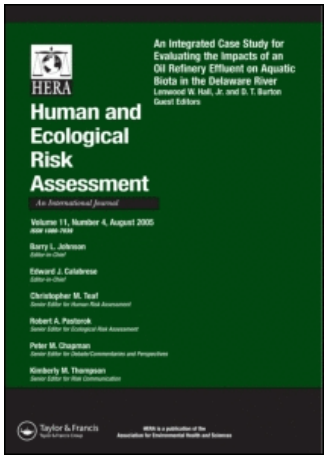

Human and Ecological Risk Assessment
An International Journal
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713400879

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Online Publication Date: 01 March 2008
To cite this Article: Schleier III, Jerome J., Davis, Ryan S., Shama, Leslie M., Macedo, Paula A. and Peterson, Robert K. D. (2008) 'Equine Risk Assessment for Insecticides Used in Adult Mosquito Management', Human and Ecological Risk Assessment, 14:2, 392 - 407
To link to this article: DOI: 10.1080/10807030801934812
URL: http://dx.doi.org/10.1080/10807030801934812

Human and Ecological Risk Assessment, 14: 392–407, 2008

ISSN: 1080-7039 print / 1549-7680 online
DOI: 10.1080/10807030801934812

Equine Risk Assessment for Insecticides Used in Adult Mosquito Management

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ABSTRACT

Since West Nile virus (WNV) was introduced to New York City in 1999, it has subsequently spread through the Americas, creating human and animal health risks. Our equine risk assessment focused on three pyrethroid insecticides (phenothrin, resmethrin, and permethrin), pyrethrins, and two organophosphate insecticides (malathion and naled). Piperonyl butoxide, a synergist commonly used in pyrethroids, was also assessed. The objective was to use deterministic and probabilistic risk assessment methodologies to evaluate health risks to horses from vector management tactics used for control of adult mosquitoes. Our exposure estimates were derived from the Kenaga nomogram for food deposition, AgDRIFT® for deposition onto soil and hair, AERMOD for ambient air concentrations, and PRZM-EXAMS for water concentrations. We used the risk quotient (RQ) method for our assessment with the RQ level of concern (LOC) set at 1.0. RQs were determined by comparing the exposure to no-observable-effect-levels. Acute deterministic RQs ranged from 0.0004 for phenothrin to 0.2 for naled. Subchronic deterministic RQs ranged from 0.001 for phenothrin to 0.6 for naled. The probabilistic assessment revealed estimates of deterministic acute and subchronic RQs were highly conservative. Our assessment revealed that risks to horses from adult mosquito insecticides are low and not likely to exceed the LOC.

Key Words: horse, mosquito control, pesticide exposure, risk analysis, West Nile virus, ultra low volume.

INTRODUCTION

West Nile virus (WNV) was first observed in New York City in 1999 and subsequently has spread through the Americas, producing thousands of West Nile disease

Received 4 April 2007; revised manuscript accepted 23 May 2007
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cases in humans and animals, and causing the largest arboviral encephalitis epidemic in U.S. history (Huhn *et al.* 2003). WNV is a Flavivirus that is mosquito-borne and transmitted in cycles between vertebrates (particularly birds) and mosquitoes (especially *Culex* spp.) (Dauphin *et al.* 2004).

In 2005 alone, there were 1,072 equine cases of WNV in 33 states (CDC 2005). Horses experimentally infected with the virus did not show sufficient viremia for transmission back to a mosquito host (Bunning *et al.* 2002). Therefore, horses, like other mammals, are most likely dead-end hosts for the virus (Dauphin *et al.* 2004). However, unlike most other mammals, and for reasons that are not well understood, horses are especially sensitive to WNV.

In horses, 10% of those infected with WNV will develop clinical signs of illness (*i.e.*, depression, reluctance to move, agitation, weakness, ataxia, tremors, recumbency, coma, and seizure) (Siger *et al.* 2006; Weese *et al.* 2003). And, of those horses that develop clinical signs of illness, the mortality rate is approximately 33% (Murgue *et al.* 2001; Ostlund *et al.* 2001; Trock *et al.* 2001; Porter *et al.* 2003; Weese *et al.* 2003; Ward *et al.* 2004, 2006).

Currently there is no effective therapeutic treatment for WNV in horses, but prevention is effective and relies on vaccination and vector management (Siger *et al.* 2006). Vector management during disease outbreaks often involves treatment of areas at dusk with insecticides to kill adult female mosquitoes, which are actively flying, seeking a bloodmeal, and may be carrying the virus. This use of insecticides is called "adulticiding" and uses ultra-low-volume (ULV) aerosol spraying. The use of this technique to control the WNV mosquito vectors has resulted in concerns by the public over risks to humans and other non-target organisms (Peterson *et al.* 2006).

Peterson *et al.* (2006) performed a human-health risk assessment for six mosquito insecticide active ingredients and piperonyl butoxide (PBO) along with WNV, which demonstrated that the risks from WNV most likely were larger than the risk from the insecticides. Other biomonitoring studies, reports, and regulatory assessments also have concluded that risks to humans and other non-target organisms from exposure to adulticides most likely are negligible (Karpati *et al.* 2004; Currier *et al.* 2005; NYCDOH 2005; O'Sullivan *et al.* 2005; Carr *et al.* 2006; Davis *et al.* 2007).

Even though it is known that equines are especially sensitive to WNV, no assessments have examined the health risks to horses from exposure to mosquito insecticides. An understanding of the equine health risks from vector management measures would allow decision-makers to weigh the multiple risks that adult horses experience in the face of WNV. Therefore, the objective of this study was to use deterministic and probabilistic risk assessment methodologies to evaluate health risks to adult horses from vector management tactics used for the control of adult mosquitoes.

MATERIALS AND METHODS

Problem Formulation

We performed a reasonable worst-case deterministic and probabilistic risk assessment of equine health risks associated with acute and sub-chronic exposures to ULV insecticide applications. Acute exposures were defined in this study as single-day

exposures after a single insecticide application. Subchronic exposures were defined as the exposure to ULV insecticides, per day over 90 days, during and after 10 spray events.

Hazard Identification

We conducted risk assessments for 6 insecticide active ingredients and 1 synergist used in ULV applications. Malathion (O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate) and naled (1,2-dibromo-2, 2-dichloroethyl dimethyl phosphate) are in the organophosphate class of insecticides. Pyrethrins ((Z)-(S)-2-methyl-4-oxo-3-(penta-2,4-dienyl)cyclopent-2-enyl (1R,3R)-2,2dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate) are insecticides derived from the Chrysanthemum species. Permethrin ((3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2dimethylcyclopropane carboxylate), resmethrin (5-benzyl-3-furylmethyl (±)-cis-trans-chrysanthemate), and phenothrin (3-phenoxybenzyl (1R)-cis/trans-chrysanthemate) are in the pyrethroid class of insecticides. The synergist, piperonyl butoxide (PBO) (2-(2-butoxyethoxy)ethyl 6-propylpiperonyl ether), is present in many pyrethroid insecticides and pyrethrin formulations. All compounds are currently registered by the United States Environmental Protection Agency (USEPA) for use in adult mosquito management in the United States.

Toxicity and Dose-response Relationships

Toxicity and dose-response information for each compound was reviewed and endpoints were chosen based on both acute and subchronic exposure durations. The toxicity endpoints used in this assessment were no-observable-adverse-effects-levels (NOAEL) for the rat (*Rattus norvegicus*) (Table 1). We used NOAELs because they are an acceptable threshold to protect horses. To our knowledge there have been no toxicity studies on horses for these chemicals. The acute LD50 for the rat for PBO, phenothrin, permethrin, resmethrin, malathion, naled, and pyrethrins is 4570, 5000, 8900, 4639, 390, 90, and 700 mg/kg, respectively (USEPA 2002, 2006a,b,c,d,e; WHO/FAO 1994).

Table 1. Regulatory no-observable-adverse-effects-level (NOAEL) and lowest-observable-adverse-effects-level (LOAEL) for the rat.

Compound	Acute NOAEL (mg/kg/day)	Acute LOAEL (mg/kg/day)	Subchronic NOAEL (mg/kg/day)	Subchronic LOAEL (mg/kg/day)	USEPA source
Malathion	25	50	2.4	29	2000a
Naled	1.0	N/A*	0.2	N/A*	2002
Permethrin	25	75	25	75	2006b
Resmethrin	105.5	205.1	35	70.8	2005b, 2006e
Phenothrin	70	216	70	216	2000b
PBO	630	1065	15.5	52.8	2006c
Pyrethrins	20	63	4.37	42.9	2006d

*N/A = Not available.

Exposure Assessment

Environmental modeling of concentrations and fate of insecticides

Insecticides were assumed to be sprayed a total of 10 times (days 1, 4, 14, 17, 27, 30, 33, 43, 46, and 56) over a 90-day period, which represents a reasonable worst-case application scenario during a WNV outbreak in humans (NYCDOH 2005; Peterson *et al.* 2006). The insecticides and synergist were assumed to be applied at the maximum label rates (Peterson *et al.* 2006). The maximum label rates for PBO, phenothrin, permethrin, resmethrin, malathion, naled, and pyrethrins are 0.0392, 0.004, 0078, 0.0078, 0.0639, 0.0224, and 0.009 kg active ingredient/ha, respectively.

We used the Kenaga nomogram (Fletcher *et al.* 1994) to predict environmental deposition of the insecticides on food sources for horses. The Kenaga nomogram is a linear model that uses application rate to predict concentrations of the insecticide on different types of food. To provide a conservative exposure value, we used short range grass as the insecticide recipient and food source.

We used AERMOD version 1.0 tier-1 air dispersion model (USEPA 1999) to predict the air concentrations at 7.62 m (25 ft) from the spray source, for the 6 active ingredients and PBO within 1- and 6-h time ranges after truck-mounted ULV application (Peterson *et al.* 2006). The assumptions included: (a) each chemical had a 24-h half-life in the air except for naled, which had a 18-h half-life; (b) the insecticides were applied at the maximum application rate as stated on each label; (c) all of the insecticides were susceptible to the same weather conditions using standardized weather data from Albany, New York, from 1988; (d) all spray events occurred at 2100 h; and (e) each spray release was at 1.5 m.

Receptors were established within the model on a Cartesian grid at 5 intervals of 7.6 m from the edge of the spray source. The receptors were at a height of 1.5 m. At each receptor, the estimated 1-h peak air concentration and the 6-h average air concentrations for each insecticide were determined for the acute and subchronic exposures. An average was then taken of the estimates from the 6 receptors at 7.6 m that were not at the edges of the spray zone.

The USEPA water quality software, EXPRESS v. 1.00.00.012, (USEPA 2005a) was used to obtain estimated environmental concentrations of the insecticides in water in a standard farm-pond scenario through the interface with the Pesticide Root Zone Model v. 3.12.3 (PRZM) (USEPA 2005c). The input parameters used for all adulticides included: (a) a 45-m buffer as the distance of the spray from the pond, (b) spray drift into the pond was 1%, reflecting the default drift percentage from a high boom, fine particle, ground sprayer, (c) applications were made on Florida turf grass, a conservative, minimal vegetative cover that would be in the mosquito control area, (d) applications began on July 1 and ended August 25 with 3- and 10-day intervals between applications, and (e) applications were made at the maximum rate listed on the label for mosquito control. PRZM-EXAMS input parameters for most of the mosquito insecticides were gathered from their respective USEPA Reregistration Eligibility Documents (RED) or other sources, which included the following inputs: molecular weight, the sorption coefficient (Koc), vapor pressure, solubility, aerobic soil half life, aerobic biolysis, anaerobic biolysis, aqueous photolysis, and hydrolysis (Table 2).

Table 2. Input parameters for PRZM-EXAMS.

Chemical	Mol. Wt. (grams/ mole)	Koc*	Vapor Pressure (mPa)	Solubility (ppm)	Aerobic Soil Half Life (days)	Foliar Half Life (days)	Aerobic Biolysis (days)	Anaerobic Biolysis (days)	Aqueous Photolysis (days)	Hydrolysis (days)		
										ph 5	ph 7	ph 9
Malathion	330.4[a]	1200[a]	0.45[a]	130[a]	1[a]	5.5[b]	3.5[b]	7.64[b]	42[b]	107[a]	6.3[a]	0.05[a]
Naled	381[a]	157[a]	26[a]	1.5[a]	4[a]	NA	1.5[c]	4.5[c]	4.4[c]	4[c]	0.642[c]	0.067[c]
Resmethrin	338.4[a]	100000[a]	0.01[a]	0.0379[a]	197.5[d]	NA	37[d]	682[d]	0.033[d]	89[d]	168[d]	127[d]
Phenothrin	350.5[e]	56000[e]	1.43E-7[e]	0.0097[e]	26[f]	NA	7.2[f]	61.9[f]	5[f]	301[e]	495[e]	120[e]
Pyrethrins	328.4[a]	100000[a]	0.001[a]	0.001[a]	10.5[g]	NA	10.5[g]	86[g]	0.5[g]	stable[g]	stable[g]	17[g]
Permethrin	391.3[a]	39300[a]	0.0029[a]	0.006[a]	30[a]	NA	38[h]	175[h]	30[a]	stable[a]	stable[a]	49.87[a]
PBO	338.5[i]	399[i]	5E-13[i]	14.3[i]	14[i]	NA	75[i]	181[i]	0.35[i]	stable[i]	stable[i]	stable[i]

[a](USDA-ARS 2005), [d](USEPA 2005f), [g](USEPA 2005e), [b](USEPA 2000a), [e](NLM 2005), [h](USEPA 2005d), [c](USEPA 1997), [f](WHO/FAO 1994), [i](USEPA 2005g), * = Sorption coefficient.

We used AgDRIFT® v. 2.00.05 (Spray Drift Task Force 2000), for estimated environmental concentration (EEC) deposited on soil and body surface. AgDRIFT® is currently the industry standard for predicting both on- and off-target spray drift deposition and is considered a conservative model. AgDRIFT® is a method for evaluating off-site deposition of pesticides applied by aerial, ground, and orchard airblast spraying (Spray Drift Task Force 2000). We used a Tier-1 ground (agricultural) application for all adulticides, and the parameters included: (a) high boom, (b) very fine to fine spray, (c) 90th percentile of the modeled data (90th percentile of the deposition data generated), (d) terrestrial field definition, (e) active ingredients were applied at the maximum rate listed on the label for mosquito control, and (f) the receptor is 7.6 m from the spray source.

Acute exposure

We assumed multi-route exposures immediately after a single-spray event were limited to 24 h. Routes of insecticide exposure to the horse were from ingestion of food, soil, water, grooming, and from inhalation. We assumed that dermal exposure would be negligible because the hair of horses would protect against any insecticide reaching and absorbing into the skin. We assumed that the horse would be in a field when the spray truck passed and remained in the field for at least 24 h. We used an overall body-weight mean of 492.05 kg for an adult horse (Tasker 1967; Glade 1983; Koterba *et al.* 1988; Lafortuna and Saibene 1991; Nyman *et al.* 2002; Sponheimer *et al.* 2003). The surface area of an adult horse was estimated by an allometric equation (USEPA 1993) to be 6.19 m^2 for a 492.05 kg horse. We assumed that a horse would groom half of its surface area (self grooming or through social grooming) in one day. Respiratory rate was assumed to be 508.05 L/min for an adult horse, which is indicative of walking activity (1.496 m/s) at a 7% incline (Lafortuna and Saibene 1991). This is a conservative value because we assumed that the horse would be respiring at a walking activity rate during the entire 7-h exposure. The respiration rate of an adult horse at rest is 89.12 L/min (Lafortuna and Saibene 1991). Food intake was assumed to be 16.1 kg dry weight (Gallagher *et al.* 1992) and soil ingestion was assumed to be 2 kg/day (Frape 2004). The water intake per day was assumed to be 24.04 L (van den Berg *et al.* 1998) (Table 3).

We assumed that the insecticide settled onto short range grass and that the horses consumed their daily amount of food (all from short range grass) in 1 night and the next day after a spray event. The amount of insecticide ingested was estimated as the amount of residue on the quantity of food consumed in dry weight. Acute insecticide exposure from ingestion of food was estimated by

$$PE = (FE^* FI)/BW \quad (1)$$

where PE is potential exposure (mg/kg BW/day), FE is total amount of insecticide deposited on dry food source (mg/kg), FI is total amount of food ingested in a day (kg), and BW is body weight (kg).

Acute insecticide exposure from ingestion of soil was estimated by

$$PE = [(DS/SW)^* SI]/BW \quad (2)$$

where PE is potential exposure (mg/kg BW/day), DS is total amount of insecticide deposited on soil (mg/m^2), SW is soil weight (kg/m^3), SI is the amount of soil

Table 3. Biological input distributions used in the probabilistic analysis.

Input variable	Distribution type	Parameter	Value	Units
Food Intake	Normal (Truncated)	Mean	16.1[a]	kg (Dry Weight)
		SD*	2.59	
		Lower Bound	0	
Surface Area	Normal (Truncated)	Mean	3.09[b]	m^2
		SD*	0.19	
		Lower Bound	0	
Respiratory Rate	Normal (Truncated)	Mean	508.05[c]	L/min
		SD*	126.8	
		Lower Bound	0	
Water Intake	Normal (Truncated)	Mean	24.04[d]	L/day
		SD*	5.84	
		Lower Bound	0	
Soil Intake	Triangular	Minimum	1[e]	kg
		Likeliest	1.5	
		Maximum	2	
Body Weight	Normal (Truncated)	Mean	492.05[f]	kg
		SD*	30.7	
		Lower Bound	0	

[a](Gallagher *et al.* 1992), [b]$SA(m^2) = 0.11\ Wt^{0.65}$(kg) (USEPA 1993), [c](Lafortuna and Saibene 1991), [d](van den Berg *et al.* 1998), [e](Frape 2004), [f](Tasker 1967; Glade 1983; Koterba *et al.* 1988; Lafortuna and Saibene 1991; Nyman *et al.* 2002; Sponheimer *et al.* 2003), *SD = Standard Deviation.

ingested (kg), and BW is body weight. Soil weight was assumed to be 481 kg/m^3 based on reported densities for Scotts® garden soil. We chose commercial garden soil because mineral soil weighs more than the commercial garden soil (Abdel-Magid *et al.* 1987); therefore more garden soil would be ingested than would be consumed if mineral soil was used. We assumed the insecticide would only be deposited in the first centimeter of soil, so the adulticide concentration (mg/m^2) would be the same for 0.01 cubic meters of soil.

Acute insecticide exposure from ingestion of water was estimated by

$$PE = (WE^* WI)/BW \tag{3}$$

where PE is potential exposure (mg/kg BW/day), WE is milligrams of insecticide per liter of water, WI is total amount of water ingested in a day (L), and BW is body weight.

Acute insecticide exposure from ingestion while grooming was estimated by

$$PE = (DG^* SA)/BW \tag{4}$$

where PE is potential exposure (mg/kg BW/day), DG is total amount of insecticide deposited on a horse (mg/m^2), SA is half the surface area of a 492.05 kg horse (m^2), and BW is body weight (kg).

Acute insecticide exposure from inhalation was estimated by

$$PE = (AR^* AC^* D)/BW \tag{5}$$

where PE is potential exposure (mg/kg BW/day), AR is total amount of air respired in a day (1-h peak plus the 6-h average in mg/L/h), AC is milligrams of insecticide per liter of air (mg/L), D is the duration of the exposure (h), and BW is body weight (kg). Duration of exposure was assumed to be 7 h. Acute insecticide inhalation at a walking pace (1.496 m/s) was estimated by the total amount of insecticide respired at the 1-h peak plus the amount of insecticide respired at the 6-h average.

Total acute exposure to active ingredients was estimated by

$$PE_{total} = PE_{food} + PE_{soil} + PE_{water} + PE_{grooming} + PE_{inhalation} \quad (6)$$

Subchronic exposure

We assumed multi-route exposures per day over 90 days after multi-spray events. Routes of insecticide exposure were from food, soil, water, and grooming in addition to inhalation. The same assumptions about body weight, surface area, inhalation rate, soil, food, and water ingestion were used as stated earlier for acute exposure.

Subchronic exposures from deposition on food, soil, and grooming were estimated by the exponential decay model (USEPA 2004) to characterize insecticide persistence on food, soil, and surface area within a spray program that included 10 sprays on days 1, 4, 14, 17, 27, 30, 40, 43, 53, and 56. Insecticide concentrations for each spray event were followed through day 90 using the following degradation model

$$D = \sum_{j=i}^{n} Pe^{(r1+r2)t} \quad (7)$$

where D is the sum of the deposition over 1 spray, P is the peak deposition after a spray event, r_1 is the rate of decay calculated by using each active ingredient's aerobic soil half-life, r_2 is the rate of decay calculated by using the soil photolysis half-life of each active ingredient, t is the time in hours, i is the spray day, and n is the decay period.

PRZM-EXAMS input parameters for all active ingredients were the same as stated earlier, except the 90-day average concentrations were used. Subchronic insecticide ingestion from food, soil, water, grooming, and total exposure was estimated by the same equations used to characterize acute risks.

Subchronic inhalation exposure was estimated by

$$PE = (PE_{acute,inhalation} * SE)/D \quad (8)$$

where PE is potential exposure (mg/kg BW/day), $PE_{acute,inhalation}$ is the acute potential exposure for inhalation (mg/kg BW/day), SE was 10 spray events, and D is the 90 day duration of the exposure (day).

Risk Characterization

We used the risk quotient (RQ) method for our risk assessment, which is calculated by dividing the total potential exposure (PE_{total}) by its ingestion toxic endpoint value. In environmental risk assessments of pesticides, RQs are often used to quantitatively express risk (Peterson 2006). Estimated RQs are compared to a RQ level of concern (LOC) that is set by USEPA or another regulatory agency to determine if regulatory

action is needed. The RQ LOC used in our assessment was 1.0. An RQ of >1.0 means that the estimated exposure is greater than the relevant NOAEL.

Probabilistic Risk Assessment

Probabilistic risk assessments differ from deterministic risk assessments by sampling values from the distributions of exposures and biological parameters. For the probabilistic risk assessment, we used Monte Carlo simulation (Crystal Ball® 2000; Decisioneering, Denver, CO) to evaluate the RQ and input variables used to calculate the RQ. Probabilities of occurrence of RQ values were determined by incorporating sampling from the statistical distribution of each input variables used to calculate the RQs. Each of the input variables was sampled so that each input variable's distribution shape was reproduced. Then, the variability for each input was propagated into the output of the model so that the model output reflected the probability of values that could occur. We performed 10,000 iterations using the assumptions outlined in Tables 2–4. The equations used to calculate acute and subchronic deterministic exposure and risk were used to calculate probabilistic potential exposure and RQs.

We used normal distributions for the physiological measurements and food and water intake of equines because the mean is the most likely value, the uncertain variable could be as likely above the mean as below, and the uncertain variable is more likely to be in the vicinity of the mean. We truncated surface area, respiratory rate, and body weight at zero, because the likelihood of selecting zero is extremely low. We truncated food and water intake at zero because a horse could not eat or drink negative amounts of food or water in a day. For soil intake, we used a triangular distribution because we used a range of 1–2 kg/day of soil ingested with the most likely being 1.5 kg/day. For concentrations of insecticide deposition, inhalation, and water we chose the distribution that best fit the data generated from the fate models. We truncated distributions if concentrations for any exposure route fell below zero.

RESULTS

Acute Deterministic Risk

Risk quotients ranged from 0.0004 for phenothrin to 0.2 for naled with none of the acute RQs exceeding the LOC (Table 5). For naled, this means that reasonable worst-case exposures would be 20% of the NOAEL. The largest contributing factor to the RQs was food ingestion, which contributed 96.9%–98.3% of the exposure while water ingestion contributed <0.01% to the potential total exposure. Soil ingestion contributed ≤0.16% to potential total exposures, grooming contributed 0.6–1.2%, and inhalation contributed 0.4–1.8% to total exposures (Table 5).

Subchronic Deterministic Risk

Risk quotients ranged from 0.001 for phenothrin to 0.6 for naled with none of the subchronic RQs exceeding the LOC (Table 5). The largest contributing factor to the RQs was food ingestion, which contributed 98.3–99.9% of the exposure whereas water ingestion contributed ≤0.01% to the potential total exposures. Soil ingestion

Table 4. Input distributions for each exposure route used for acute and *subchronic* probabilistic analysis.

Input distribution	Distribution type	Parameter	PBO	Phenothrin	Permethrin	Resmethrin	Malathion	Naled	Pyrethrins	Units
Food Deposition	Uniform	Minimum	3.85 *8.27*	0.4 *1.31*	0.77 *2.78*	0.77 *4.77*	6.27 *1.39*	2.2 *1.54*	0.88 *1.36*	mg/kg (Dry Weight)
		Maximum	8.4 *18.04*	0.86 *2.85*	1.68 *6.07*	1.68 *10.41*	13.68 *3.04*	4.8 *3.36*	1.92 *2.96*	
Water Deposition	Pareto	Location	0.152 *0.152*	0.0097 *0.0097*	0.0038 *0.0038*	0.0038 *0.0038*	0.0308 *0.0308*	0.0109 *0.0109*	0.0039 *0.0039*	μg/L
		Shape	0.739 *0.739*	34.61 *34.61*	1.0238 *1.0238*	1.0238 *1.0238*	1.4931 *1.4931*	1.6017 *1.6017*	0.783 *0.783*	
Soil and Grooming Deposition	Gamma	Location	0.01 *0.0202*	0.0016 *0.0004*	0.0032 *0.0057*	0.0019 *0.0002*	0.0153 *0.0034*	0.0090 *0.0038*	0.0022 *0.0002*	mg/m^2
		Scale	0.01 *0.0146*	0.001 *0.0003*	0.001 *0.004*	0.0014 *0.0001*	0.011 *0.0023*	0.001 *0.0026*	0.0016 *0.0002*	
		Shape	0.9393 *0.9489*	0.9393 *0.9912*	0.9393 *0.9533*	0.9393 *0.9529*	0.9393 *0.953*	0.9393 *0.9653*	0.9393 *0.9654*	
6 Hour Average Aerial Concentrations	Extreme Value (Truncated)	Mode	0.5286	0.0895	0.1285	0.1285	0.876	0.876	0.1871	μg/m^3
		Scale	0.1172	0.0198	0.0285	0.0285	0.1842	0.18418	0.0415	
1 Hour Peak Aerial Concentrations	Weibull (Truncated)	Location	2.24	−2.04	−3.14	−3.14	3.91	−7.95	0.79	μg/m^3
		Scale	1.50	2.64	4.07	4.07	2.19	10.01	0.53	
		Shape	1.46	15	15	15	1.21	15	1.46	

Table 5. Acute and *subchronic* deterministic potential exposure for food, soil, water, grooming, respiration and total potential exposure (PE_{total})[1] (mg/kg BW/day) and risk quotients (RQ)[2] for each active ingredient.

Active	Food	Soil	Water	Grooming	Respiration	PE_{total}	RQ
PBO	0.2749	0.0004	0.0001	0.0033	0.0052	0.2839	0.0005
	0.5903	*0.001*	*<0.0001*	*0.0071*	*0.0006*	*0.5989*	*0.04*
Phenothrin	0.0283	0.0001	< 0.0001	0.0003	0.0003	0.0292	0.0004
	0.0933	*<0.0001*	*<0.0001*	*0.0001*	*<0.0001*	*0.0935*	*0.001*
Permethrin	0.055	0.0001	< 0.0001	0.0003	0.0006	0.0564	0.002
	0.1986	*0.0001*	*<0.0001*	*0.001*	*0.0001*	*0.1999*	*0.008*
Resmethrin	0.055	0.0001	< 0.0001	0.0007	0.0006	0.0569	0.0005
	0.3406	*<0.0001*	*<0.0001*	*0.0001*	*0.0001*	*0.3408*	*0.01*
Malathion	0.4476	0.0007	0.0001	0.0054	0.0036	0.4574	0.02
	0.0995	*0.0002*	*<0.0001*	*0.0012*	*0.0004*	*0.1016*	*0.04*
Naled	0.1571	0.0003	< 0.0001	0.0019	0.0006	0.1610	0.2
	0.1099	*0.0002*	*<0.0001*	*0.0013*	*0.0001*	*0.1116*	*0.6*
Pyrethrins	0.0628	0.0001	< 0.0001	0.0008	0.0006	0.0649	0.003
	0.0969	*<0.0001*	*<0.0001*	*0.0001*	*0.0001*	*0.0971*	*0.02*

[1] $PE_{total} = PE_{food} + PE_{soil} + PE_{water} + PE_{grooming} + PE_{inhalation}$.
[2] $RQ = PE_{total}$/toxic endpoint.

contributed ≤0.16%, grooming contributed 0.02–1.2% and inhalation contributed 0.02–0.4% to potential exposures (Table 5).

Acute and Subchronic Probabilistic Risk

The probabilistic risk assessment revealed that estimates of all deterministic acute and subchronic RQs were conservative. All deterministic RQs were greater than the 85th percentile of probabilistic occurrence (Table 6). Sensitivity analysis using Crystal Ball showed that the amount of insecticide deposition on food (64.7%) contributed the greatest variance to the RQs for acute risk, followed by the amount of food ingested (30.9%) and body weight (4.3%). That is, insecticide deposition on food contributed 64.7% of the variability to the output of the model. The analyses for subchronic risks were similar, with the amount of insecticide deposited on food (64.4%) contributing the majority of variance to the RQs, followed by the amount of food ingested (31.1%) and body weight (4.5%).

DISCUSSION

Based on the results of the probabilistic risk assessment, we believe that our exposure and toxicity assumptions were sufficiently conservative and most likely overestimated risk. For example, we used a respiration rate of 508.05 L/min at a walking pace with a 7% incline, which is considerably more than Lafortuna *et al.* (2003), who found 248.7 L/min at a walking pace with no incline. Yearling horses could also be in the spray zone but they weigh less and eat less so their exposure would not be proportionally more than an adult horse (Grace *et al.* 2002).

Table 6. Acute and *subchronic* probabilistic total potential exposure (PE_{total})[1] (mg/kg BW/day) and risk quotients (RQ)[2] at the 50th, 90th, and 95th percentiles.

	Percentile	50th	90th	95th	*50th*	*90th*	*95th*
PBO	PE_{total}	0.1976	0.2785	0.3021	*0.42*	*0.6*	*0.65*
	RQ	0.0003	0.0004	0.0005	*0.0273*	*0.0384*	*0.0415*
Phenothrin	PE_{total}	0.0202	0.0286	0.0308	*0.07*	*0.09*	*0.1*
	RQ	0.0003	0.0004	0.0004	*0.0009*	*0.0014*	*0.0015*
Permethrin	PE_{total}	0.0395	0.0562	0.0605	*0.141*	*0.2*	*0.216*
	RQ	0.0395	0.0562	0.0605	*0.0057*	*0.008*	*0.008*
Resmethrin	PE_{total}	0.0395	0.0555	0.0601	*0.244*	*0.345*	*0.374*
	RQ	0.0004	0.0005	0.0006	*0.007*	*0.0098*	*0.0107*
Malathion	PE_{total}	0.3223	0.4549	0.4916	*0.071*	*0.101*	*0.109*
	RQ	0.013	0.0184	0.0199	*0.0296*	*0.0419*	*0.0455*
Naled	PE_{total}	0.1128	0.1587	0.1724	*0.08*	*0.11*	*0.12*
	RQ	0.1136	0.16	0.1725	*0.3934*	*0.5574*	*0.60023*
Pyrethrins	PE_{total}	0.0451	0.0635	0.0687	*0.07*	*0.098*	*0.106*
	RQ	0.0023	0.0032	0.0035	*0.016*	*0.0226*	*0.0244*

[1] $PE_{total} = PE_{food} + PE_{soil} + PE_{water} + PE_{grooming} + PE_{inhalation}$.
[2] $RQ = PE_{total}$/toxic endpoint.

Deposition on food sources contributed >96.9% to the total exposure and approximately 65% to the variability of the total exposure. The Kenaga nomogram predicts pesticide deposition after agricultural applications, which typically use larger amounts of insecticide and water per hectare and are sprayed directly onto the food source compared to ULV applications. Therefore, the Kenaga nomogram most likely overestimates ULV insecticide deposition on food.

Currently, there are limited data on airborne and surface deposition of insecticides applied with ULV equipment (Moore *et al.* 1993; Tietze *et al.* 1994, 1996; Knepper *et al.* 1996). Previous studies have found 1–22.3% of the insecticide sprayed during ULV application settled onto the ground, with concentrations decreasing over 36 h (Tucker *et al.* 1987; Moore *et al.* 1993; Tietze *et al.* 1994; Knepper *et al.* 1996). For deposition onto soil and hair, AgDRIFT® predicted about 6% of the insecticide sprayed landed 7.6 m from the spray source, which is comparable with the studies listed earlier. Also, none of the models has a scenario for ULV spray applications, which limits their ability to accurately predict EECs. Measurements of actual environmental concentrations of mosquito insecticides would result in more realistic estimates of exposure and risk to horses and other non-target organisms.

The toxicological uncertainties include extrapolation of mammalian toxicities from rat and dog to horse for each active ingredient. Many of the pyrethroid formulations are synergized with PBO. PBO has been shown to increase the toxicity of pyrethroids to trout, but there is no indication that PBO acts as a synergist in mammals (Paul *et al.* 2005; USEPA 2006c). Based on the toxicological uncertainties,

if a 10-fold uncertainty factor was applied to the NOAELs, naled would be the only insecticide to exceed the LOC.

The Centers for Disease Control and Prevention (CDC) currently recommends that mosquito adulticides be used in conjunction with surveillance data (CDC 2003). Adulticides are used to reduce the density of adult mosquitoes, and therefore most likely reduce the risk of WNV transmission (CDC 2003). In our reasonable worst-case risk assessment, the acute and subchronic exposure for naled resulted in exposures of 20% and 60% of the NOAEL, respectively, the highest risk values of any insecticide we evaluated (Tables 5–6). Therefore, our risk assessment reveals that the acute and subchronic risks to horses from insecticides used to control adult mosquitoes are low and not likely to exceed levels of concern established by regulatory agencies, such as USEPA. The probabilistic assessment revealed that our deterministic exposure estimates were conservative with exposures between the 85th to 95th percentile of exposures. Because of the conservative exposure assumptions used in this study, it is likely that more realistic environmental concentrations are much lower than those presented here.

ACKNOWLEDGMENTS

This study was funded by a grant from the U.S. Armed Forces Pest Management Board's Deployed War Fighter Protection Research Program and by the Montana Agricultural Experiment Station, Montana State University.

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