A Protocol for Measuring Population Exposures to Malathion during Mosquito Adulticiding using Ground-Based ULV Spraying

Report to the BC Centre for Disease Control
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by Adrian Hingston[1], Tracy Kirkham, Josephine Rekart, Kay Teschke[2]

[1] contact Adrian Hingston when Kay Teschke is not available (ahingsto@interchange.ubc.ca)
[2] primary contact (kay.teschke@ubc.ca, 604 822-2041)

School of Occupational and Environmental Hygiene
Faculty of Graduate Studies
University of British Columbia
3rd Floor – 2206 East Mall
Vancouver, BC
V6T 1Z3

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1.0 Overview of Study

This study was designed to understand population exposures to insecticides during adulticiding of two mosquito species (*Culex tarsalis* and *Culex Pipiens*) that are known carriers of the West Nile virus.

The protocol was designed before final decisions were made about *where* spraying will be done. These decisions await evidence of human cases of West Nile virus, evidence of infected birds, or evidence of mosquitos carrying the virus. The Study was also designed before final decisions were made about the *insecticide* to be used and the *method* of spraying. To make the protocol design as parsimonious as possible, we have made assumptions about these latter issues, with guidance from BC Centre for Disease Control personnel about the most likely options. Therefore this protocol assumes that *malathion* will be used as the insecticide and that it will be applied using *ultra-low-volume (ULV) ground-based spraying*.

The questions that are the focus of this study were selected to allow public health officials to guide members of the public about how to minimize their exposures, and to answer exposure-related queries that they are likely to receive and that do not appear to have been answered in previous research:

A. Do people need to leave the spray area during spraying?
B. Does staying indoors during spraying reduce airborne exposures?
C. How far away from the spray area does one need to go to minimize exposure?
D. How long after the spraying stops does the malathion stay in the air?
E. Does spraying increase the body burden of malathion metabolites in members of the public who live in the spray area? What about those who live nearby, but outside the spray area?
F. Are members of the public still being exposed (e.g., via touching contaminated surfaces) after the spray has settled out of the air?

While these questions and the resulting study design seem very straightforward, the conduct of this study will be extraordinarily difficult logistically, for three main reasons: the uncertainty about the spray locations; the likely short notice between decisions about the spray location and the start of spraying; and the likelihood that the study will be conducted during one short adulticiding campaign. We have tried to design a protocol that is manageable, by having three levels of personnel: a study coordinator who trains and coordinates trainers; trainers who train and coordinate study participants; and study participants who carry out the sample collection protocol.

The protocol that follows outlines the staff and training, participant selection, participant recruitment, and the process and timeline, and then describes in more detail the exposure questions, their rationale, and the resulting sampling strategies. Accompanying the protocol are drafts of an *Exposure Assessment Training Manual* for the trainers, and a *Participant Guide* for the participants. Finally, an estimated study *Budget* is also included.

2.0 Staffing and Training

In order to handle the size and scope of the study, there are many people who will work on this study, each with their own set of responsibilities. The following is a list of the staff and their tasks.

2.1 Study Coordinator

The *Study Coordinator* will oversee the study from start to finish. It is the study coordinator's responsibility to ensure that all of the study personnel are aware of their individual jobs and that these jobs are completed.

Prior to the start of the study, the *Study Coordinator* will ensure that the *Environmental Health Officers* (EHOs) in the Interior Health Authority and the *West Nile Virus Area Coordinators* throughout the province are aware of the study design. It will also be the Study Coordinator's job to

- hire and train the *Trainers*, likely from the University of British Columbia (Okanagan site) or Okanagan College, and
- order, organize and prepare study equipment and documentation.

Once the announcement of intent to spray is made, the *Study Coordinator* will oversee the selection and recruitment of participant households. The *Study Coordinator* will rent a truck, load it with the equipment and documentation, and travel to the study area.

Throughout the duration of the spray period and the 10 days following, the *Study Coordinator* will remain in the spray area in order to oversee the *Trainers* and respond to any pressing questions or equipment needs. During this period the *Study Coordinator* will be in contact with the *Trainers*, ensuring that participant households are being reminded of the scheduled sampling events. The *Study Coordinator* will also be responsible for shipping samples to the laboratory once they have been collected from the participating households by the *Trainers*.

After the spraying and 10-day exposure sampling period, the *Study Coordinator* will be responsible for ensuring that the *Trainers* have collected all of the sampling equipment and documentation from the participating households. Once this collection is complete, the rental truck will be loaded and driven back to the lower mainland.

After sampling, the *Study Coordinator* will be responsible for communicating with the laboratories to ensure that the samples are analyzed, for taking all forms and questionnaires to a data entry firm, for sending thank you cards with the study incentive to the participating households, for cleaning and analyzing the data, for writing reports, and for sending individual results to all study participants.

2.2 GIS Technician

Once the announcement of intent to spray is made, the *GIS Technician* from the BC Centre for Disease Control will be responsible for randomly selecting households from inside and outside of the spraying swath area following the methods in section 3.0 of this protocol: Participant Selection.

2.3 Trainers

Prior to the start of the study, a team of 12 individuals (likely from the University of British Columbia, Okanagan site, or Okanagan College) will be hired to be *Trainers*. It is also possible that some local EHOs will be part of the *Trainer* team. The *Trainers* will be trained by the *Study Coordinator* prior to the announcement of the intent to spray, so that they are comfortable with the study design and the sampling equipment and materials. Twelve will be trained initially in the expectation that up to 33% may be unavailable during the spray period, because it will be unknown at the time of hiring. This should allow for as few as 8 and as many as 12 *Trainers* during the active measurement period of the study.

Once the announcement of the intent to spray has been issued, the *Trainers* will be responsible for telephone recruitment of the participating households. This will be done following the methods in section 4.0 of this protocol: Participant Recruitment.

The *Trainers* will also be responsible for training between 5 and 8 participant households each. This will involve traveling to the residence and training the participants so that they are able to take the required samples and fill out the appropriate paper work. The *Trainers* will walk the participants through the *Participant Guide* in a training session that will last between one and two hours. During this training session with the participant, the *Trainer* will explain the sampling procedures as indicated in section 6.0 of this protocol on Air and Urine Sampling.

The *Trainers* will also be responsible for maintaining contact with the participants that they train throughout the sampling period. This will involve telephone reminders as well as physically delivering study equipment to and collecting samples from the participant households. Also, the *Trainers* will act as a contact for the participants if there are any questions or problems during the study period. Finally, the *Trainers* will be required to pick up the study materials and equipment from the participants' residence at the conclusion of the study.

2.4 Responsible Adult Study Participants

A highly unusual feature of this study is that one *Responsible Adult Study Participant* from each study household will themselves be responsible for collecting all the study air and urine samples, and completing data forms and a questionnaire.

This is necessary to allow this logistically difficult study to be feasible. In addition, we expect this method to make the study results, especially those of indoor air samples, to be more representative of normal household conditions than if study personnel were required to go in and out of the household during the study period, potentially creating more indoor contamination than otherwise would occur.

3.0 Participant Selection

The goal is to recruit a total of 60 sampling sites: 30 residences from within the malathion spray swath; and 30 from outside the swath (Figure 1). The details of this design and its rationale are included in section 6.0 of the protocol.

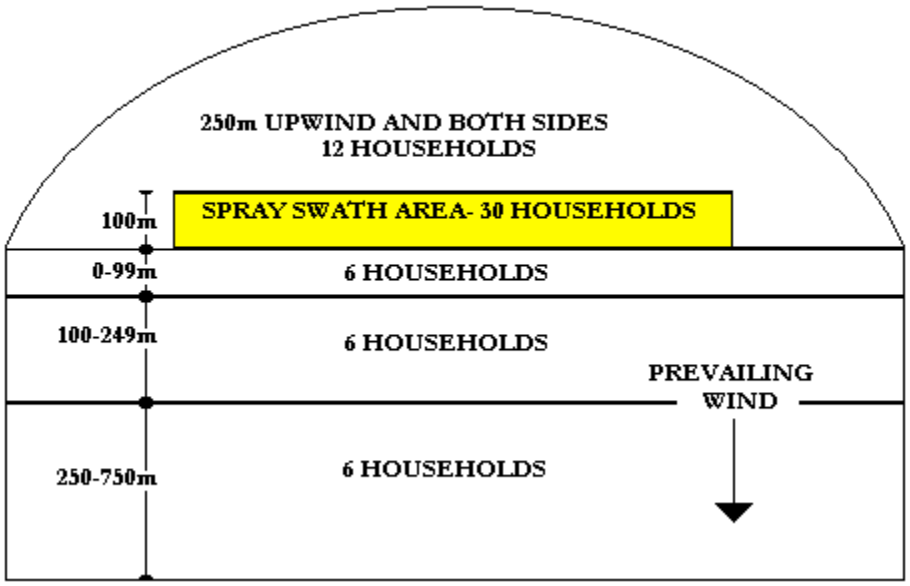

Figure 1. *Illustration of number of participating households in each selection area.*

Participation in the study is expected to present a burden to study participants, since they will be required to collect all the study air and urine samples and complete data forms, but we also expect there will be benefits to their participation, including receiving results of measurements of the malathion air concentrations inside and outside their homes, results of measurements of malathion urinary metabolites in one or two household members, and an incentive payment of $100 per household participant.

Based on the burden and benefits of participation, we have conservatively estimated that approximately one third of the households contacted will agree to take part in the study, so three times the number of households needed will initially be selected. Therefore, a total of 180 residences will be identified:

- 90 randomly selected residences within the spray swath
- 90 additional residences outside the swath, as follows:
  - 54 selected from locations downwind of the spray swath, but within 750 meters of the swath boundary
  - 36 selected from locations on either side or upwind of the spray swath, but within 250 meters of the swath boundary,

All households will be selected randomly by the *GIS Technician,* in coordination with the *Study Coordinator,* from a list of residences in the areas defined above, located using GIS

technologies available at the BC Centre for Disease Control. The samples should be selected from a list in the public domain, such as Telus' listed residential phone numbers. This will allow direct contact of the households via mail and phone, while abiding by required ethics standards for studies of human subjects conducted by universities and health facilities.

Contact information including name, address, and phone number of the randomly selected households will be provided to the Study Coordinator who will coordinate participant recruitment. A letter will be sent to each household, briefly describing the study and indicating that they will receive a telephone call within a day or two to invite their participation.

4.0 Participant Recruitment

The *Trainers* will each be given 15 to 24 households to contact for recruitment. Because of the likely short timeline necessary to recruit participants, only three attempts will be made to contact each selected household by telephone.

Upon making contact, the *Trainers* will

- describe the work involved in the study
- describe the benefits to the participant and the incentives for participating in the study
- ask for verbal agreement from an adult resident to participate in the study
- request a second participant from their household (a person with the largest possible age difference from the initial participant)
- arrange attendance at a central training session, if possible, or arrange for the *Trainer* to train the participants individually.

During the recruitment process, the *Trainers* will need to report the name and address of each participant who has agreed to participate to the *Study Coordinator* who will coordinate with the *GIS Technician* to ensure that the numbers sampled in each area follow the protocol. Adjustments will be made where necessary (e.g., selecting more samples, or ending recruitment in an area whose quota has been reached).

5.0 Process Overview & Timeline

Timeline	Who	Role
Preparation for Study	*Study Coordinator*	Recruit and train *Trainers* (12)
		Inform *EHOs* and *WNV Area Coordinators* of the study
		Arrange for buying and renting study equipment, print participant package documentation
	Trainers, EHOs, West Nile Virus Area Coordinators	Become familiar with the study design and responsibilities
ID of Vectors or Human Cases	*Study Coordinator*	Make travel arrangements
		Finalize all arrangements for study equipment
		Make arrangements for area residential household list from Telus
Announcement of Intent to Spray	*GIS Technician and Study Coordinator*	Map area of spray and take random sample of households from Telus list
	Trainers	Recruiting of study participants
	Study Coordinator	Gather equipment and travel to spray area
	Study Coordinator and Trainers	Put together study packages for participant
	Trainers	Train participants
Spray Date	*Trainers*	Notify participants of spray start
		Be available for questions from participants
	Study Coordinator	Be available for questions from *Trainers*
During 10 Days Following Spray Date	*Trainers*	Telephone participants to remind them about sampling times
		Pick up equipment and samples
	Study Coordinator	Pick up equipment and samples
		Ship samples to laboratories for analysis
After Sampling is Completed	*Trainers, GIS Technician, Study Participants*	Participate in debriefing
	Study Coordinator	Debrief *Trainers*
		Take all data forms to firm for data entry
		Send thank you letters to participants
		Receive and clean data for analysis
		Analyze data
		Send results to study participants
		Write report

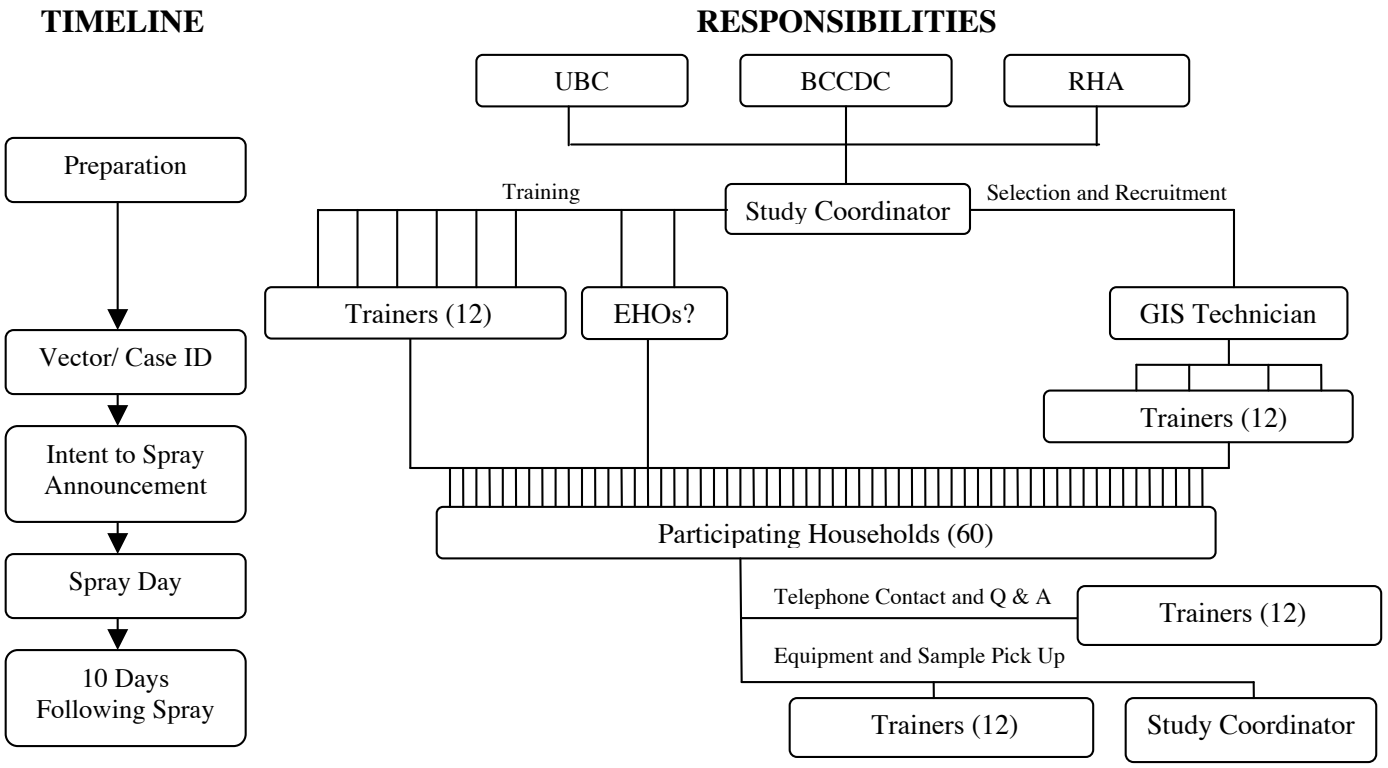

Figure 2. *A diagram of the timeline, responsibilities, and system of communication and activities for the exposure measurement period.*

6.0 Exposure Questions and Sampling Strategy

6.1 Study Questions

The specific questions that this study is designed to answer are listed below. The rationale and methods for each is described in the sections that follow.

A. What are airborne levels of malathion outdoors in the spray swath during spraying?
B. Is there a difference in airborne concentrations between indoor and outdoor locations in the spray swath?
C. Does the airborne malathion drift outside the spray swath?
D. What is the temporal gradient of airborne concentrations of malathion?
E. Does spraying increase the body burden of malathion metabolites in individuals who live inside or outside the spray zone?
F. Do exposures from other routes (e.g., dermal absorption, ingestion) continue after malathion concentrations in the air have declined?

6.2 Routes of Exposure

To answer these questions, we propose that both air and urine samples be taken.

Air samples are required to understand the pattern of malathion concentrations in air during and after spraying, indoors and outdoors, and inside and outside the spray area (questions A to D above). This information can be used to give guidance to individuals about where and when airborne exposures resulting directly from the adulticiding program might occur.

Urine samples will be used to address questions E and F and will provide information regarding exposures from all sources, including inhalation of malathion from the spray and other airborne sources (e.g., hobby or work-related), and dermal absorption and ingestion. It will also account for patterns of behaviour during and after the spray (e.g., an individual may spend the evening and night in the spray zone, but their work day may be spent elsewhere). Alternatives to urine sampling include swab sampling of surfaces, patch sampling of body parts likely to contact contaminated surfaces, food samples, and extensive daily diaries. Given the already difficult logistics of this study, urine sampling was seen as a more feasible way to assess exposures from other routes and sources. In addition, it is the most accurate method to quantify body burden from all sources.

6.3 Air Sampling

Rationale for air sampling measurement method: We reviewed the scientific literature (using Medline and Web of Science bibliographic databases) and found three main sampling methods for malathion in air, including methods using polyurethane foam plugs, XAD-2 media, and quartz/glass fibre filters to capture the malathion; all involve "active" sampling with pumps.[3, 7, 10, 22, 25, 27, 28, 30] Although the ideal method for a study asking participants to collect the samples would be a "passive" method that does not use pumps to pull air through the sampling train, no such method was found.

For this study, the US National Institute for Occupational Safety and Health (NIOSH) Analytical Method 5600, Organophosphates in Air[27] was selected, because the research literature indicated that the XAD-2 media used in NIOSH method is the most commonly used,[7, 22, 30] meaning that results from this study will be comparable to those of others. In fact, the NIOSH method uses OVS-2 tubes which incorporate all three of the media listed above since the sampler is made up of XAD-2 media, with a quartz fiber filter and PUF plug.[27] Finally, this is a standard method that has developed protocols and known operating characteristics.

Summary of air sampling method:

- NIOSH Analytical Method 5600, Organophosphorus Pesticides in Air.
- Air will be pulled through OVS-2 tubes using sampling pumps calibrated to ~1 L/min for the times specified in the sections below.
- The analysis of malathion in air will be done by gas chromatography, with flame photometric detection. The Department of Environmental and Occupational Health Sciences Laboratory at the University of Washington has extensive experience with these methods and can perform the analyses for the study.
- For every 10 samples, 1 field blank will be taken as a quality control measure to detect any contamination of samples during storage or transport between the sample site and the laboratory.

Summary of air sampling equipment:

- OVS-2 tubes (SKC # 226-58); can be ordered through Integra or another SKC supplier.
- Tube holders (SKC # 224-29V); can be ordered through Integra or another SKC supplier.
- Calibration chambers (e.g., SKC #225-111); can be fabricated or ordered through Integra or another SKC supplier.
- Pumps capable of sampling @ 1 L/min, with chargers/AC adapters and a calibration device; can be rented from the School of Occupational and Environmental Hygiene (UBC); BCIT; PHH Environmental; Pine Environmental; Levitt Safety; Concept Controls; and EnviroRentals. More than one source will likely be necessary to locate sufficient pumps for the study.

6.3.1 A. What are the airborne levels of malathion outdoors in the spray swath during spraying?

Rationale for measuring air concentrations during spraying: This information will provide data on what are expected to the highest airborne concentrations to which residents of the area might be exposed. This information also provides the basis for the comparisons in the following three questions, i.e., comparisons to indoor air concentrations, to concentrations in areas outside the spray swath, and to concentrations at times following the spraying.

Rationale for selection of sample duration: A 4-hour duration was selected for sampling during spraying. The sample duration was based primarily on logistical simplicity. Spraying will occur at dusk and there are concerns that it will be difficult for residents to anticipate when the spray trucks will be passing, especially on nearby streets (rather than their own street), and therefore will not know when to start sampling. To solve this logistical problem it was decided that the "during spraying"

sample will take place during the 4-hour window when spraying is expected to take place (5 pm - 9 pm). Since the spray trucks will be mounted with GPS locators, the exact time spraying begins within a given distance of each residence can be determined. With this information, the air volume collected by the pumps can be adjusted to reflect the volume collected from the time spraying began, providing a more accurate "during spraying" concentration.

This sampling duration is also expected to be sufficient so that most or all of the samples should have concentrations above the limit of detection of the analytical method.

Sampling strategy:

- 30 houses inside the spray swath will be selected, as described in section 4 of the protocol.
- One sample will be taken outside each house.
- Sampling will begin at 5 pm, the approximate time when spraying will begin, and continue until 9 pm, a four-hour sample.
- All samples will be area samples, taken near an entrance to the house, at a height of about 1 m above the ground.
- A form will be completed describing the location and home characteristics (apartment, house, townhouse; indoor entrance or direct entrance from outdoors; number of storeys; surrounding tree cover).
- Wind speed and direction data for each location will be taken from the nearest Environment Canada meteorological station.

6.3.2 B. Is there a difference in airborne concentrations between indoor and outdoor locations in the spray swath, during spraying?

Rationale for comparison of indoor and outdoor concentrations: Information on the differences between indoor and outdoor locations will indicate whether staying indoors during spraying is an effective method to reduce exposures, since this advice is currently given by public health authorities to residents in pesticide spray zones. There is little data on the effectiveness of such a strategy. It was successful during aerial *Btk* spraying in Victoria, though the reduction in exposure compared to outdoors was not maintained after spraying.[33]

Sampling strategy:

- The same 30 houses inside the spray swath described in section 6.3.1 above will also have one indoor sample taken during the spraying.
- This sample size should be able to discriminate an indoor/outdoor difference of 1.5-fold with geometric standard deviations of air concentrations of 2.0 or less, as expected based on results of other studies.[30]
- Sampling will begin at 5 pm, the approximate time when spraying will begin, and continue until 9 pm, a four-hour sample.
- All samples will be area samples, taken in a commonly used room in the house that is not the sleeping area (because of the noise of the pump), at a height of about 1 m above the floor.

Data analysis:

- A simple t-test will be used to compare the mean concentrations indoors and outdoors.
- Data will be transformed (e.g., natural log) as indicated by histograms and other descriptive analyses.

6.3.3 C. Does the airborne malathion drift outside the spray swath?

Rationale for expected drift out of prescribed spray swath: Although provincial health personnel advise residents within pesticide spray zones that spraying will occur and recommend that they remain indoors during spraying, residents outside of the spray zone are not usually notified and little is known about their potential for exposure. Determining if malathion drifts out of the spray swath will provide evidence about whether the advice should be extended outside of this zone, and if so how far.

Studies of airplane-mounted aerial spraying have shown pesticide drift outside of spray zones in the range of 1 to 5 km downwind,[12, 24, 33, 35] but evidence from ground-based spraying is less extensive. Two studies using ULV truck-mounted spraying examined pesticide (malathion and permethrin) drift up to 100 meters downwind from the spray swath in open and forested areas, and found evidence of mosquito or honeybee mortality to that distance.[17, 24] A study of short-range transport of pesticides (lindane, parathion and pirimicarb) applied from tractor-mounted boom showed concentrations above the limit of detection reaching 200 m downwind.[31] Information on drift beyond this distance and upwind of the spray zone does not appear to be available, underscoring the importance of gathering such data.

Note that this portion of the protocol would need to be altered if adulticiding were done using airplanes for aerial spraying (i.e., the distances over which drift was checked would be considerably greater).

Sampling strategy:

- 30 houses outside the spray swath will be selected, as described in section 4 of the protocol (see Figure 1).
  - 18 of the 30 samples will be in locations in prevailing downwind locations, 6 in each of three distance categories: 0 – 99, 100 – 249, and 250 – 749 m from the spray swath boundary.
  - 12 of the 30 samples will be in locations on either side of the spray swath and/or in prevailing upwind locations, ranging from 0 – 250 m from the spray boundary.
- This sample size should be able to discriminate an inside the spray swath to outside the spray swath difference in malathion concentration of 1.5-fold with geometric standard deviations of 2.0 or less.
- Two area samples will be taken at each location during spraying, one indoors and one outdoors, as described in sections 6.3.1 and 6.3.2 above.
- Sampling will begin at 5 pm, the approximate time when spraying will begin, and continue until 9 pm, a four-hour sample.

- A form will be completed describing the location, and home characteristics (apartment, house, townhouse; indoor entrance or direct entrance from outdoors; number of storeys; surrounding tree cover).
- Wind speed and direction data for each location will be taken from the nearest Environment Canada meteorological station.

Data analysis:

- A simple t-test will be used to compare the mean concentrations inside and outside the spray swath.
- A multiple regression analysis will examine the effect of the distance from spray swath boundary and wind speed (as a vector) on air concentrations.
- Data will be transformed (e.g., natural log) as indicated by histograms and other descriptive analyses.

6.3.4 D. What is the temporal gradient of airborne concentrations of malathion?

Rationale for examining the temporal gradient of air concentrations: Performing repeat samples over time will provide information on how quickly malathion concentrations in air decline after spraying. Given the target size of the malathion aerosol (in the range of 5 to 10 microns in aerodynamic diameter), it is expected that the aerosol may stay airborne for hours to days. Several studies have demonstrated prolonged presence of pesticides in air after completion of spraying.[7, 29, 33] Brown *et al.* reported that malathion was still present in the air nine days after spraying.[7]

This part of the study will also assess whether indoor air concentrations decline at the same rate as outdoor concentrations, and help public health authorities determine whether and how long they should advise residents to remain indoors after spraying. During the Victoria *Btk* sampling, indoor levels increased 3 hours after spraying and exceeded outdoor levels 5-6 hours after spraying.[33]

Rationale for selection of sample duration: For these "post spraying" samples, a 24-hour sample duration was chosen to maximize the chance that the mass of malathion collected will be greater than the limit of detection of the analytical method. Similar long-duration sampling was also done in a study evaluating pesticide exposures in agriculture areas.[20]

Sampling strategy:

- Each of the 60 homes sampled during spraying will have air samples taken on 4 additional occasions.
- Samples will be taken outdoors and indoors at each of 4 periods post spraying (day 0 to 1, day 2 to 3, day 4 to 5, and day 8 to 9 after completion of spraying).
- Post spray samples will be 24 hours in duration. The first post-spray samples will begin immediately following the completion of the during-spray samples at 9 pm (i.e., all samples will be 9 pm to 9 pm samples).
- This will give a total of 120 samples at each time post spraying for a total of 480 post-spraying malathion samples (i.e., 240 outdoor and 240 indoor), allowing regression analysis with time and location as independent variables.

Data analysis:

- A multiple regression analysis will examine the effect of time and location as independent variables on air concentrations.
- Data will be transformed (e.g., natural log) as indicated by histograms and other descriptive analyses, and by theoretical considerations about the likely decay over time.

6.4 Urine Sampling

Rationale for urine sampling measurement method: Once absorbed into the body, malathion is metabolized into several different metabolites and excreted in urine. There are two main types of metabolites: alkyl phosphates (dimethyl phosphate – DMP, dimethyl thiophosphate – DMTP, and dimethyl dithiophosphate – DMDTP); and carboxylic acids (monocarboxylic acid – MCA, and dicarboxylic acid – DCA). Alkyl phosphates are non-specific metabolites of organophosphate pesticides, whereas MCA and DCA are specific metabolites of malathion.[5, 9, 10, 34] The analysis of MCA and DCA is more complicated because it more difficult to obtain them in pure form for preparation of standards, and they can be unstable.[10]

Many studies have used measures of metabolites to evaluate exposures to malathion and other pesticides; the majority have focused on the dialkyl phosphate metabolites (DMP, DMTP, DMDTP).[1, 4, 5, 8-11, 13-16, 18-21, 23, 26, 30, 32, 34] Because results for alkyl phosphates have been reported more frequently than for the acids, and because analysis of the alkyl phosphates is more readily available, the metabolites proposed for analysis in this study are the alkyl phosphates.

Rationale for selection of urine collection time: Collection of spot urine samples is logistically much simpler than 24-hour urine samples. Given the burden of sample collection in this study, this seems a prudent course. University of Washington researchers have conducted several studies on malathion exposures in the general population, using urinary metabolites.[8, 11, 14-16, 18, 20, 23] They recommend early morning spot urine samples, since this is when urine is the most concentrated, allowing for detection of metabolites when exposures are low.[16]

Summary of urine sampling method:

- First morning void urine samples will be collected using standard collection practices.
- Samples will be shipped on dry ice to the laboratory for analysis of alkyl phosphates by gas chromatography, with flame photometric detection by the Jaffe method. [23] Creatinine correction will be done. The Department of Environmental and Occupational Health Sciences Laboratory at the University of Washington has extensive experience with these methods and can perform the analyses for the study.
- For every 10 samples, 1 field blank will be taken as a quality control measure to detect any contamination of samples during storage or transport between the sample site and the laboratory.

Summary of urine sampling equipment:

- Standard urine collection cups (volume ~120 mL); can be ordered from Canadian Hospital Specialties Ltd., or any other desired vender.

- Commode inserts; can be ordered from Canadian Hospital Specialties Ltd., or any other desired vender.

6.4.1 E. Does spraying increase the body burden of malathion metabolites in individuals who live inside or outside the spray zone?

Rationale for measuring malathion metabolites in urine before and after spraying: Given that malathion insecticides are used for applications other than mosquito adulticiding (e.g., some study participants may work in agriculture or in pesticide application), and given that the alkyl phosphate metabolites are not specific to malathion, it is prudent to assess the metabolite concentrations prior to spraying. In addition, an essential public health question is whether the spraying results in body burdens greater than background levels, in individuals living both within and outside the spray swath.

Rationale for measuring malathion metabolites in different age groups: It is likely that opportunities for exposure will differ between individuals, and this may be affected to some extent by the age of the participants. For example, children are more likely to consume dirt and to play outdoors and on the ground. Adults of working age are more likely to have opportunities for work site exposures. The elderly may be more housebound that other adults.

Sampling strategy:

- One or two residents (as different as possible in age) of each of the participating households will be asked to submit urine samples.
- Each participant will provide a pre-spraying urine sample collected on the morning prior to spraying to measure their background levels of metabolites.
- Each participant will provide a post-spraying urine sample collected on the morning immediately after spraying.
- Each participant will complete a diary indicating the locations at which they spent the evening of spraying.
- This will give a total of 60 to 120 each of pre- and post-spray samples, which should be able to discriminate an exposure difference of less than 1.5-fold with a geometric standard deviation in urinary metabolites of 2.0 or less.

Data analysis:

- A simple t-test will be used to compare the mean metabolite concentrations before and after spraying, inside and outside the spray swath.
- Data will be transformed (e.g., natural log) as indicated by histograms and other descriptive analyses.

6.4.2 F. Do exposures from other routes (e.g., dermal absorption, ingestion) continue after malathion concentrations in the air have declined?

Rationale for Repeat Sampling: Analyzing metabolite concentrations in urine over time, after spraying, will allow examination of whether people are being exposed to malathion by routes of exposure other than air. If exposures are solely from air, metabolite burdens should decline in parallel to declines in air concentrations, since

the biological half-life of malathion in humans is relatively short, on the order of 4 to 12 hours.[6]

Other routes of exposure may include dermal absorption and ingestion. Ingestion exposures may result from consumption of vegetables from home gardens, and hand to mouth transfer from surfaces. Dermal exposures may arise from deposition on a wide variety of surfaces (e.g., cars, household items, playgrounds, toys, and animals) and during household cleaning and gardening. Ando *et al.* examined the persistence of malathion and its degradation product, malaoxon, over a 32-day period, and found that the environmental half-life was 6 days on sand and 4 days on steel sheet surfaces.[2]

Sampling strategy:

- Each participant who submitted a pre- and post-spray urine sample will be asked to submit urine samples on three additional mornings post exposure (days 3, 5, and 9).
- This will provide 60 to 120 samples on each day, with a total of 180 to 360 samples in total, which should provide sufficient samples to allow regression analysis with time and location as independent variables.

Data analysis:

- A multiple regression analysis will examine the effect of time and location as independent variables on concentrations of urinary metabolites.
- The rates of decline in air and urine concentrations will be compared.
- Data will be transformed (e.g., natural log) as indicated by histograms and other descriptive analyses, and by theoretical considerations about the likely decay over time.

7.0 References

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