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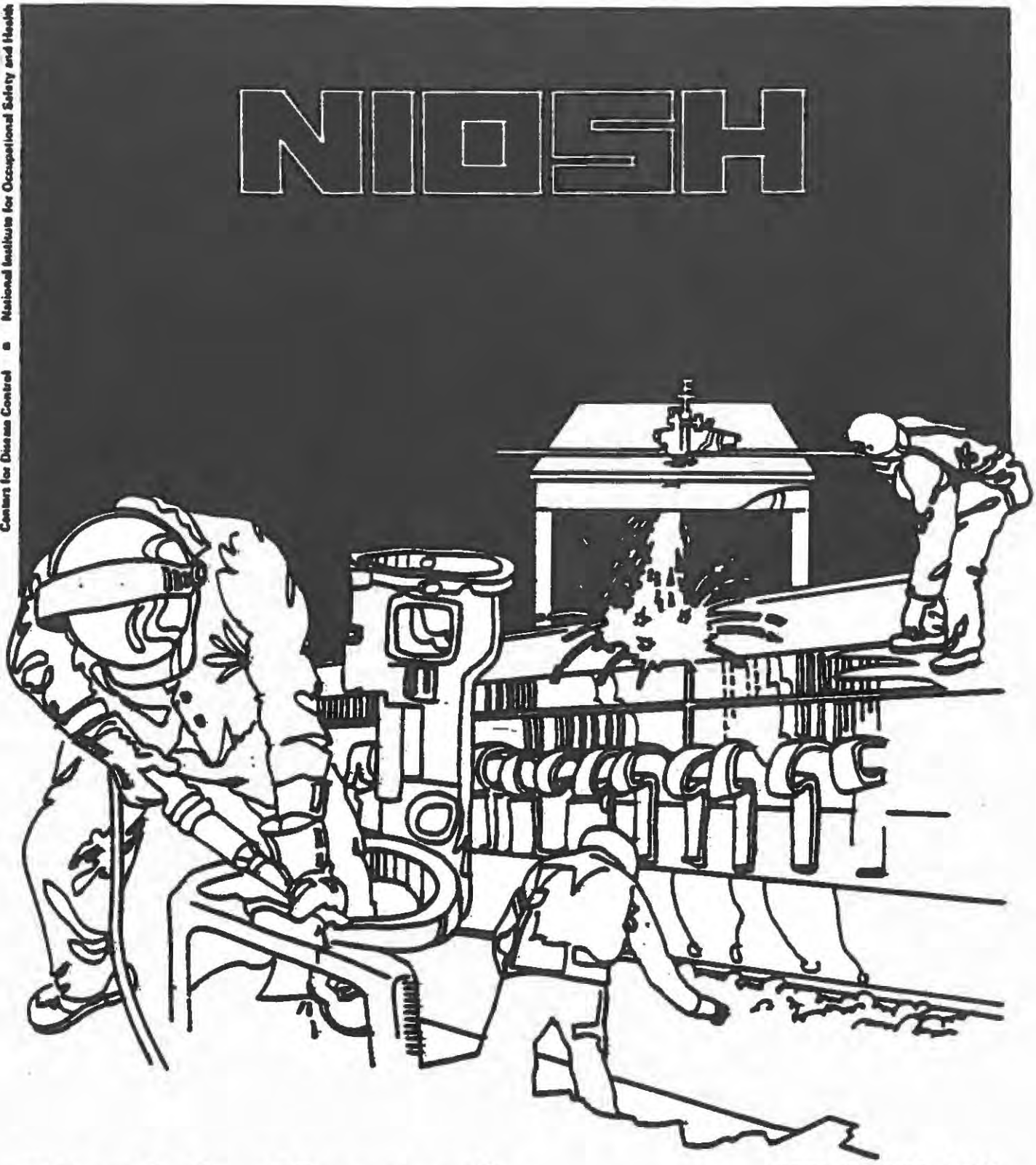

Health Hazard Evaluation Report

HETA 85-309-1739
OREGON DEPARTMENT OF HUMAN RESOURCES
HEALTH DIVISION
GYPSY MOTH CONTROL PROJECT
EUGENE, OREGON

PREFACE

The Hazard Evaluations and Technical Assistance Branch of NIOSH conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employer or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

The Hazard Evaluations and Technical Assistance Branch also provides, upon request, medical, nursing, and industrial hygiene technical and consultative assistance (TA) to Federal, state, and local agencies; labor; industry and other groups or individuals to control occupational health hazards and to prevent related trauma and disease.

HETA 85-309-1739
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OREGON DEPARTMENT OF HUMAN RESOURCES
HEALTH DIVISION
GYPSY MOTH CONTROL PROJECT
EUGENE, OREGON

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I. SUMMARY

On April 10, 1985, the National Institute for Occupational Safety and Health (NIOSH) was requested by the State Department of Human Resources, Health Division, Portland, Oregon to assist in the evaluation of occupational and general public exposure to Bacillus thuringiensis (Bt). Bt is a microbial agent used as an insecticide in the suppression and control of the gypsy moth, Lymantria dispar.

Approximately 250,000 acres of forest, rural, and urban areas were to be sprayed with Bt. The public health surveillance and infection monitoring programs, and safety procedures of the mixing and spraying application were reviewed on April 29 through May 1, 1985. A follow up survey was conducted June 2 through 6, 1986 during the second year spray application. Air sampling was conducted during both years to determine the occupational and general public exposure potential to the Bt during these spray operations.

The air sampling for Bt was conducted using MSA Model G and DuPont P-200 portable sampling pumps with 37-mm (0.8 micron pore size) cellulose ester filters as the collection media. The sampling duration ranged from 15 minutes to 4 hours at flow rates of 0.1 to 2.0 liters/minute with an open-face filter technique. The filter samples were analyzed for Bt by microbial culture technique. The sample results for the 1985 survey were deficient due to the inability to quantitate the positive cultures, and a blank sample contamination problem; however, all personal breathing zone samples were Bt positive. The sampling methodology and technique was modified for the 1986 survey to improve quantitation and eliminate sample contamination. Air samples collected before the 1986 application were all Bt negative. Air sample results collected in the personal breathing zone of individuals within the spray boundaries in 1986 ranged from 0 to 11,000 colony forming units of Bt/m^3.

Based on the air monitoring data collected, Bt exposure potential existed for various occupations and the general public within the spray area during, and for some time after, Bt application. The Oregon State Health Division should be consulted for results of the ongoing medical surveillance. Recommendations are offered in Section VIII concerning safety aspects of the mixing and application of Bt, and for future studies concerning biological pesticides.

Key Words: SIC 0851, Bacillus thuringiensis, microbiological insecticide, Gypsy Moth Control

II. INTRODUCTION

On April 10, 1985, the National Institute for Occupational Safety and Health (NIOSH) received a request for technical assistance from the Oregon State Department of Human Resources, Health Division, in Portland, Oregon. The Oregon Department of Agriculture was preparing to spray approximately 250,000 acres of forested and urban areas of Lane County Oregon with the biological insecticide, Bacillus thuringiensis (Bt), in an attempt to suppress and control the gypsy moth, Lymantria dispar. The Oregon Health Division had assumed the task of providing information to health providers and the general public regarding the spraying of the insecticide, as well as monitoring for attributable adverse health effects in the area. NIOSH was requested to assist in the planning and implementation of surveillance and monitoring activities of workers and the general public in the spray area. NIOSH researchers are interested in the use of microbial pesticides with respect to characterizing and documenting exposure potential to these agents. There are no regulatory standards or exposure evaluation criteria for microorganisms, therefore it is important to document exposure potential and determine if adverse health effects occur as a result of exposure to these agents.

A review of health surveillance and infection monitoring programs, safety procedures, and observations of the mixing preparation and application of the microbial pesticide was conducted on April 29 through May 1 and on May 13 through May 16, 1985. A follow-up survey was conducted June 2 through 6, 1986 during the second year of Bt spray application. The spray boundaries were slightly different the second year, although the acreage to be sprayed remained approximately 250,000 acres. Air sampling was conducted during both years to determine the occupational and general public exposure potential to the Bt during the mixing preparations and application of Bt. An interim report was provided to the Oregon Department of Human Resources, Health Division in February, 1986.

III. BACKGROUND

The gypsy moth is one of the most destructive insect pests for trees and shrubs in the United States. Since its introduction in the United States in 1869, the gypsy moth has extensively infested areas of 13 northeastern and mid-atlantic states. These infestations are of large proportions. Smaller, localized, infestations have been found in recent years in 17 other states across the nation. When the chemical insecticide DDT was available, the gypsy moth was almost eradicated from the northeastern United States, but as DDT use was curtailed, gypsy moth infestations became widespread.[1]

Currently, there are three EPA approved treatment procedures effective for the eradication of the gypsy moth. These consist of mass trapping and removal of the male moth, the use of biological insecticides, and the use of synthetic chemical insecticides. The state of Oregon chose, over the other options, to primarily use the biological insecticide Bt for its gypsy moth control project. Mass trapping was decided to be an ineffective alternative due to the size of the infected area. The use of synthetic chemical insecticide was limited because of obvious public health and environmental concerns. Bt was the selected biological insecticide because of its apparent low level toxicity to humans and fauna and its proposed effectiveness in large scale applications. Bt is a naturally occuering organism which is not prominently indigenous to all soils or environments. After approximately two decades of use as an insecticide, only two case reports of human infection by this organism have been reported; a corneal ulcer as a result of Bt, Kurstaki solution splashed in the eye and a localized infection of the hand as a result of an accidental innoculation with Bt, israelinsis and Acinetobacter calcoaceticus suspension.[2,3] Bt has been reported to be a mammalian pathogen in only one case where it was identified as the causal agent in a fatal case of bovine mastitis.[4]

Bt is very specific in its insecticidal activity affecting only insects having a caterpillar stage in their developmental cycle. The most destructive stage of the gypsy moth is the larvae or caterpillar. The distinctive feature of Bt is the presence of a bipyramidal crystal produced in the endospores during the dormant stage of the organism's life cycle. This crystal, delta endotoxin, is the principal active ingredient in Bt insecticidal formulations. To produce the formulation Bt, also known as Dipel®, is mixed with water and Plyac®, an emulsifier and dispersion adjuvant. The Bt formulation is applied by aerial spraying as a mist which settles on the foliage of the infested area. The Bt, and the toxin crystal, is ingested by the larvae as it feeds on treated foliage. The toxin crystal is comprised of a protein which is insoluble in acidic, but soluble in alkaline conditions. The alkalinity of the insect's mid-gut dissolves the protein resulting in paralysis and death of the insect.[5]

The Oregon Department of Agriculture, as part of its Integrated Pest Management Program (IPM) of 1984, trapped 16,000 male gypsy moths within Lane County, Oregon. This was evidence of a well established infestation of sufficient magnitude that officials felt control was in order. Failure to prevent the spread of the infestation would precipitate quarantine, inspection, and possible substantial economic loss of forest and agricultural products. Therefore, the Gypsy Moth Control Program for Lane County was developed and initiated during the months of April, May, and June, 1985. This program was staffed and operated by personnel from 3 state agencies, 4 federal agencies, and 2 aerial application contractors. The 1985 program's strategy to control the gypsy moth consisted of treating 34 acres with the

chemical insecticide orthene, 4800 acres with the chemical insecticide dimilin, and 250,000 acres with Bt. In 1986, Bt was selected as the only pesticide for use over approximately 250,000 acres. The Bt was applied using helicopters in three separate applications (approximately 7-10 days apart) over approximately 250,000 acres of forest, rural, and urban areas. The applications were made in the early morning hours from daybreak until about 10:00 am. This was the optimum time for application because meteorological conditions were best to prevent spray drift, as well as reduce exposure potential to the general public.

The 1985 Bt application was considered 97% effective based upon comparison of mass trapping studies before and after the application. The boundaries of the spray area were redefined accordingly to conduct the 1986 follow-up spray application.

IV. EVALUATION DESIGN AND METHODS

A complete review and evaluation of the project operational plans, accident prevention and safety plans, personal protection equipment usage, and work practices was conducted. The medical surveillance program of occupational and public health, including laboratory identification of infection isolates, was reviewed and discussed with representatives of the Oregon State Health Division, the Center for Infectious Diseases of the Centers for Disease Control (CID), and the Division of Surveillance, Hazard Evaluations, and Field Studies, NIOSH.

Personal exposure and area air monitoring for Bt was conducted during the mixing and aerial application procedures. This air monitoring was conducted using MSA Model G and DuPont P-200 portable sampling pumps with 37-mm (0.8 micron pore size) cellulose ester membrane filters (MF) as the collection media. The MF were contained in pre-sterilized plastic cassettes which were kept closed until the sampling was conducted; the sampling was conducted with an open-face filter technique. The pumps were calibrated, with the filters in line, at various flow rates of 0.1 to 2.0 liters/minute. Sampling duration ranged from 15 minutes to 4 hours depending on job operation or activity and was conducted to characterize exposure (occupational and/or general public) potential to Bt. The filter samples were analyzed for Bt by microbial culture technique.[6,7] The filter cassette samples received by the laboratory from the 1986 surveys, were individually packed in whirl pack bags. They were removed from the bags, dipped into a solution of bleach (1 part commercial bleach, 1 part water), and dried with a towel. Inside a biological safety cabinet, a cassette was opened and the filter was removed with sterile forceps. The filter cassette samples from the 1985 surveys were not individually wrapped, decontaminated with bleach, nor transferred inside a biological safety cabinet. Filters from the 1985 survey were removed with sterile forceps. The filter, from both surveys, was then placed face up in the center of a nutrient agar (Difco) Petri plate (150 mm).

All plates were incubated, inverted, for 24 hours at 30°C. Colonies were counted and the plates were left at room temperature for an additional 5 days incubation. Colony numbers were again checked.

Smears were made, stained, and examined.[7] Briefly, smear slides were made with distilled water, air dried, and lightly heat fixed. The slides were flooded with methanol for 30 seconds. The methanol was poured off and the slides were dried throughly by passing them through the flame of a Bunsen burner. The slides were flooded with carbolfuchsin Ziehl-Neelsen stain and heated from below with an alcohol lamp until steam appears. This step was repeated after waiting about 1 minute. The slides were washed in running tap water and air dried (without blotting).

All slides were examined under oil (1000x), for the darkly stained diamond-shaped toxin crystals. The presence of toxin crystals confirms identification as Bt.

V. EVALUATION CRITERIA

As a guide to the evaluation of the hazards posed by workplace exposures, NIOSH field staff employ environmental evaluation criteria for assessment of a number of chemical and physical agents. These criteria are intended to suggest levels of exposure to which most workers may be exposed up to 10 hours per day, 40 hours per week for a working lifetime without experiencing adverse health effects. It is, however, important to note that not all workers will be protected from adverse health effects if their exposures are maintained below these levels. A small percentage may experience adverse health effects because of individual susceptability, a pre-existing medical condition, and/or a hypersensitivity (e.g. allergy).

In addition, some hazardous substances may act in combination with other workplace exposures, the general environment, or with medications or personal habits of the worker to produce health effects even if the occupational exposures are controlled at the level set by the evaluation criterion. These combined effects are often not considered in the evaluation criteria.

Workplace evaluation criteria for microbiological agents do not exist as for chemical or physical hazards, however, interest has been heightened during the past two decades on problems caused by microorganisms in the work environment.[8] It is well known that high concentrations of inhaled microorganisms can cause an allergic lung disease called extrinsic allergic alveolitis, also termed hypersensitivity pneumonitis.[9,10] Certain microorganisms are known pathogens (they have the ability to produce disease) and are grouped according to virulence (degree of pathogenicity). Bt has not been known to cause allergic alveolitis and is not considered a virulent pathogen.[2,3]

NIOSH is interested in the use of microbiological insecticides with respect to characterizing and documenting exposure potential to these agents. This interest involves efforts to identify adverse health effects in susceptible and/or healthy individuals attributable to exposure to such microorganisms or their products. Environmental, occupational, and public health concerns relative to the use of synthetic chemical pesticides are prompting an increased interest in the use of microbiological insecticides. As part of this interest, biotechnology (especially genetic engineering and recombinant DNA) is playing an important role in the development and proposed use of biological pesticides.[11] Therefore, it is important to explore ways to characterize exposure (sampling and analysis methods) as well as document the exposure potential, and determine if adverse health effects occur as a result of exposure.

VI. RESULTS AND DISCUSSION

The gypsy moth control project operational plans, safety objectives and plans, and the passive medical surveillance program instituted by the State Health Division were reviewed. These efforts were found to be comprehensive, well formulated, and properly directed. Several suggestions and recommendations were offered to improve safety conditions and/or reduce accident potential (these are listed in the recommendations section of this report). The safety and health attitude of, and effort exhibited by, the project personnel was excellent.

Twenty-four laboratory environment cultures and 7 spill sample cultures were submitted for Bt analysis during the 1985 application. Twenty-three of the 24 (95.8%) environmental isolates, and 3 of the 7 spill sample cultures obtained during the 1985 application were confirmed positive for Bt. The medical surveillance and environmental isolates monitoring for the 1986 application were underway at the time of this report. Results of this monitoring may be obtained from the Oregon Department of Human Resources, Health Division, Portland, Oregon.

Laboratory environmental culture samples were obtained in laboratories and/or hospitals in the spray area using settling plates containing culture media which were left open for 10 minutes. The results of this sampling (95.8% positive for Bt) during the 1985 application indicate the ubiquitous distribution of Bt throughout the environment of the Eugene, Oregon area after the spray application. These results could also indicate poor aseptic transfer technique (i.e., introduction of Bt from a source external to the sampling/analysis effort) although these laboratories were not transferring or handling stock cultures of Bt and thus the organism as sampled was probably the result of environmental contamination.

The 7 spill samples from 1985 consisted of soil and water samples from Bt spill sites. The 4 confirmed Bt negative samples indicate the effective effort to decontaminate the areas where the spills occurred. Sodium hypochlorite solution was used as the decontaminating agent. A comprehensive spill management program had been developed by the project safety staff to include proper reporting of the spill incident, location, cause, and extent of corrective action taken. Spill kits consisting of personal protective equipment and the necessary items to contain and clean up contaminated material were supplied to all project teams.

Table 1 presents the 1985 personal Bt exposure monitoring data by job operation and title. The results are reported as positive (POS) or negative (NEG) for Bt. Confirmed positive Bt cultures were counted (when possible) and reported as colony-forming-units (CFU). Generally, any plate that had more than six CFUs were overgrown to the point that individual colonies were indistinguishable. In these cases the samples were graded as: POS-A (1/2 the plate was covered), POS-B (3/4 the plate was covered), and POS-C (the entire plate was overgrown). Normally, sampling results of this type are reported as a concentration, CFU/cubic meters (m^3) of air. This reporting approach was not used for the 1985 results due to the limitations of the method in this particular circumstance. While this method appeared to be less than an optimal means of quantifying exposure, it at least provided a qualitative indication of Bt exposure during the sampling period. These results show that all individuals in the job titles sampled experienced airborne Bt (inhalation) exposure. The average sampling collection time was 50 minutes at an average air flow rate of 1.7 liters/minute. Samples were collected during the application of the spray (direct exposure), as in the cases of the batch truck driver and the load checker, and under non-spray conditions (indirect exposure), as in the cases of the security guard and the service station attendant. Samples collected on the lead mixer and his helper show that they were exposed to Bt even though they conducted the mixing using closed system pumping controls. Possibly, their exposure resulted from the open hatches on the storage tank and truck tank. Incidental Bt exposure was noted in the cases of the safety chief and safety officer during early morning preparations for work while in their hotel room. This environment was probably contaminated with Bt via the officers' contaminated clothing and equipment. The helicopter pilot and observers inside the ship were exposed to Bt because the spray drifted into the flight path of the observation helicopter. The helicopter pilot in the application ship was exposed in the same manner.

Table 2 depicts the results of the 1985 ambient air monitoring for Bt by date and location. The results of this sampling are reported in the same fashion as the personal breathing zone results. These results also show the ubiquitous distribution of the organism. Bt was found upwind and downwind of the mixing operation, and during the loading of the batch truck. It was also found throughout the various offices of the Project Camp. Bt was found inside and outside a hotel

room, on a high school campus in Eugene which were within the spray boundary. Bt was found in the residence of the safety chief in Corvallis, Oregon. Bt was not found in one office trailer at the Project Camp, on the campus of the University of Oregon, outside the home of the safety chief, nor inside or outside the Prineville, Oregon home of the safety officer. Bt found in the sample from the safety chief's home may have resulted from contaminated clothing or equipment since his home is approximately 50 miles outside the spray area.

Along with the quantitative limitation previously noted, a secondary problem regarding contamination of control (blank) filter samples was also observed during the 1985 surveys. As shown in Table 3, of 11 control samples submitted, 7 had confirmed Bt colony growth. Two controls which were prepared in the hotel room on the night before the first sampling and Bt spraying occurred (April 29), were negative for Bt. Six control samples, all found to be Bt contaminated, were prepared in the hotel room after spraying had begun (May 15). Three other controls were prepared in Cincinnati in a "Bt-free environment" on May 23, one of which was found to be contaminated with Bt; while the other two were Bt negative. Additional precautions were employed during the 1986 survey to reduce contamination as a result of poor aseptic technique during the preparation and handling of the blanks in the field or during laboratory analysis. These precautions included: assembling the filter/cassettes and placing them in individual sterile whirl pack bags, sterilizing all individually wrapped filter/cassettes with ethylene oxide, decontaminating the outside of the cassette with bleach solution after sampling, and placing each sample in a sterile whirl pack bag for shipment to the laboratory. Additional aseptic precautionary technique was employed at the laboratory as previously mentioned. As Table 3 indicates, controls generated in the hotel room after the 1985 spraying had begun, were probably contaminated by that environment. This was substantiated by the control sample results for controls from the 1986 survey; one control sample (opened within the hotel room on the third day of the survey) was contaminated with Bt. Contamination of the 1985 control sample prepared in Cincinnati possibly occurred due to shipment with field samples or during laboratory procedures in Portland. This information indicates the extent of distribution of the Bt organism throughout the spray area, the robust viability of this sporeforming bacillus, and the caution required to conduct good aseptic sampling/analysis technique.

Ambient air monitoring results obtained prior to the 1986 Bt application are presented in Table 4. The first six samples listed were collected at locations within the 1985 spray boundary. These locations were selected to determine if Bt organisms were present from the previous years application. The last four samples in this table were collected in locations outside of the 1985 spray boundary for comparison with the first six sample locations. Considerable volumes of air were sampled and Bt was not found on any of these pre-application samples.

Table 5 presents the personal Bt exposure monitoring results from the 1986 survey. All of these samples were collected using a flow rate of 100 cc/min. This flow rate, as compared to that of the 1985 survey, brought into focus the quantitative range of the membrane filter method for this sampling circumstance. Sample results are reported in CFU/filter and then interpreted to CFU/m^3 of air. These results indicate Bt exposure potential for Bt application personnel as well as the general public. Breathing zone (BZ) samples for a safety officer, helicopter spray pilot, aerial observer, card checkers, and security guard indicated Bt exposure ranged from no exposure to 11,000 CFU/m^3. The highest exposure was that of a card checker who was in brief direct contact with the spray; this was the only sample in which direct contact with the spray was observed. All other samples represent collection periods after the spray application had ceased or the individual was removed from direct contact with the spray. General public BZ samples represented by a church custodian, a U.S. Postal Service employee, a grocery store clerk, and a service station attendant ranged from no Bt exposure to 1600 CFU/m^3. Samples for the church custodian and postal employee were collected in an area which had been sprayed with Bt 3-4 days prior to the sampling. The positive Bt exposure for the grocery clerk and the service station attendant represent sampling on the same day Bt application occurred. These two individuals were not observed to have been in direct contact with the spray.

General area Bt air monitoring results are presented in Table 6. In comparison with area air monitoring results for 1985 (Table 2), the hotel was not within the 1986 application boundary. On June 2 and 3, the hotel room was apparently not Bt contaminated; yet on June 4, 1900 CFU/m^3 were detected in this room. Results of 500 CFU/m^3, 800 CFU/m^3, and 50 CFU/m^3 at restaurant locations within the application boundary also indicate general public exposure potential.

A summary of the membrane filter sample results from both the 1985 and 1986 surveys is presented in Table 7. These results are listed as Bt positive and negative results by location (within versus outside the spray application boundaries).

VII. CONCLUSIONS

The Gypsy Moth Control Project conducted by the state of Oregon using the biological pesticide Bacillus thuringiensis appeared to be effective; the 1985 control project was determined to be 97% effective based on mass trapping studies before and after Bt application. This is the largest application and most effective kill rate of its kind documented in the United States.

Results of the air sampling for Bt indicated widespread exposure potential. The sampling method as utilized in 1985 was not an entirely effective method for quantitative determination of Bt environmental levels. It was, however, an effective qualitative tool to determine the presence of Bt. Method modifications (flow rate and

sample volume) along with improvements in aseptic sampling and analytical technique improved the quantitative ability of the method and reduced potential for sample contamination. Caution, with regard to aseptic technique during field sampling and laboratory preparation/analysis, is warranted because of Bt's viability and widespread dissemination in the spray area.

Effects of Bt exposure on immuno-suppressed or hypersensitive individuals have not been conclusively addressed to date by this surveillance. It is apparent from the infection isolates and indications of the personal breathing zone/environmental sampling results, that a Bt application of this magnitude promotes dissemination of the organism throughout the environment raising the exposure potential for the general public and across all occupations. It is prudent to continue the surveillance program and attempt to identify, and follow, individuals who may be at an increased risk.

Indications of the air sampling results, and observations made during the air sampling, for this project indicate that microbial insecticides of this type can be transmitted throughout the environment. Future sampling efforts and results should indicate the prominence and proliferation these organisms can gain in the environment after such wide scale application. Therefore, careful consideration should be given prior to the deliberate release (field tests) of genetically modified microorganisms. The public health, ecological, and occupational health consequences must be carefully explored and evaluated before these novel organisms are used.

Further survey efforts are required to fully evaluate the exposure potential in the use of biological pesticides and to evaluate microbial sampling/analysis procedures.

VIII. RECOMMENDATIONS

The following recommendations were offered to the Safety Chief, the Project Director, and the Oregon State Health Division after the project safety programs and procedures were reviewed and the Bt mixing and application procedures were observed. These recommendations are based on good industrial hygiene practices or cited references and should reduce the potential for an accident or exposure.

1. To prevent splash of Bt to the eye, all personnel handling (transferring) Bt in solution should be required to wear eye protection. Bt has been documented to cause a corneal ulcer.[2]

2. Because of the magnitude of these applications, and the fact that small (5 gallon) containers present a hazardous waste disposal problem, the dispersing agent (Plyac®) should be purchased in bulk. This would also alleviate handling problems and reduce potential for Bt exposure. The use of a fork truck to raise

plyac containers to the top of the batch truck for dumping should be evaluated for accident potential. Perhaps a scaffold would be safer and provide an easier means to accomplish this task.

3. Non-essential personnel activity in the mixing and application areas should be restricted, isolated, and if possible reduced. Visits by the news media and other interested individuals should be controlled to reduce accident (helicopter safety) and exposure potential. All visitors should be issued and required to wear passes identifying them as visitors.

4. Follow-up on accident and/or spill reporting is an important component of these programs. Failure to do so will result in a poor safety attitude of the personnel involved and the subsequent downfall of the reporting program.

5. Safety personnel should be involved in contract review prior to letting contracts. This would grant them the opportunity to provide input regarding safety issues, hazard concerns, and the ability to implement safety guidelines in the contract.

6. A field expedient eye wash station should be provided at the mixing operation and all aerial loading sites (heliports) in case of Bt, Plyac®, or gasoline splash. Contractors should be required to wear eye protection and rubber gloves when handling these materials.

7. A first aid kit should be provided at the mixing site. Water should be provided at the aerial loading sites for hand washing and sanitation purposes. Bt solution splashed on the skin should be washed with soap and water. All cuts and abrasions must be thoroughly cleaned and a topical antiseptic applied.

8. Exposure sampling of synthetic chemical insecticides used to eradicate the gypsy moth should be conducted for individuals performing the application, ground crews, and the general public in the vicinity of the application.

9. The ground crews at the heliports should be required to wear hearing protection during the landing, loading of Bt into the helicopter, and take-off.

The following recommendations are based on the results of the air sampling for Bt and are offered for consideration in future studies and sampling efforts concerning biological pesticides.

1. Research should be conducted to further improve the quantitative ability of the cellulose ester filter sampling method in its use as a microbial sampling method for sporulating organisms. The method, because of Bt's toxin production ability, is very sensitive (specific for Bt). This method is applicable for use in the evaluation of environmental microbiology in "clean rooms",

yet it must be improved upon for use in environments where high microbial aerosol concentrations are expected.

2. Extreme care, with regard to aseptic technique during sample collection and analysis, must be exercised to prevent sample contamination. The sample filter and cassettes should be prepared in a controlled environment or sterilized after they are assembled. The sample filter cassettes should be protected from contamination while in the field. The outside of the cassettes should be decontaminated using a suitable solution before they are opened to remove the filter. The filters should be aseptically removed from the cassette holder within a laminar-flow clean air bench.

3. Pre-application measurements (air sampling) for Bt presence should be conducted to determine background levels in the spray area. Air sampling in a non-application area should be conducted concurrently with sampling in the spray area. These sampling results would indicate background levels and possible meteorological dissemination of Bt; and, in comparison would give more meaning to the sample results from the spray area.

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TABLE 1
PERSONAL EXPOSURE TO *BACILLUS THURINGIENSIS*
GYSPY MOTH CONTROL PROJECT
EUGENE, OREGON
APRIL-JUNE, 1985

DATE	JOB OPERATION, JOB TITLE	SAMPLING RESULT*	COMMENTS
4-30-85	*Bt* Mixing Operation		
	Lead Mixer	Pos.-A	Mixing conducted using closed system pumping controls. Exposure must have resulted from open hatches of storage tank and truck tank.
	Helper	Pos.-A	
5-1-85	*Bt* Application		
	Batch Truck Driver	Pos.-B	Driver experienced direct spray.
5-14-85	*Bt* Application		
	Batch Truck Driver	Pos.-C	Heliport area was in direct path of spray.
	Load Checker	Pos.-C	
	Observer/Visitor	Pos.-C	
	Safety Officer	Pos.-A	Safety officer remained in vehicle during spray.
	Helicopter Pilot	Pos.-B	
	Security Guard for Project Area	Pos.-2CFU	Upwind from mixing operation.
5-15-85	*Bt* Application		
	Helicopter Pilot	Pos.-A	*Bt* spray noted on windshield of helicopter during spray.
	Air Observer in Helicopter	Pos.-A	
	Air Observer in Helicopter	Pos.-B	
	Service Station Attendant in Eugene	Pos.-6CFU	Worksite within spray area.
5-16-85	*Bt* Application		
	Application Team Leader	Pos.-C	During spray application.
5-17-85	*Bt* Application		
	Fuel Truck Driver	Pos.-B	Heliport in spray area.
	Batch Truck Driver	Pos.-C	

TABLE 1 (continued)
PERSONAL EXPOSURE TO BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
EUGENE, OREGON
APRIL-JUNE, 1985

DATE	JOB OPERATION, JOB TITLE	SAMPLING RESULT*	COMMENTS
5-31-85	Safety Chief	Pos.-C	Early morning samples in hotel room.
	Safety Officer	Pos.-C	
6-1-85	Bt Application		
	Load Checker	Pos.-C	Not in direct contact with spray.
	Helicopter Pilot	Pos.-C	
6-3-85	Bt Application		
	Team Leader	Pos.-A	Not in direct contact with spray.
	Fuel Truck Driver	Pos.-C	

* Sampling results are reported as Pos. (positive) or Neg. (negative) for Bt. Positive Bt colonies were counted and reported as colony forming units (CFU). Generally, any plate that had more than six CFUs were overgrown to the point that individual colonies were indistinguishable. In these cases the samples were graded as: A=1/2 the plate covered, B=3/4 of the plate covered, and C=the entire plate was covered with Bt.

TABLE 2
AREA AIR MONITORING FOR BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
EUGENE, OREGON
APRIL-JUNE, 1985

DATE	JOB OPERATION, JOB TITLE	SAMPLING RESULT*	COMMENTS
4-30-85	Mixing Area, Project Camp	Pos.-A	Downwind during mixing operation.
	Mixing Area, Project Camp	Pos.-5 CFU	Upwind during mixing operation.
5-1-85	Heliport L-16 During Application		
	Next to Helicopter During Loading	Pos.-A	
	Next to Batch Truck	Pos.-B	
	Upwind from Batch Truck	Pos.-A	
	Spray Area P-2 During Spray	Pos.-C	
5-14-85	Mixing Area, Project Camp		
	Downwind-Unloading of Bt	Pos.-6 CFU	
	Upwind Loading of Batch Truck During Mixing	Pos.-B	
	Between Storage Tanks	Pos.-A	
	Gypsy Moth Camp Offices		
	Public Information Receptionists Desk	Pos.-B	4-6 personnel in office.
	Administration Office	Pos.-A	3-4 personnel in office.
	Safety Office	Pos.-A	2-4 personnel in office.
	Bulletin Board Area (Outside)	Pos.-2 CFU	
	Bulletin Board Area (Outside)	Pos.-6 CFU	During spray of Bt.
	Hotel in Eugene		
	Inside Room	Pos.-2 CFU	
	Parking Lot	Pos.-2 CFU	
5-15-85	Roadside of Spray Area	Pos.-4 CFU	Sample 2 hour after spray, no traffic.
	Roadside of Spray Area	Pos.-B	During traffic conditions.
	Gypsy Moth Camp Offices		
	Planning Chief Trailer	Pos.-A	3-4 personnel in office.
	Operations (north) Trailer	Neg.	2-3 personnel in office.
	On Campus of Univ. of Oregon	Neg.	Non-spray area.
	On High School Campus	Pos.-A	Spray area/4 hours after spray.

TABLE 2 (continued)
AREA AIR MONITORING FOR BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
EUGENE, OREGON
APRIL-JUNE, 1985

DATE	JOB OPERATION, JOB TITLE	SAMPLING RESULT*	COMMENTS
5-31-85	Safety Chief's Residence-Corvallis		
	Outside of Home	Neg.	Approximately 50 miles from spray
	Inside of Home	Pos.-B	area.
6-1-85	Safety Officer's Residence-Prineville		
	Outside of Home	Neg.	Approximately 160 miles from spray
	Inside of Home	Neg.	area.
6-3-85	Hotel in Eugene		
	Porch Area in Front of Safety Officer's Room	Pos.-A	Within spray area.

* Sampling results are reported as Pos. (positive) or Neg. (negative) for Bt. Positive Bt colonies were counted and reported as colony forming units (CFU). Generally, any plate that had more than six CFUs were overgrown to the point that individual colonies were indistinguishable. In these cases the samples were graded as: A=1/2 the plate covered, B=3/4 of the plate covered, and C=the entire plate was covered with Bt.

TABLE 3
MEMBRANCE FILTER CONTROL SAMPLES
BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
LANE COUNTY, OREGON
APRIL, 1985 THROUGH JUNE, 1986

DATE	CONTROL SAMPLE TREATMENT	Bt CONTAMINATION Pos.	Neg.	REMARKS
April 29, 1985	2 pre-assembled and pre-sterilized filter/cassettes were labeled, opened and immediately re-closed in the hotel room.		2	Bt spraying had not yet begun.
May 15, 1985	6 pre-assembled and pre-sterilized filter/cassettes were labeled, opened and immediately re-closed in the hotel room.	6		Bt spraying had been occurring for 2 weeks. A filter sample collected in the room on May 14, 1985 was Bt positive.
May 23, 1985	3 pre-assembled and pre-sterilized filter/cassettes were labeled, opened and immediately re-closed in NIOSH's Cincinnati, Ohio Laboratory.	1	2	Contamination probably occurred due to shipment with field samples or poor aseptic technique in opening the cassettes for analysis.
April 22, 1986	1 pre-assembled and pre-sterilized filter/cassette was labeled and placed in sterile whirl pack bag at the Gypsy Moth Control Camp.		1	
June 3, 1986	1 pre-assembled and pre-sterilized filter/cassette was labeled, opened and immediately re-closed, and placed in sterile whirl pack bag in hotel room. A second filter/cassette received the same treatment except the cassette was not opened.		2	
June 4, 1986	2 pre-assembled and pre-sterilized filter/cassettes were labeled, not opened, and were placed in sterile whirl pack bags in the hotel room.		2	
	2 pre-assembled and pre-sterilized filter/cassettes were labeled, opened and immediately re-closed, and placed in sterile whirl pack bags in the hotel room.	1	1	A filter sample collected in this room on this date indicated the room was contaminated with Bt. See Table 6.
June 5, 1986	2 pre-assembled and pre-sterilized filter/cassettes were labeled by marking the outside of the whirl pack bag in which they were sterilized. These samples were removed from the original whirl pack bag at the laboratory for analysis.		2	

TABLE 4
PRE-APPLICATION AREA AIR SAMPLES
BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
LANE COUNTY, OREGON
1986

DATE	LOCATION	SAMPLE VOLUME (LITERS)	COLONY FORMING UNITS/m^3	REMARKS
April 18, 1986	Mixing/Storage Area of Project Camp#	59	0	During receipt of Bt delivery from supplier.
April 21, 1986	Window (open) Sill Hotel Room#	77	0	
April 22, 1986	School Campus#	203	0	
April 26, 1986	At 1985 Bt Spill Site#	82	0	Spill area had been decontaminated immediately after spill.
April 29, 1986	Service Station in Eugene#	78	0	
May 4, 1986	Mobile Home of Mixing Operator#	61	0	Mobile home was set-up on site at Project Camp.
April 19, 1986	Personal Home of Safety Chief+	71	0	Home is located 60 miles north of spray area.
April 23, 1986	Service Station in Eugene+	200	0	
April 27, 1986	Oregon State Department of Forestry Eugene Office+	188	0	
April 28, 1986	Hotel next to Interstate I-5+	124	0	

* These membrane filter samples were collected at the noted locations before any 1986 Bt spray application occurred. These locations which were within the 1985 Bt spray application area are indicated with #, and locations outside of the 1985 Bt spray area are so indicated with +.

TABLE 5
PERSONAL BZ AIR SAMPLES
BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
LANE COUNTY, OREGON
JUNE, 1986

DATE	JOB TITLE	COLONY FORMING UNITS/FILTER	SAMPLE VOLUME (L)	COLONY FORMING UNITS/m^3	REMARKS
6-3-86	Safety Officer	53	14.6	3600	Sample taken during office activities.
	Helicopter Pilot-Spray Ship	6	4.5	1300	Air vent on ship-open
	Aerial Observer	9	6.7	1300	Air vent on ship-open
	Helicopter Pilot-Spray Ship	0	6.5	0	Air vent on ship-closed
	Aerial Observer	4	5.3	800	Air vent on ship-closed
	Church Custodian	0	8.7	0	During mowing the church yard. Area had been sprayed 3 days prior.
	Letter Carrier US Postal Service	0	6.7	0	Route had been sprayed 4 days prior.
6-4-86	Card Checker #3	0	14.7	0	Prior to placing cards.
	Card Checker #1	5	11.7	400	During placement of cards.
	Card Checker #2	66	11.7	5600	During placement of cards.
	Card Checker #1	12	21.1	600	During Bt application. Card Checker stayed out of spray.
	Card Checker #2	0	21.2	0	During Bt application. Card Checker stayed out of spray.

TABLE 5 (continued)
PERSONAL BZ AIR SAMPLES
BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
LANE COUNTY, OREGON
JUNE, 1986

DATE	JOB TITLE	COLONY FORMING UNITS/FILTER	SAMPLE VOLUME (L)	COLONY FORMING UNITS/m^3	REMARKS
	Card Checker #3	0	19.9	0	During Bt application. Card Check stayed out of spray.
	Card Checker #1	90	8.6	11,000	During Card Retrieval. Card Checker was in brief direct contact with spray.
	Card Checker #2	6	8.4	700	During Card Retrieval. Card Checker stayed out of spray.
	Card Checker #3	0	8.5	0	During Card Retrieval Card Checker stayed out of spray.
6 4 86	Grocery Store Clerk-Saginaw	5	4.9	1000	Store within same spray area days. Individual not in-direct contact with spray.
	Service Station Attendant-Creswell	7	4.3	1600	Station within spray area same day. Individual not in direct contact with spray.
6 5 86	Security Guard at Gypsy Moth Project Site	11	8.8	1300	Individual not in direct contact with spray.

TABLE 6
GENERAL AREA AIR SAMPLES
BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
LANE COUNTY, OREGON
JUNE, 1986

DATE	JOB TITLE	COLONY FORMING UNITS/SAMPLE	SAMPLE VOLUME (L)	COLONY FORMING UNITS/m^3	REMARKS
6-2-86	Hotel Room	0	5.5	0	Hotel not in 1986 spray area.
6-3-86	Mixing Tank and Pumping Station Project Camp Area	2	10.7	200	Mixing and pumping was not being conducted during sampling.
	Batch Truck at Heliport Loading Area	0	6.1	0	
	Restaurant Parking Area, Creswell	2	4.0	500	Restaurant area had been sprayed 3 days prior.
	Hotel Room	0	15.5	0	Same location as on 6-2-86
	Hotel Parking Lot	0	5.7	0	
	School Campus South Eugene	0	8.5	0	School was within spray boundary.
6-4-86	Intersection of England and Turkey Run Roads, Saginaw	57	13.5	4200	Sample collected after spraying was concluded.
	Intersection of Sears Road and I-5, Saginaw	0	8.9	0	Sample collected during spray period.
	Intersection of Sears Road and I-5, Saginaw	0	13.2	0	Sample collected after spray period.
	Intersection of Meyers Road and Witcher Gateway Road, Saginaw	3	6.2	500	Sample collected after spray period.
	Hotel Room	7	3.7	1900	Same location as 6-2-86.

TABLE 6 (continued)
GENERAL AREA AIR SAMPLES
BACILLUS THURINGIENSIS
GYSPY MOTH CONTROL PROJECT
LANE COUNTY, OREGON
JUNE, 1986

DATE	JOB TITLE	COLONY FORMING UNITS/SAMPLE	SAMPLE VOLUME (L)	COLONY FORMING UNITS/m^3	REMARKS
6-5-86	Hotel at I-5	0	13.9	0	Outside of spray area, same location as noted in Table 4.
	Restaurant Parking Lot, Springfield	16	20.2	800	Restaurant was within spray boundary.
	Inside Restaurant, Springfield	1	20.1	50	

TABLE 7

BACILLUS THURINGIENSIS (Bt)
SUMMARY OF Bt SAMPLE RESULTS*
GYSPY MOTH CONTROL PROJECT
LANE COUNTY, OREGON
1985 and 1986

	No. of Samples for 1985 Application		No. of Pre-Application Samples (1986)		No. of Samples for 1986 Application		
	Inside Spray Area	Outside Spray Area	Inside 1985 Spray Area	Outside 1985 Spray Area	Inside Spray Area	Outside Spray Area	Total
Bt Positive	42	1	0	0	18	1	62
Bt Negative	1	4	6	4	11	4	30
Total	43	5	6	4	29	5	92

*Numbers includes personal breathing zone and general area air sampling results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
CENTERS FOR DISEASE CONTROL
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