 1995). Sawfly NPVs (including NeabNPV) are ingested by host larvae. Polyhedrin is dissolved in the gut releasing the virions. The virions fuse with the microvilli of the midgut epithelial cells and nucleocapsids are transported into the nucleus where the virus uncoats, undergoes replication and morphogenesis. Ultimately, the host cell dies and lyses releasing PIBs into the gut lumen of the host and PIBs pass out with the frass and may then be consumed by other host larvae. Infected host larvae usually die with 7 – 14 days. NeabNPV was isolated from balsam fir sawfly (*Neodiprion abietis*) larvae collected near Corner Brook, Newfoundland in 1997.
- c) The recommended application rate of NeabNPV and other sawfly NPVs is 1-5 x 10⁹ PIBs/ha (see also Wallace and Cunningham 1995).
- d) Bioinsecticide (larvicide).
- e) Restricted for use in forestry.
- f) NeabNPV (4 x 10⁹ PIBs/mL) suspended in water. To be diluted 1:2,000 to 1:10,000 with 20% aqueous molasses.
- g) *Neodiprion abietis* (Diprionidae: Symphyta), the balsam fir sawfly.
- h) Normally 1-5 x 10⁹ PIBs/ha (but, up to 1 x 10¹⁰ PIBs/ha for the purpose of NeabNPV field production) suspended in 20% aqueous molasses and applied at a volume of 2.5L/ha.
- i) Single application to coincide with first and second larval instars. NeabNPV must be ingested by feeding larvae to be effective.
- j) Fix-winged aircraft or helicopter equipped with Micronair® AU 4000 nozzles or equivalent. May also be applied from the ground using powered ground sprayers or backpack sprayers.
- k) Standard procedures for aerial applications (protective clothing, eyewear, etc.) to be followed.
- l) Sawfly NPVs are only known to infect and replicate in their specific sawfly host. Use buffer and exclusion zones as designated by federal and provincial environmental agencies.

Biological Properties of NeabNPV

- a) NeabNPV has been reported in populations of balsam fir sawfly from Alberta, Saskatchewan, Manitoba, Ontario, Quebec, Ontario (see Olofsson 1972) and Newfoundland (Campbell et al. 2004). Balsam fir sawfly feed on one-year old and older foliage of balsam fir (*Abietis balsamea*). NeabNPV is the principle cause of balsam fir population declines (Moreau et al. 2004) and would be pervasive in the environment surrounding these declining populations.

- b) Balsam fir sawfly, *Neodiprion abietis* (Hymenoptera: Diprionidae). Larval infection is *per os*. NeabNPV virions infect epithelial cells of the larval midgut. Viral replication is only known to occur in the nucleus of midgut epithelial cells of larval sawflies (Federici 1993). The LD₅₀ for NeabNPV against balsam fir sawflies at 7 days post-inoculation (95% confidence limits) for second instars was 87.4 PIBs (7.1-304.9) and 2074.8 PIBs (813.1-3999.0) for third instars. Sawfly larvae tend to feed gregariously. This combined with high virulence contribute to sawfly NPVs being highly contagious amongst host larvae.
- c) NeabNPV is known to only infect and replicate in the midgut cells of balsam fir sawfly larvae. NeabNPV may cause mortality in other sawfly larvae, specifically *Acantholyda erythrocephala* (pine false webworm, Pamphiliidae), *Diprion similis* (introduced pine sawfly, Diprionidae), *Gilpinia hercyniae* (European spruce sawfly, Diprionidae), *Pristiphora geniculata* (mountain ash sawfly, Tenthredinidae). NeabNPV does not appear to replicate in these other sawfly species.
- d) Sawfly NPVs only infect the midgut epithelium of host sawflies (Federici 1993). Following the virus replicative cycle, infected cells, containing PIBs, are sloughed off into the frass and out of the body where they can be ingested and subsequently infect other host insects. Host death normally occurs within a one to two weeks but, during that time, the host produces infective units of the disease.
- e) No plasmids or extra chromosomal DNA. NeabNPV is a naturally occurring pathogen of the balsam fir sawfly and was isolated from that host in Newfoundland in 1997. NeabNPV genome is a covalently closed, double stranded DNA approximately 95 kb in size. This virus has not been subjected to any type of nucleic acid recombination.
- f) NeabNPV can only be produced *in vivo* in balsam fir sawfly larvae. Currently, there are no tissue culture systems available for the production of NeabNPV. Like most baculoviruses, NeabNPV is probably sensitive to UV radiation.
- g) Not known to have any unusual morphological, physiological or biochemical characteristics.
- h) Experimental ground applications of NeabNPV were made against balsam fir sawfly in Ontario in the early 1970s (Olofsson 1972) and extensive field trials have been carried by the Canadian Forest Service in Newfoundland in 2000-2003. Other sawfly NPVs have been used successfully in trials against pest sawflies (Wallace and Cunningham 1995). Registration for two sawfly NPVs for use to control sawfly pests have previously been sought in Canada: Sertifervirus (*Neodiprion sertifer Nucleopolyhedrovirus*, NeseNPV) for European spruce sawfly (*Neodiprion sertifer*) and Lecontvirus (*Neodiprion lecontei Nucleopolyhedrovirus*, NeleNPV) for redheaded pine sawfly (*Neodiprion lecontei*). (See Wallace and Cunningham 1995).
- i) No known relationship to any pathogen of plants or vertebrates. NeabNPV belongs to the Baculoviridae; a family of viruses known only to infect arthropods, mostly insects. (See, Gröner 1986, 1990, OECD 2002 for reviews of the scientific literature).
- j) No known relationship to any known human dermatophyte.
- k) NeabNPV is not related to any toxigenic human pathogen. (See Gröner 1986, OECD 2002 for reviews of the scientific literature).
- l) No adverse effects by baculoviruses to humans or other vertebrates. (See, Gröner 1986, OECD 2002 for reviews of the scientific literature).

NeabNPV Product Characterization and Analysis

Product Manufacture and Formulation

Natural Resources Canada
Canadian Forest Service – Atlantic Forestry Centre
1350 Regent Street
P.O. Box 4000
Fredericton, New Brunswick, E3B 5P7

Proposed Trade Name: Abietiv

Origin, Derivation and Identification of NeabNPV

- a) *Neodiprion abietis Nucleopolyhedrovirus* (NeabNPV, Baculoviridae) (Volkman et al. 1995).
- b) In August 1997, balsam fir sawfly larvae were collected from two plots near Corner Brook, Newfoundland (Ecozone 5). These insects were reared in a laboratory at the Canadian Forest Service in Fredericton, New Brunswick and larvae that died in rearing were examined for the presence of NeabNPV. This virus was found in a number of larvae and was isolated. Amplification of NeabNPV was carried out at the Pasadena Field Station in July and August in 1998 and 1999. Here, larvae were reared on balsam fir foliage in 5-L plastic tubs. NeabNPV was applied to the foliage and dead insects were picked from the foliage, by hand, and were frozen. Since 1999, NeabNPV has been mass produced according to the method described below.
- c) Stock isolates of NeabNPV are held at either 4°C or -20°C.

Manufacturing Methods and Quality Assurance

Preservation and Maintenance of the Productive Strain

Balsam fir sawfly larvae infected with NeabNPV from the original collection area have been frozen and stored at -20°C.

Manufacturing Processes

Semi-purified NeabNPV PIBs in 20% aqueous molasses (commercial grade) are applied to balsam fir trees infested with balsam fir sawfly larvae using fixed-wing aircraft, helicopters, motorized ground-sprayers and/or backpack sprayers up to the equivalent concentration of 1×10^{10} PIBs/ha. Collections of larvae begin at the first sign of larval mortality (about 7 days after application) and continue for the next 10 days. Larvae are knocked onto tarpaulins placed under balsam fir trees by beating the tree branches with a 2-m length of pruning pole. Collected larvae are transferred to 50-lb brown paper bags so that the bags are one-third filled with larvae and fir needles. Three, 30-cm, branch tips cut from balsam fir trees are added as a source of food and an additional 2-3 mL of NeabNPV PIBs suspended in water (1×10^7 PIBs/mL) is misted onto the foliage. The bag tops are folded over and stapled shut. The larvae are left in the bags to die or finish their development at ambient laboratory temperatures (18-20°C). Following the death of the larvae, the branch tips are removed and the contents of three bags are placed into a single, clean 50-lb brown paper bag. These bags are stapled shut and are stored in the laboratory at ambient temperature (18-20°C). By this time the needles and dead larvae are quite dry. Dead larvae, from these bags, are picked out from the needles,

by hand, and are placed into 50-mL centrifuge tubes and frozen at -20°C. NeabNPV from the dead larvae are purified using the method described below.

NeabNPV Isolation Protocol - Large Scale.

1. Thaw and re-hydrate NeabNPV-infected balsam fir larvae in water, overnight.
2. Homogenize larvae in a 1000 mL beaker using a hand held blender.
3. Dilute with water and add 1% SDS to a concentration of 0.3% (final volume approximately 10 times the volume of dead larvae).
4. Add magnetic stirrer bar and stir for 60 min.
5. Filter through plastic mesh, save filtrate (contains NeabNPV).
6. Resuspend solid debris in 0.3% SDS and stir as in step 4 for 5 min.
7. Filter again through plastic mesh and repeat until clear filtrate is obtained.
8. Filter NeabNPV suspension through 8 layers of cheesecloth.
9. Centrifuge for 15 min in a Sorvall RC 28S centrifuge and HS-4 rotor (or equivalent at approximately 2000 x g).
10. Discard supernatant and add more of the NeabNPV suspension to centrifuge tubes, repeat steps 9 and 10 until all the NeabNPV suspension has been used.
11. Resuspend NeabNPV PIB pellets in 0.3% SDS and vortex.
12. Repeat centrifugation and resuspended until a clear supernatant is obtained.
13. Resuspend and pool NeabNPV PIB pellets.
14. Resuspend pellet in 0.5M NaCl. Centrifuge.
15. Resuspend pellet in water (5 – 10 x volume of pellet).

NeabNPV suspensions are stored in water at 4°C to inhibit growth of contaminating bacteria. To further reduce unwanted bacterial propagation, NeabNPV suspensions are only added to the hooper on the aircraft containing the 20% aqueous solution of molasses immediately prior to the aircraft taking-off to the spray site in field applications.

Quality Assurance

NeabNPV PIBs are too small (0.5-1.0 µm) to be counted accurately using a hemocytometer. Instead, PIBs are quantified by combining a known volume of an unknown concentration of NeabNPV PIBs with a known volume of a known concentration of latex beads (2.97 µm diameter, SD 0.04). Latex beads and PIBs from 50 fields of view on each of four slides are counted under the 100X oil lens of a compound microscope. The concentration of PIBs is determined as a proportion of the number of latex beads counted. The volume of the supernatant is adjusted to give a final concentration of 1.4×10^9 PIBs/mL. When a 40 mL volume of NeabNPV at this concentration is placed in a 50-mL centrifuge tube, there is sufficient virus to spray 160 ha at an application rate of 1.0×10^9 PIBs/ha in a mix volume of 2.5L/ha (400 L total mixture volume).

Potency Estimation

The potency of NPVs is expressed in terms of the number of PIBs that provide a lethal dose to 50 percent of test insects (LD_{50}). The LD_{50} at 7 days post-inoculation (95% confidence limits) for second instars was 87.4 PIBs (7.1-304.9) and 2074.8 PIBs (813.1-3999.0) for third instars. In aerial application trials against lepidopteran larvae, the range has been in the order of 1.6×10^{10} to 4.4×10^{12} PIBs/ha (Cunningham and Kaupp 1995, Wallace and Cunningham 1995).

For sawfly NPVs the range has been 1.3×10^9 to 3.9×10^{11} PIBs/ha (Cunningham and Kaupp 1995, Wallace and Cunningham 1995). In field efficacy trials of NeabNPV, we have found that applications of $1-3 \times 10^9$ PIBs/ha can result in significant declines in sawfly populations in spray blocks compared to controls, in the year following NeabNPV application. In the year of the application, numbers of balsam fir sawfly larvae/m² foliage do not necessarily decline to a greater extent in the application versus control blocks but, the levels of NeabNPV infection is generally much higher in the application blocks following the spray. Timing of the applications is important. Earlier instars (first and second) are more susceptible to the virus than are the later instars. Also the stage of the population cycle at the time of the application can also affect NeabNPV efficacy. Generally, it can be said that, applications against first and second instar balsam fir sawfly larvae prior to peak population levels will be more effective than at any later stage.

Unintentional Ingredients

Baculoviruses are obligate pathogens of insects and can only be produced in the host species, a susceptible insect usually closely related to the host or in an *in vitro* insect cell line. Sawfly NPVs are highly host specific and no *in vitro* cell line has been developed for any sawfly or sawfly NPV. Thus, sawfly NPVs must be produced in the host sawfly. Sawfly NPVs only replicate in the midgut epithelium of the host species. Lepidopteran NPVs, on the other hand, initially infect the larval midgut but, then the infection spreads to other tissues such as the fat body and hemocytes. Many lepidopterans can be reared on artificial diets, this is not true of sawflies. The inability to rear sawfly larvae on artificial diets means that the larvae must be reared on host plant foliage and limited tissue tropism means that the amount of virus produced per larva is lower than what one would get from a lepidopteran larva. The advantage of sawfly NPVs is that they are highly communicable between their hosts and aerial application rates can be one or two orders of magnitude lower than those for lepidopterans (Cunningham and Kaupp 1995, Wallace and Cunningham 1995). To produce sawfly NPVs economically, it must be done in the field (Cunningham and MacPhee 1986). We have reared balsam fir sawfly larvae, on foliage, in the laboratory, intensively for up to six months and have produced sufficient NeabNPV to spray 2,000 ha at a rate of 1.0×10^9 PIBs/ha. Using aerial applications in the field, we have produced this much from collecting NeabNPV-infected larvae in one week from 1 ha. Field production, however, does result in contamination by microbes that are part of the microflora of the environment from which the larvae were collected. Also, since the aircraft used to apply NeabNPV are also used to spray *Bacillus thuringiensis*, this bacterium may also contaminate the final product. The current mixing procedure for NeabNPV is 1 ml of NeabNPV at 4×10^9 PIBs/mL in 2-10 L 20% aqueous molasses. Thus, the dilution factor for the NeabNPV suspension and any contaminating microbes is 2,000 to 10,000 times and 2.5 L of this are applied to a hectare of forest. NeabNPV is only added to the molasses carrier immediately before the aircraft taxis for take-off to the spray block. Waiting to add NeabNPV to the molasses just before take-off reduces the time during which any contaminating bacteria would have to reproduce.

As part of the manufacturing process, filtration and centrifugation are used to remove as much insect tissue and other contaminating debris as possible. However, cells, tissues, fats, proteins, nucleic acids and other materials derived from the insect host may remain in the product following these steps in the manufacturing process. As a precaution against the inadvertant

inclusion of vertebrate pathogenic microbes and/or metabolites, each batch of NeabNPV product is sent to accredited laboratories for bacterial screening and mouse intraperitoneal injection assays.

Analysis for Microbial Contaminants

Microbial contaminant analyses have most recently been conducted by IG MicroMed Environmental Inc., Richmond, B.C. This company is accredited by Standards Council of Canada (SCC) for most of the test methods of interest (DACO). We will continue to use them or an equivalent company to test for microbial contaminants. The references for the methods used are listed below.

Total Aerobic Bacteria

MFHPB-18. Determination of the aerobic colony count in foods. October 2001. Health Products and Food Branch, Ottawa, ON. In: *Compendium of Analytical Methods*. http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_2/e_mfhp1801.html

Total Coliforms, Fecal Coliforms, *Escherichia coli*

MFHPB-19. Enumeration of coliforms, faecal coliforms and of *E. coli* in foods using the MPN Method. April 2002. In: *Compendium of Analytical Methods*. Health Products and Food Branch, Ottawa, ON. http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_2/e_mfhp1901.html

Fecal *Streptococcus/Enterococcus*

Standard Methods for the Examination of Water and Wastewater, 20th Ed. 1998. Section 9230 Fecal *Streptococcus* and *Enterococcus* Groups. Part 9000, Microbiological examination.

Salmonella spp.

MFHPB-20. Isolation and identification of *Salmonella* from foods. April 1998. In: *Compendium of Analytical Methods*. Health Protection Branch, Ottawa, ON. http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_2/e_mfhp2001.html

Shigella spp.

BAM Chapter 6, *Shigella*. January 2001. In: *U.S. Food and Drug Administration – Bacteriological Analytical Manual (BAM)*. Center for Food Safety & Applied Nutrition.

Aerobic Sporeformers

MFLP-44. Determination of aerobic and anaerobic sporeformers. April 1998. In: *Compendium of Analytical Methods*. Health Protection Branch, Ottawa, ON. http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_3/e_mflp4401.html

Bacillus cereus

MFLP-42. Isolation and enumeration of *Bacillus cereus* in foods. April 2003. In: *Compendium of Analytical Methods*. Health Products and Food Branch, Ottawa, ON. http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_3/e_mflp4201.html

Staphylococcus aureus

MFHPB-21. Enumeration of *Staphylococcus aureus* in foods. November 2000. In: *Compendium of Analytical Methods*. Health Protection Branch, Ottawa, ON. http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_2/e_mfhp21e01.html

Vibrio sp. (*cholerae*)

BAM Chapter 9, *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and other *Vibrio* spp. January 2001. In: *U.S. Food and Drug Administration – Bacteriological Analytical Manual (BAM)*. Center for Food Safety & Applied Nutrition. <http://www.cfsan.fda.gov/~ebam/bam-toc.html>

Molds and Yeast

MFHPB-22. Enumeration of yeasts and moulds in foods. March 2002. In: *Compendium of Analytical Methods*. Health Protection Branch, Ottawa, ON. http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_2/e_mfhp2201.html

Vertebrate Pathogens

Mouse IP injections are conducted according to Good Laboratory Practices (GLP) at an accredited laboratory. Observation period, seven days. Currently carried out by Alberta Research Council, Hwy 16A & 75 Street, Postal Bag 4000, Vegreville, AB.

Summary of Physical and Chemical Properties

- a) Physical state; solids (NeabNPV PIBs, approximately 0.5-1.0 µm diameter) suspended in water.
- b) Density, bulk density of specific gravity; not applicable.
- c) Viscosity; water, 1.0
- d) Corrosion character; not corrosive
- e) Suspendibility; PIBs suspended in water.

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Wallace, D. R. and J. C. Cunningham. 1995. Diprionid sawflies. *In*: J. A. Armstrong and W. G. H. Ives (editors). Forest Pest Insects in Canada. Natural Resources Canada. Ottawa, Ontario. Pp. 193-232.

DRAFT MATERIAL SAFETY DATA SHEET

Product name: Abietivirus

Chemical name: Balsam fir sawfly (*Neodiprion abietis*) nucleopolyhedrovirus (NeabNPV)

Formula: Biological organism, virus, Baculoviridae: Nucleopolyhedrovirus

Molecular weight: Not applicable

Synonyms: Balsam fir sawfly nuclear polyhedrosis virus

Chemical family: Not applicable

I. Physical data

Boiling point: Not applicable

Freezing point: Not applicable

Specific gravity: Not applicable

Vapour pressure: Not applicable

Vapour density: Not applicable

Evaporation rate: Not applicable

% volatiles by volume: None

Solubility in water (% by wt): Insoluble

Appearance and colour: brownish suspension, musty odor.

II. Ingredients

Balsam fir sawfly nucleopolyhedrovirus (NeabNPV) polyhedral inclusion bodies (PIBs) 1.5%
Water 98.5%

Impurities: Proteins, fats, cells, tissues, cuticle from host insect (balsam fir sawfly).

This nucleopolyhedrovirus is non-toxic to vertebrate animals. Impurities may cause eye irritation.

III. Fire and explosion hazard data

Flash point: Not applicable

Flammable limits: Not applicable

Extinguishing media: Water

Special fire fighting procedures: None except to avoid inhalation of particulates released by fire.

Unusual fire and explosion hazards: Not applicable

IV. Health hazard data

Oral: A single dose of 1×10^8 PIBs by oral gavage showed no evidence of acute oral toxicity or pathogenicity to Sprague-Dawley[®] rats (average initial weights 101-124 g).

Intravenous injection: A single dose of 1×10^7 PIBs by intravenous injection showed no evidence of acute injection toxicity or pathogenicity to Sprague-Dawley[®] rats (average initial weights 112-129 g).

Inhalation: A single dose of 1×10^8 PIBs by intratracheal instillation showed no evidence of acute pulmonary toxicity or pathogenicity to Sprague-Dawley[®] rats (average initial weights 123-148 g).

Dermal: A single topical dose of 2 g NeabNPV/kg body weight showed no evidence of acute

dermal toxicity or pathogenicity to New Zealand white rabbits.

EMERGENCY AND FIRST AID PROCEDURES:

Remove from exposure situation. If in eyes, flush with plenty of water. If irritation persists, get medical attention. If on skin, wash with soap and water.

NOTES TO PHYSICIAN

Prolonged exposure may cause allergies and hypersensitivity in some individuals.

V. Reactivity data

Stability: Stable

Conditions to avoid: Do not store in direct sunlight (ultraviolet sensitive) or at temperatures above 30°C.

Incompatibility: Not applicable

Hazardous decomposition products: May contain bacteria

VI. Spill or leak procedures

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED

Containment and cleanup by placing in a sealable container for transport to an approved disposal site.

WASTE DISPOSAL METHOD

Triple rinse containers and dispose of them at an approved site. Washing waste from this product may be disposed of on site or at an approved disposal site.

VII. Special protection information

Respiratory protection: Medical face mask as appropriate.

Ventilation: Use in areas with good ventilation

Protective clothing: Coveralls, gloves, safety goggles and medical face mask are required for mixers.

Other protective equipment: Have eyewash, soap and water available at project location.

VIII. Special precautions

KEEP OUT OF REACH OF CHILDREN.

1. Avoid direct application to bodies of water.
2. Do not contaminate water, food or feed by inappropriate storage and disposal.
3. Only for use as a biological insecticide for balsam fir sawfly control programs limited to forestry.
4. Avoid heat and direct sunlight.
5. Other handling and storage conditions: Wastes from this product may be disposed of on site or at an approved waste disposal facility.
6. Do not reuse empty containers but arrange for disposal in a sanitary landfill or by incineration.

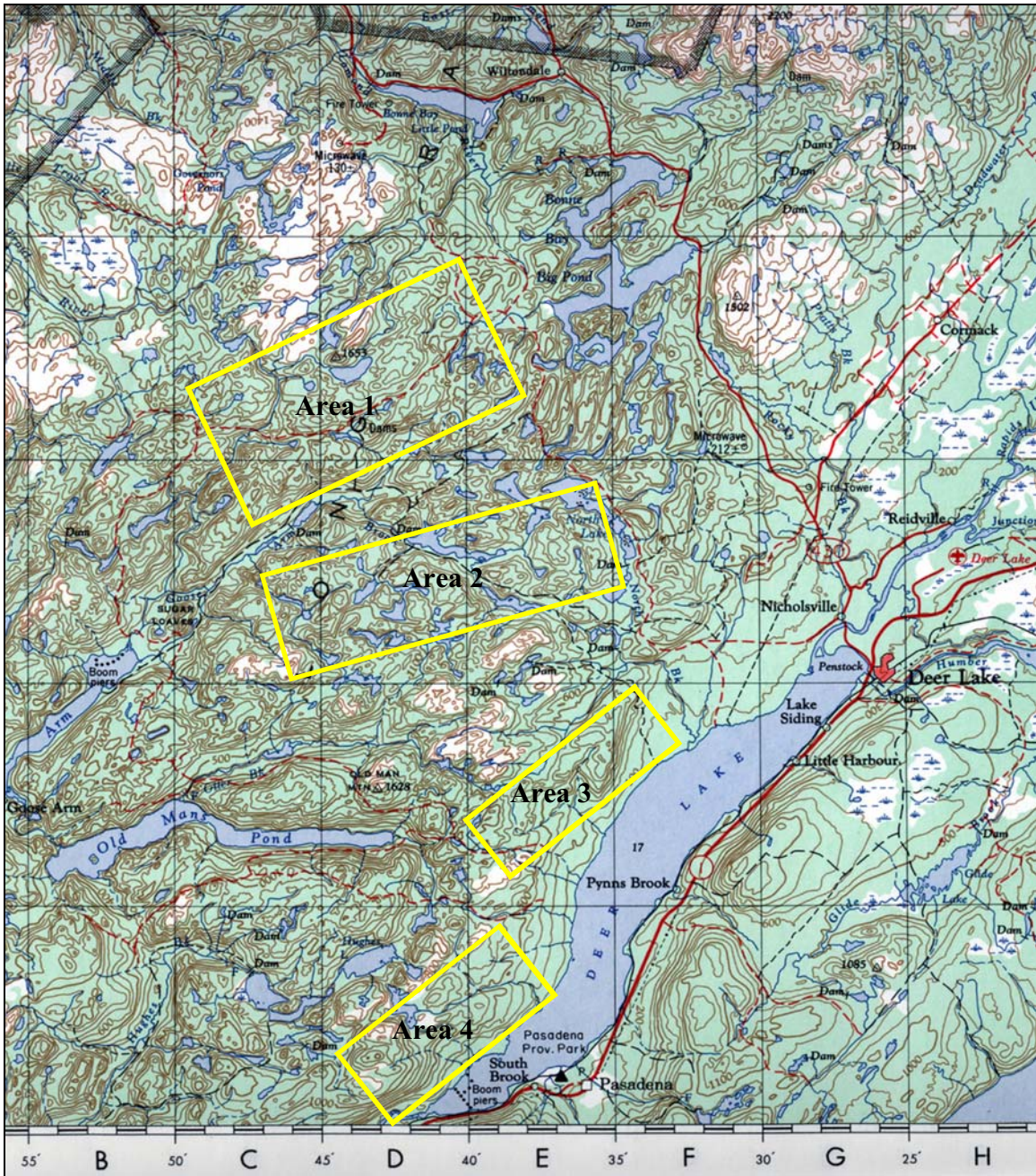


Figure 1. Map showing the four areas in which trial spray blocks to be treated with NeabNPV (balsam fir sawfly nucleopolyhedrovirus) will be established. Spray blocks will be established within these areas based on balsam fir sawfly egg surveys carried out by the NL Department of Natural Resources, restrictions as dictated by the NL Department of Environment and the Pest Management Regulatory Agency (Health Canada) and accessibility. The total area to be sprayed will not exceed 5,000 hectares.

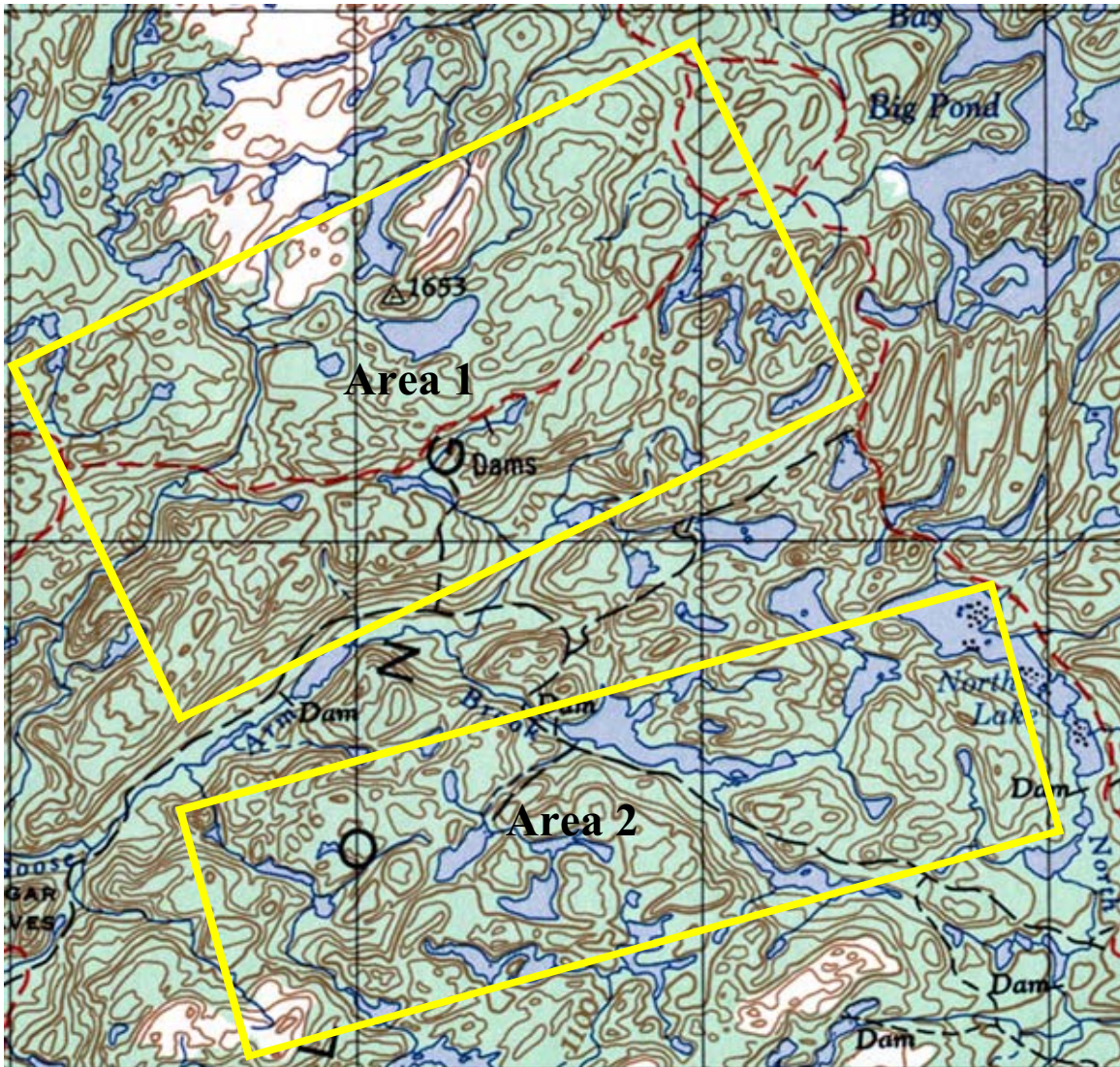


Figure 2. Proposed NeabNPV spray areas 1 and 2. (See figure 1 for locations on a larger map).

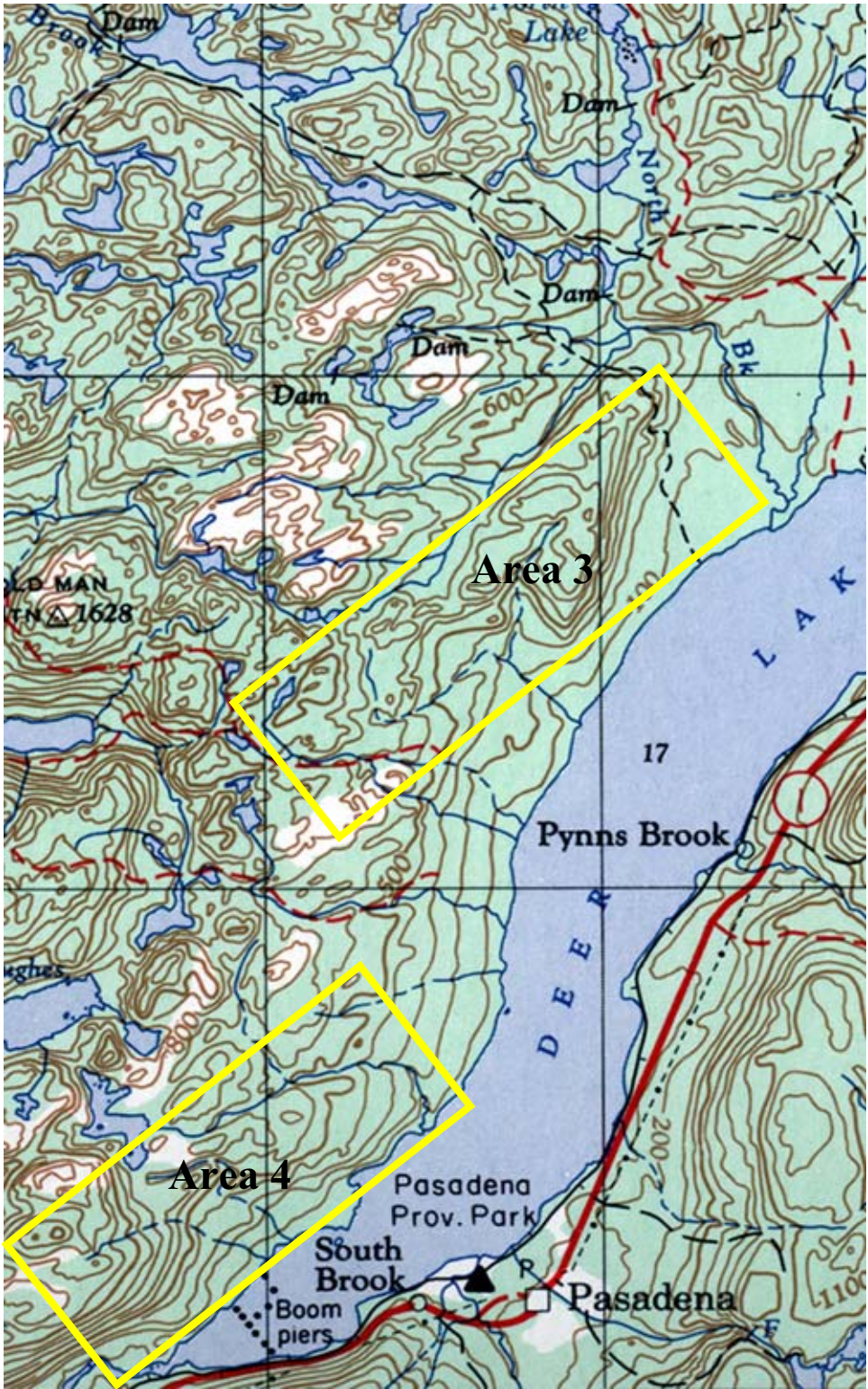


Figure 3. Proposed NeabNPV spray areas 3 and 4. (See figure 1 for locations on a larger map).



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GOVERNMENT OF NEWFOUNDLAND AND LABRADOR
Department of Environment
Pesticides Control Section

Pesticide Operator Licence

Pursuant to *The Environmental Protection Act SNL 2002 cE-14.2*
and *The Pesticides Control Regulations, O.C. 96-957*
and subject to the attached stipulations, this licence
is issued to :

Forest Protection Limited

This Licence is valid from

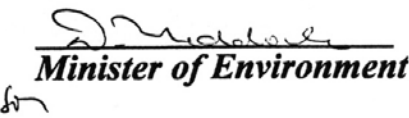
January 20, 2004

to

December 31, 2004

Valid For:
Aerial

Licence No. 04-086


Minister of Environment



Health
Canada

Santé
Canada

Pest
Management
Regulatory
Agency

Agence de
réglementation
de la lutte
antiparasitaire

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834.1 RP
Sub. No. 2003-3040

May 7, 2004

by fax: (506) 452-3525 (original by mail)

Dr. Chris Lucarotti
Natural Resources Canada
Canadian Forest Service - Atlantic Forestry Centre
1350 Regent Street, P.O. Box 4000
Fredericton, NB E3B 5P7

Dear Dr. Lucarotti:

Re: **Sub. No. 2003-3040 Research Permit for Abietivirus containing the active ingredient Neodiprion abietis nucleopolyhedrovirus (NDP) for the control of Balsalm fir sawfly on Balsalm fir**

The Pest Management Regulatory Agency (PMRA) has now completed the review of your application for a Research Permit of November 20, 2003 which was received on December 1, 2003. Your application is acceptable and Research Permit Number 35-RP-04 has been assigned to Research Permit Submission Number 2003-3040.

The following conditions and comments apply to this research permit:

Label Revisions:

- On the principal display panel, add the strain of the active ingredient to the GUARANTEE statement.
- Under DIRECTIONS, delete the current statement "Apply 1-5 billion (10^9) PIBs/hectare of Abietivirus in 2.5 L of aqueous solution of molasses per hectare" and replace with the following statement:
Aerial application. Apply 1-5 billion (10^9) PIBs of Abietivirus in 2.5 L of aqueous solution of molasses per hectare.
- Under PRECAUTIONS, delete the word "THE" in the statement "KEEP OUT OF THE REACH OF CHILDREN" statement.

Comments on Quality of Submission:

- For future submissions, please ensure that all studies include the appropriate controls and control data. Please also include all relevant information regarding microbial contaminant analysis testing (e.g., describe methods, raw data).

.../2

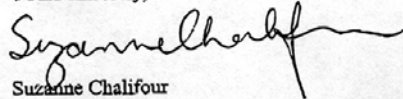
Canada 

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Dr. Chris Lucarotti
May 7, 2004

A copy of your experimental label is enclosed along with the Research Permit. Posters listing warnings, permit number and a contact person must be posted at each research site (refer to the attached pages for further information on this).

Should you have any questions or comments regarding this review please do not hesitate to contact the Administrative Coordinator for this submission, Catharine Hooper, at 613 736-3469.

Yours sincerely,



Suzanne Chalifour
Acting Director
Efficacy and Sustainability Assessment Division

Attachments

cc Barb Szegvary, CROS, Compliance, Lab Services and Regional Operations Division (CLSROD)
Neil McTiernan, Regional Manager, Atlantic Region, CISROD (*for use in Newfoundland*)



Pest Management Regulatory Agency
Agence de réglementation de la lutte antiparasitaire

APPLICATION FOR RESEARCH PERMIT
Under the Pest Control Products Act

DEMANDE DE PERMIS DE RECHERCHE
Aux termes de la Loi sur les produits antiparasitaires

NOTE: TYPE OR PRINT CLEARLY.
LEAVE SHADED AREAS BLANK.

NOTA: ÉCRIRE LISÈLEMENT OU DACTYLOGRAPHIER.
NE PAS ÉCRIRE DANS LES ESPACES OMBRÉS.

1. Product name or experimental no./Marque de commerce ou n° d'essai du produit Abietivirus	2. Reg. No. if appl./N° d'homol. si appl.	Submission no. N° de la demande 3003-3090 Date Received Date de réception 01 DEC 2003 Research permit no. N° permis de rech. 35-PP-04 Registrant code Code du titulaire FPM Active ingred. code Code matière active NDP
3. CSA common name - Chemical name and percent for each active ingredient Nom commun (CSA), formule chimique et pourcentage de chaque matière active Neodiprion abietis nucleopolyhedrovirus - (NeabNPV)		
4. Name of applicant/Nom du demandeur Natural Resources Canada Canadian Forest Service Address/Adresse 1350 Regent Street P.O. Box 4000 Fredericton NB Canada E3B5P7	Tel. no./N° de tél. (506)-452-3538 Fax. no./N° de télécopieur (506)-452-3525	
5. Name of research coordinator/Nom du coordonnateur de la recherche Dr. Christopher Lucarotti Address/Adresse as above	Tel. no./N° de tél. (506)-452-3538 Fax. no./N° de télécopieur (506)-452-3525	
6. Name of supplier/Nom du fournisseur Dr. Christopher Lucarotti Address/Adresse as above	Tel. no./N° de tél. (506)-452-3538 Fax. no./N° de télécopieur (506)-452-3525	

7A. Quantity of product distributed or obtained by region/Quantité de produit distribuée ou obtenue par région

7B. Points of entry/Points d'entrée
NA

8A. Host or crop being treated/Hôte ou culture traitée Balsam fir (Abies balsamea)	8F. Additional directions/Directives supplémentaires
8B. Pest/Agent nuisible Balsam fir sawfly (Neodiprion abietis)	
8C. Rate/Taux 1 billion NeabNPV occlusion bodies per hectare (a 25-50 ha area will be treated at 3 billion occlusion bodies per hectare in order to produce more NeabNPV for later use).	9. Preharvest interval/Délai d'attente avant récolte
8D. Timing and number of applications (testing dates where possible) Calendrier et nombre d'applications (dates des applications si possible) Single application mid- to late July	10. Purpose/But Residue <input type="checkbox"/> Résidus <input checked="" type="checkbox"/> Efficacy <input checked="" type="checkbox"/> Efficacité <input type="checkbox"/> Environment <input type="checkbox"/> Environnement <input type="checkbox"/> Other / Autre <input type="checkbox"/> Autre
8E. Application equipment to be used/Équipement d'application à être utilisé aircraft	11. Exact location, size & number/Région exacte, grandeur et numéro western Newfoundland between Corner Brook and Deer Lake Total 5000
13. Indicate if the following have been submitted/Indiquer si les renseignements suivants ont été soumis	12. Type of research/Genre de recherche <input type="checkbox"/> New use of reg. product <input type="checkbox"/> Additional Residue Data Nouvel emploi d'un <input type="checkbox"/> Données additionnelles sur <input type="checkbox"/> New Formulation <input checked="" type="checkbox"/> New active/New Source Nouvelle Formulation <input checked="" type="checkbox"/> Nouvelle matière active/Nouvelle source

A. Product Specification form <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N	C. Material Safety Data Sheets <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N	E. List of cooperators and researchers <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N	G. Data (specify) <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N
B. Experimental label <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N	D. Maps/Cartes <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N	F. Protocols/Protocoles <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N	H. Other (specify)/Autres (spécifier) <input checked="" type="radio"/> Y/O <input checked="" type="radio"/> N/N

14. I hereby specify that the above information is correct in all respects. I understand that the grant of a permit does not create any liability on the Crown and the applicant remains totally liable for such things as damage to treated crops or properties on the crops or property of others and for such matters as occupational health and safety and environmental impact as a result of this research being performed.

Nous attestons que les renseignements sur cette formule sont exacts sous tous les rapports. Nous comprenons que la délivrance d'un permis ne constitue aucune responsabilité de la part de la Couronne et que le demandeur est totalement responsable pour certaines répercussions de cette recherche telles que les dommages infligés aux cultures ou aux propriétés traitées ou aux cultures ou propriétés appartenant à une autre personne ainsi que pour d'autres sujets tels que la santé professionnelle, l'innocuité et l'impact sur l'environnement.

Signature of Name of Applicant/Signature ou nom du demandeur: Lucarotti

Email Address/cour élec.: clucarot@nrccan.gc.ca

15. Date of application/Date de la demande: 03/11/20

16. Comments (reserved for use of Federal Government Officials)/Commentaires (réservé à l'usage des agents du gouvernement fédéral)

Please refer to accompanying letter and experimental label for conditions pertaining to this research permit.

Pursuant to the application and attachments and subject to amendments (if any) made therein, authorization is granted to proceed with research involving the specified pest control product. This issuance of this federal permit does not imply provincial approval. It is the applicant's responsibility to obtain provincial approval of pesticides research trials where this may be required. Crops harvested from treated plots should not be sold for food purposes unless written authorization is obtained from the PMRA.

Sur la foi des renseignements contenus dans la formule et les documents en annexe et sous réserve, (s'il y a lieu) des modifications qui leur sont apportées, le demandeur est autorisé à entreprendre la recherche sur le produit antiparasitaire susmentionné. La délivrance de ce permis n'implique pas l'approbation provinciale. Le demandeur a la responsabilité d'obtenir l'approbation de la province pour les essais de recherche sur les pesticides lorsque cela est nécessaire. Les cultures vivrières récoltées sur les parcelles traitées ne devraient pas être vendues comme aliment à moins qu'une permission écrite ne soit obtenue de l'ARLA.

This authorization of research is for the year ending: 31 DEC 2004

La présente autorisation prend fin le: 31 DEC 2004

Reviewing Officer/Agent responsable: Suzanne...

Code: 1

Date: 17 May 2002