Prequalification Team Vector Control Decision Document

Royal Guard

(Long Lasting Mosquito Net Treated with Alpha-cypermethrin and Pyriproxyfen)

Prequalification Team–Vector Control Group (PQT-VC)

Access to Medicines, Vaccines and Pharmaceuticals (MVP)

World Health Organization

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1 Introduction

WHO's Prequalification Team-vector control (PQT-VC) ensures that vector control products and public health pesticide active ingredients are safe, effective and manufactured to a high-quality standard. This is done by assessing product dossiers, inspecting manufacturing sites, and supporting quality-control testing of products. Products and manufacturing sites that meet prequalification requirements are added to (a) the WHO list of vector control products or (b) the WHO list of manufacturing sites for public health pesticide active ingredients, respectively.

WHO prequalification of vector control products primarily benefits populations most affected by major vector-borne (often also neglected tropical) diseases such as malaria, dengue fever and other arboviral diseases (Chikungunya, Zika virus), Chagas disease, lymphatic filariasis, visceral leishmaniasis, and human African trypanosomiasis.

This document presents the results of the safety, efficacy and quality (product chemistry and manufacturing process) assessments for the product Royal Guard which provide the basis for the prequalification listing decision.

2 Product Identification

Royal Guard is a combination long lasting insecticide treated bednet (LLIN) incorporated with alpha-cypermethrin and pyriproxyfen. The insecticidal treatment is incorporated into the high density polyethylene monofilament yarn during the extrusion process by addition of a proprietary low density polyethylene alpha-cypermethrin masterbatch and proprietary low density polyethylene pyriproxyfen masterbatch. The product is available in two deniers, 120D and 150D yarn. The 120D product has a fabric weight of 38 GSM and declared content of 5.5 g/kg for both alpha-cypermethrin and pyriproxyfen which corresponds to 208 mg/m^2 for each active ingredient. The 150D product has a fabric weight of 45 GSM and declared content of 5.0 g/kg for both alpha-cypermethrin and pyriproxyfen which corresponds to 225 mg/m^2 for each active ingredient.

The fabric construction relies on a warp knit using a 4-lock stitch. The product is available in rectangular and conical shapes in a variety of sizes and colors.

The source active ingredient and the declared minimum content is:

Alpha-cypermethrin, declared minimum content 930 g/kg

Pyriproxyfen, declared minimum content 970 g/kg

The applicant, Disease Control Technologies LLC, Greer, South Carolina, USA submitted a dossier containing supporting data on the product to PQT-VC on 1 June 2018 and requested a PQ listing for the product. The dossier was updated on 20 July 2018. The product is formulated in Jiangxi Province, China.

3 Assessment of Quality

3.1 Chemical and Physical Properties

Data on the chemical and physical properties of the active ingredients and the product Royal Guard were provided. Product specific properties are summarized in Table 1 for the 120 denier version and Table 2 for the 150 denier version. These data were obtained from studies conducted according to established standards and/or Good Laboratory Practices (GLP) and are complete[1].

Table 1. Chemical & Physical Properties - Royal Guard 120 denier

Title	Study Number	Test method ID	Result
Colour	NA	NA	Standard colours include white, blue, light blue, green, and light green. Custom colours are available upon request. All colours are incorporated via masterbatch pigments and have no effect on insecticidal properties.
Active ingredient content before washing (5 replicates were tested) Batch:120250250	71191123968-CHM15/11A-TQY-CR1	CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/pyriproxyfen content (average of five replicates): 5.36/4.83 g/kg
Active ingredient content before washing Batch:120250351	71191123968-CHM15/11A-TQY-CR1	CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/ pyriproxyfen content (average of five replicates): 5.35/4.81 g/kg
Active ingredient content before washing (5 replicates were tested) Batch:120250452	71191123968-CHM15/11A-TQY-CR1	CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/ pyriproxyfen content (average of five replicates): 5.12/4.98 g/kg
			Nominal content of Active Ingredient Alpha-cypermethrin/pyriproxyfen 5.5/5.5 g/Kg Acceptable range alpha-cypermethrin: 4.1-6.8 g/kg Acceptable range pyriproxyfen 4.1-6.8 g/kg
Wash resistance index (after 4 washing, 1 batch in 3 replicates) Batch:120250452	71191123968-CHM15/11A-TQY-CR1	MT 195 CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/pyriproxyfen average wash resistance index: 100/97.3% Acceptable range: Alpha-cypermethrin/pyriproxyfen more than 90% and 87%, respectively.
Wash resistance index (after storage at 54 °C for 14 days and after 4 washing, 1 batch 3 replicates) Batch120250351	7191123968CHM15/11A-TQY-CR1	MT 195 CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/pyriproxyfen average wash resistance index: 94/90% Acceptable range: Alpha cypermethrin/ pyriproxyfen more than 90% and 87%, respectively.
Storage stability data Batch:120250250 Batch:120250351	7191123968-CHM1511A-TQY-CR1	MT 46.3.4	Percentage of active ingredient present in the net after 14 days at 54 °C: More than 95% of Active ingredient remaining Requirement: More than 95%
Mass per unit area Batch: 120275300 Batch: 120275401 Batch: 120275502	719114040625-ECC16/07-CSL	ISO 3801/EN 12127	Mean: 37 g/square meters Acceptable range: 34.2-41.8 g/square meter

[1] Some data were generated prior to the implementation of the GLP requirement.

Table 1. Chemical & Physical Properties - Royal Guard 120 denier			
Title	Study Number	Test method ID	Result
Mesh size Batch: 120250250	7191123968-ECC15/09A	ISO 7211/2	Mean: 24.5 holes/cm^2 Acceptable value: Minimum average 20 holes/cm^2 No less than 18 holes/cm^2
Bursting strength of fabric (before storage stability) Batch: 120250250 Batch: 120250351	71991123968-EEC15/03A-CSL	ISO 13398-2:1999 (pneumatic method)	Average: 471 KPa Minimum: 400 KPa
Bursting strength of fabric (after storage stability) Batch: 120250250 Batch: 120250351	7191123968-EEC15/04A-CSL	ISO 13398-2:1999 (pneumatic method)	Average: 457 KPa Minimum: 400 KPa
Dimensional stability (before storage stability) Batch: 120250250	7191123968-EEC15/07A-CSLss	ISO 3759 ISO 6330 ISO 5077	shrinkage values: less than 10% expansion values: less than 5%
Dimensional stability (after storage stability at 54±2 °C for 2 weeks) Batch: 120250250	7191123968-EEC15/08A-CSL	ISO 3759 ISO 6330 ISO 5077	shrinkage values: less than 10% expansion values: less than 5%
Dimensional stability			Acceptable value: expansion no more than 5%. Shrinkage no more than 10%
Flammability Batch: 120275300	7191140652-EEC16/09-CS	EN 1102	No surface flash or ignition of filter paper. Not flammable

Table 2. Chemical & Physical Properties - Royal Guard 150 denier			
Title	Study Number	Test method ID	Result
Colour	NA	NA	Standard colours include white, blue, light blue, green, and light green. Custom colours are available upon request. All colours are incorporated via masterbatch pigments and have no effect on insecticidal properties.
Active ingredient content before washing (5 replicates were tested) Batch:150230230	71191123968-CHM15/11B-TQY-CR1	CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/pyriproxyfen content (average of five replicates): 4.96/4.77 g/kg
Active ingredient content before washing Batch:150230331	71191123968-CHM15/11B-TQY-CR1	CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/pyriproxyfen content (average of five replicates): 4.78/4.46 g/kg
Active ingredient content before washing (5 replicates were tested) Batch:150230432	71191123968-CHM15/11B-TQY-CR1	CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/pyriproxyfen content (average of five replicates): 4.80/4.47 g/kg
			Nominal content of Active Ingredient Alpha-cypermethrin/pyriproxyfen 5.0/5.0 g/Kg Acceptable range alpha-cypermethrin 3.75-6.25 g/kg Acceptable range pyriproxyfen 3.75-6.25 g/kg
Wash resistance index (after 4 washing, 1batch in 3replicates)	71191123968-CHM15/11B-TQY-CR1	MT 195 CIPAC 5043 for alpha-cypermethrin	alpha-cypermethrin/pyriproxyfen average wash resistance index: 98/97%

Table 2. Chemical & Physical Properties - Royal Guard 150 denier			
Title	Study Number	Test method ID	Result
Batch:150230432		CIPAC 4887 for pyriproxyfen	
Wash resistance index (after storage at 54 °C for 14 days and after 4 washing,1 batch 3 replicates) Batch150230331	7191123968CHM15/11B-TQY-CR1	MT 195 CIPAC 5043 for alpha-cypermethrin CIPAC 4887 for pyriproxyfen	alpha-cypermethrin/pyriproxyfen average wash resistance index: 95/90% Acceptable range: Alpha-cypermethrin/pyriproxyfen more than 90% and 87%, respectively.
Storage stability data, Batch:150230230 Batch:150230331	7191123968-CHM1511B-TQY-CR1	MT 46.3.4	Percentage of active ingredient present in the net after 14 days at 54 °C: More than 95%. Required more than 95%.
Mass per unit area Batch: 150260275 Batch: 150260376 Batch: 150260477	719114040625-ECC16/08-CSL	ISO 3801	Mean of the batches: 45 g/m^2 Acceptable range: 40.5-49.5 g/m^2
Mesh size Batch: 150230331	7191123968-ECC15/09B	ISO7211/2	Average: 22.5 holes/cm^2 Minimum: Average minimum 20 holes/cm^2 No less than 18 holes/cm^2
Bursting strength of fabric (before storage stability) Batch: 150230230 Batch: 150230331	71991123968-EEC15/03B-CSL	ISO 13398-2:1999 (pneumatic method)	Average: 531 KPa Minimum: 450 KPa
Bursting strength of fabric (after storage stability) Batch: 150230230 Batch: 150230331	7191123968-EEC15/04B-CSL	ISO 13398-2:1999 (pneumatic method)	Average: 457 KPa Minimum: 450 KPa
Dimensional stability (before storage stability) Batch: 150230331	7191123968-EEC15/07B-CSL	ISO 3759 ISO 6330 ISO 5077	shrinkage values: less than 10% expansion values: less than 5%
Dimensional stability (after storage stability at 54±2 °C for 2 weeks) Batch: 150230331	7191123968-EEC15/08B-CSL	ISO 3759 ISO 6330 ISO 5077	shrinkage values: less than 10% expansion values: less than 5%
Dimensional stability			Acceptable value: Expansion no more than 5%. Shrinkage no more than 10%
Flammability Batch: 150260275	7191140652-EEC16/10-CSL	EN 1102	No surface flash or ignition of filter paper. Not flammable

3.2 Manufacturing, Composition and Formulant Information

Data on the manufacturing process and product composition have been provided and are adequate. A summary is presented in Table 3. Detailed information on the manufacturing process and product formulation is considered Confidential Business Information (CBI) and is presented in Appendix A.

Table 3. Data Submitted for Royal Guard		
Description of Starting Material	Confidential Information of Disease Control Technologies, LLC, Royal Guard Statement of Formulation	The manufacturing process starts with mixing HDPE granules with a proprietary mixture of alpha-cypermethrin and pyriproxyfen masterbatch.
Production / Formulation Process	Confidential Information of Disease Control Technologies, LLC, Royal Guard Manufacturing Process	Included in the Confidential Business Information.
Discussion of Impurities	Confidential Information of Disease Control Technologies, LLC, Royal Guard Statement of Formulation	There are no known relevant impurities in the inert ingredients and in the product.
Control Product Specification Form / Confidential Statement of Formula	Quality Dossiers	Included in the Confidential Business Information.
Certification of Limits	Quality Dossiers	120 Denier alpha-cypermethrin/pyriproxyfen 5.5/5.5 g/kg Acceptable range 4.12-6.87 g/Kg for both active ingredients 150 Denier alpha-cypermethrin/pyriproxyfen 5.0/5.0 g/kg Acceptable range 3.75-6.25 g/Kg for both active ingredients
Enforcement Analytical Method	alpha-cypermethrin pyriproxyfen	CIPAC 5043 Extension for CIPAC 454/LN/(3.2 CIPAC 4887 Extension for CIPAC 715/TC/(M)3

3.3 Enforcement Analytical Method

Table 3. Details of the analytical method used to determine alpha-cypermethrin/pyriproxyfen in Royal Guard	
Method ID	**Alpha-cypermethrin** CIPAC 5043 Extension for CIPAC 454/LN/3.2 for quantification in LLIN CIPAC 454/LN/M2.1 for identification in LLIN
Method ID	**Pyriproxyfen** CIPAC 4887 Extension for CIPAC 715/TC/M3 for quantification in LLIN CIPAC 715/LN/2.1 for identification in LLIN

The method is appropriate for the determination of the active ingredient content of the product. This method was validated through the inter-laboratory CIPAC process. Relative standard deviation in the measurements were lower than 2% for both active ingredients in the LLIN.

3.4 Specifications

The source of active ingredient is supported by existing WHO specifications.

The specification for the formulated product has been established through the procedures of the WHO/FAO Joint Meeting on Pesticide Specifications (JMPS).

3.5 Impurities of Toxicological Concern

No impurities of toxicological concern were found in the technical active ingredient and inert ingredients.

3.6 Quality Conclusions

According to the studies presented all physical-chemical properties of the product were in accordance with the specifications. The proposed methods for assessing the physical-chemical properties of the

product were CIPAC methods and/or validated methods. The physical-chemical data were generated in accordance with GLP.

The quality component of the dossier is complete. The assessment of the submitted information on quality supports the prequalification of the product.

4 Assessment of Safety

The applicant submitted an exposure and risk assessment for Royal Guard conducted by Toxicology Regulatory Services (Charlottesville, VA 22911, USA) on behalf of Clariant International Ltd., Switzerland (TRS, 2016) based on the WHO 2012 "Generic Risk Assessment Model for Insecticide Treated Nets (GRAM)".

In 2018, the WHO published a 2nd edition of the GRAM for insecticide treated nets. This document presents the exposure and risk assessment conducted by the PQT-VC according to the more recent 2018 Generic Risk Assessment Model.

Alpha-cypermethrin is a non-systemic broad-spectrum type II synthetic pyrethroid insecticide effective against target pests through contact and ingestion. Pyriproxyfen is a pyridine based, juvenile hormone mimic, insect growth regulator, which affects the reproductive, fecundity and oviposition, and reduces offspring in surviving blood fed mosquitoes.

There is sufficient information on the toxicity of the two active ingredients to assess the human health safety of Royal Guard. This information was obtained through human safety evaluations and risk assessments conducted by regulatory agencies (USEPA, JMPR, JECFA).

The existing toxicology data base of Alpha-cypermethrin and Pyriproxyfen are adequate to support the proposed labelled uses of Royal Guard as an LLIN.

4.1 Product Specific Toxicity Data

Acute toxicity data for the end-use product (Royal Guard) was not submitted and a waiver was requested. Based on the low acute toxicity profile of the components, it is not expected that the acute toxicity of Royal Guard would be different from that of each ingredient or from the combined toxicity of the ingredients since synergistic effect is not expected due to different mechanisms of action. Therefore, the waivers were granted.

The carrier, high density polyethylene (HDPE), is a non-toxic, non-hazardous material and can be considered as safe for contact with humans, animals, and the environment. This carrier is not subject to evaluation in this human health risk assessment.

The toxicity profile of the two active ingredients, alpha-cypermethrin and pyriproxyfen, is shown below.

4.2 Summary of the Available Toxicity Data on the Active Ingredient: Alpha-cypermethrin

Pyrethroids have historically been classified into two groups, Type I and Type II, based on chemical structure and toxicological effects. Pyrethroids disrupt the voltage-gated sodium channels in the nervous system, resulting in neurotoxicity. Alpha-cypermethrin is a Type II pyrethroid. Neurotoxicity

was observed throughout the database and clinical signs characteristic of Type II pyrethroids, such as increased salivation, altered mobility/gait, and tremors, were the most common effects observed. In repeated dose studies with rodents, the main toxicological findings were reduced body weight gain, reduced food consumption, and at higher doses, signs of neurotoxicity (convulsions, tremors, hypersensitivity to touch and sound). Dogs appeared to be the most sensitive species, with clinical signs of neurotoxicity (tremors, gait abnormalities, ataxia, agitation, head nodding, and lip licking) being observed in the absence of body-weight loss. There is no evidence for genotoxic, developmental, reproductive, immunotoxic or carcinogenic potential.

Points of Departures (PODs) based on the most sensitive endpoints in the toxicity database are available for alpha-cypermethrin. The PODs and toxicological endpoints of concern selected for dietary and non-dietary risk assessment are considered protective of any potential adverse effects, including neuro-, developmental, reproductive, immune, and systemic toxicity as well as carcinogenicity for all populations including infants and children.

It should be noted that toxicology studies on cypermethrin, alpha-cypermethrin and zeta-cypermethrin were included in the referenced reviews to evaluate the hazard potentials and inform the selection of PODs for alpha-cypermethrin.

4.2.1 Mammalian Toxicity

- **Acute Toxicity**

Table 4. Acute Toxicity of Alpha-cypermethrin

Route of Exposure	Species	Toxicity	GHS Category	Reference
Oral	Rat	>2000 mg/kg >5000 mg/kg bw (Aqueous suspension) >57 mg/kg bw (Corn oil)	5	USEPA, 2017
			3	TRS, 2018
Dermal	Rat	>2000 mg/kg	5	USEPA, 2017
Inhalation	Rat	>2.79 mg/L	3	USEPA, 2017
Eye Irritation	Rabbit	Mild irritant	3	USEPA, 2017
Skin Irritation	Rabbit	Mild irritant	3	USEPA, 2017
Dermal Sensitization	Guinea pig	Non-sensitizer	--	USEPA, 2017

- **Subchronic Toxicity**

In a subchronic toxicity with rats, the NOAEL was 9.3 mg/kg/day and the LOAEL was 29.6 mg/kg/day based on reduced body weights, body weight gains and decreased food consumption in both sexes and gait changes in males (USEPA, 2012; 2017).

In a subchronic toxicity with dogs, the NOAEL was 2.25 mg/kg/day and the LOAEL was 6.75 mg/kg/day based on significant clinical signs characterized by tremors, gait abnormalities, ataxia, agitation, head nodding and lip licking in both sexes (USEPA, 2012; 2017).

In a 21-day inhalation (nose-only) toxicity with rats, the NOAEL was 0.01 mg/L and the LOAEL was 0.05 mg/L based on increased salivation (USEPA, 2012; 2017).

- **Chronic Toxicity/Carcinogenicity**

In a chronic study with dogs, the NOAEL was 1.5 mg/kg/day and the LOAEL was 3 mg/kg/day based on reddening of the skin and hair loss and associated irritation. No systemic toxicity was seen (JMPR, 2006).

In another chronic toxicity study with dogs, the NOAEL was 4.11 mg/kg/day in males and 4.29 mg/kg/day in females and the LOAEL was 7.9 mg/kg/day in males and 8.45 mg/kg/day in females based on clinical signs, skin reddening, hair loss and tail irritation (USEPA, 2012; 2017).

In a chronic toxicity/carcinogenicity study in rats with cypermethrin, the systemic NOAEL was 75 mg/kg/day; a LOAEL was not established. There was no evidence of carcinogenicity (USEPA, 2012)

In a carcinogenicity study in mice with alpha-cypermethrin, the NOAEL was 3 mg/kg/day and the LOAEL was 11 mg/kg/day based on decreases in body weight, body weight gains, and food efficiency and clinical signs indicative of poor health. In females, the NOAEL was 12 mg/kg/day and the LOAEL was 38 mg/kg/day based on decreased body weight and weight gain (USEPA, 2012; 2017).

In a carcinogenicity study in mice with cypermethrin, the systemic NOAEL was 57 mg/kg/day and the systemic LOAEL was 229 mg/kg/day based on alterations in hematology parameters. Benign lung tumors in females were seen at 229 mg/kg/day dose level (USEPA, 2012; 2017).

- **Developmental Toxicity**

In a pre-natal developmental toxicity study in rats, for maternal toxicity, the NOAEL was 9 mg/kg/day and the LOAEL was 15 mg/kg/day based on decreased body weight gain and food consumption and increased incidence of clinical signs such as unsteady gait, piloerection, limb splay, and hypersensitivity to sound and touch. For developmental toxicity, the NOAEL was 9 mg/kg/day and the LOAEL was 15 mg/kg/day based on decreased fetal weights (USEPA, 2012, 2017).

In a pre-natal developmental toxicity study with rabbits, for maternal toxicity, the NOAEL was 15 mg/kg/day and the LOAEL was 30 mg/kg/day based on decreased body weight gain and food consumption. For developmental toxicity, the NOAEL was 30 mg/kg/day; a LOAEL was not established (USEPA, 2012; 2017).

- **Reproductive Toxicity**

In a reproduction and fertility study in rats with cypermethrin, for parental toxicity the NOAEL was 7.5 mg/kg/day and the LOAEL was 37.5 mg/kg/day based on clinical signs and decreased body weight in both sexes. For offspring toxicity, the NOAEL 7.5 mg/kg/day and the LOAEL was 37.5 mg/kg/day based on decreased mean litter weight gain during lactation (USEPA, 2017).

- **Neurotoxicity**

In an acute neurotoxicity study with rats, the NOAEL was 4 mg/kg/day and the LOAEL was 20 mg/kg/day based on gait abnormalities, prostration, thrashing, vocalization, piloerection, unkept appearance, limb abnormalities and increased reactivity. At 40 mg/kg/day, mortality, neurotoxic clinical signs, FOB effects and slight nerve degeneration were noted (USEPA, 2017).

In a subchronic toxicity study in rats with cypermethrin, the NOAEL was 31 mg/kg/day and the LOAEL was 77 mg/kg/day based on decreased body weight gain, increased landing splay, ataxia, splayed hindlimbs, impaired gait and decreased feces (USEPA, 2017).

- **Genotoxicity**

Alpha-cypermethrin was non-mutagenic in a battery of *in vivo* and *in vitro* assays (JMPR, 2006; USEPA, 2012; 2017).

- **Immunotoxicity**

There was no evidence of immunotoxicity in rats at the highest dose tested (USEPA, 2012; 2017).

- **Absorption/Distribution/Metabolism/Elimination**

Several studies with rats, dogs and mice are available to support the requirement for metabolism in mammals. Some of these studies assess individual cis and *trans* radiolabeled isomers and other studies assess the metabolism of cypermethrin with the label in either the cyclopropyl of the phenoxybenzyl ring. In general, the following has been demonstrated from these studies: cypermethrin is readily absorbed from the gastrointestinal tract and extensively metabolized. It is mostly excreted in the urine that contains several characterized metabolites derived from conjugation of the hydrolysis products of the parent compound following cleavage of the esteratic linkage site (USEPA, 2012, 2017).

- **Dermal Absorption**

Following dermal application, a portion of the applied dose (0.76-0.78%) remained in the skin at the application site and surrounding skin at 120 hours post-application, with the low dose providing a conservative estimate of dermal absorption factor (DAF) of 13.4% (USEPA, 2012).

- **Cancer Classification**

The USEPA has classified alpha-cypermethrin as a Group C "Possible human carcinogen" based on increased incidence of lung adenomas and adenomas plus carcinomas combined in females in the carcinogenicity with CD-1 mice (USEPA, 2012; 2017).

4.3 Summary of the Available Toxicity Data on the Active Ingredient: Pyriproxyfen

Pyriproxyfen is a broad-spectrum pyridine based, juvenile hormone mimic, insect growth regulator, which acts by suppressing embryogenesis within the insect egg and inhibiting metamorphosis and adult emergence of target insects. It also affects oviposition, hence reduces offspring in surviving blood fed mosquitoes. There is no evidence for genotoxicity, developmental toxicity, reproductive toxicity, immunotoxicity or carcinogenicity. Points of Departures (PODs) based on the most sensitive endpoints in the toxicity database are available for Pyriproxyfen. The PODs and toxicological endpoints of concern selected for dietary and non-dietary risk assessment are considered protective of any potential adverse effects, including neurotoxicity, developmental toxicity reproductive toxicity, immunotoxicity, systemic toxicity as well as carcinogenicity for all populations including infants and children.

4.3.1 Mammalian Toxicity

- **Acute Toxicity**

Pyriproxyfen is not toxic by oral ingestion (LD50 rats > 5000mg/kg - GHS Category 5), slightly toxic by inhalation (LC50 > 1.3 mg/L/4 hours - GHS Category 3). It is not toxic by dermal application (dermal LD50 > 2000 mg/kg in rats – GHS Category 5) and is not a skin sensitizer in guinea pigs (GHS Category = Not Applicable). It is minimally irritating to the skin after dermal application and not irritating to the eyes (GHS Category = Not Classified) (USEPA, 2017).

Table 5. Acute Toxicity of pyriproxyfen

Route of Exposure	Species	Toxicity	GHS Category	Reference
Oral	Rat	>5000 mg/kg	5	USEPA, 2017
Dermal	Rat	>2000 mg/kg	5	USEPA, 2017
Inhalation	Rat	> 1.3 mg/L/4 hrs	3	USEPA, 2017
Eye Irritation	Rabbit	Non-irritant	Not Classified	USEPA, 2017
Skin Irritation	Rabbit	Minimally irritant	Not Classified	USEPA, 2017
Dermal Sensitization	Guinea pig	Non-sensitizer	N/A	USEPA, 2017

- **Subchronic Toxicity**

In a 90-day toxicity study, Pyriproxyfen was administered to 10 rats/sex/group in the diet at dose levels of 0, 400, 2000, 5000, or 10000 ppm (equivalent to 0, 24, 118, 309, or 642 mg/kg bw/day for males and 0, 28, 141, 356, or 784 mg/kg bw/day for females) for 90 consecutive days. There was no treatment-related mortality or clinical signs of toxicity in either sex nor substantial changes in food consumption or urinalysis. Body weight was significantly depressed in both sexes after 13 weeks of exposure. The LOAEL is 118 mg/kg/day and 356 mg/kg/day in males and females, respectively, based on decreased mean red blood cell count, hemoglobin, and hematocrit, and elevated total cholesterol and phospholipid levels accompanied by increased liver weight and increased incidence of hepatocyte hypertrophy in both sexes. The NOAEL is 24 mg/kg/day and 141 mg/kg/day in males and females, respectively (USEPA, 2017).

In a 90-day oral dietary study in CD-1 mice, Pyriproxyfen was administered at dietary levels of 0, 200, 1000, 5000, or 10000 ppm (approximately mg/kg/day calculated equivalents: 0, 28.2, 149.4, 838.1, and 2034.5 for males, and 0, 37.9, 196.5, 963.9, and 2345.4 for females). There was nephrosis with renal tubular dilation and dilation and mineralization of the renal pelvises at the two highest doses in conjunction with decreased body weight gain in both sexes. The NOAEL was 1000 ppm (149.4 and 196.5 mg/kg/day in males and females, respectively). The LOAEL was 5000 ppm (838.1 and 963.9 mg/kg/day in males and females, respectively) based on renal pathology, statistically significant increases in liver weights and in liver:body weight ratios, and statistically significant decreases in red blood cell parameters and in body weight gain in males (USEPA, 2017).

In a subchronic toxicity study, Pyriproxyfen was administered in gelatin capsules to 4 beagle/sex at doses of 0, 30, 100, 300, and 1000 mg/kg/day for 90 days. The NOAEL was 100 mg/kg/day. The LOAEL was 300 mg/kg/day based on significantly higher absolute liver weights and liver-to- body weight ratios in the

males, and enlargement of hepatocytes observed in females at that concentration, compared with dogs on the control diet (USEPA, 2017).

In a 28-day inhalation toxicity study, Pyriproxyfen was administered to 10 Sprague-Dawley rats/sex/group in whole body exposure chambers at concentration levels of 0 (air only), 0 (vehicle control), 269, 482, and 1000 mg/m3 for 4 hours/day, 7 days/week for 4 weeks. The NOAEC is 1000 mg/m3 (or 1 mg/L). The LOAEC could not be established (USEPA, 2017).

In a 21-day repeated dose dermal toxicity study, Sprague Dawley rats/sex/dose received dermal application at dose levels of 0, 100, 300, or 1000 mg/kg/day for 6 hours/day, 5 days/week, for 3 weeks. There were no treatment-related differences in findings, organ weight, or macroscopic or microscopic organ morphology between rats in the treated and control groups. The dermal NOAEL is 300 mg/kg/day. The dermal LOAEL for this study is 1000 mg/kg/day, based on the presence of treatment-related dermal irritation in the treated skin of rats in this treatment group. The systemic NOAEL was 1000 mg/kg/day (USEPA, 2017).

- **Chronic Toxicity/Carcinogenicity**

In a combined chronic/carcinogenicity study, Pyriproxyfen was administered to 50 Sprague-Dawley rats/sex/group in diet at dose levels of 0, 120, 600, or 3000 ppm (equivalent to 0, 5.42, 27.3, or 138 mg/kg bw/day for males and 0, 7.04, 35.1, or 183 mg/kg bw/day for females) for 104 weeks. A satellite group of 30 Sprague-Dawley rats/sex/dose were exposed at the same dose levels. After 52 weeks of treatment, 10 animals/sex from the satellite group were weighed, sacrificed, and necropsied. The remaining satellite animals were treated for another 52 weeks and all surviving animals were sacrificed on Week 104 and discarded without necropsy. No treatment-related clinical signs of toxicity or sustained adverse changes in clinical chemistry, hematology, urinalysis, organ weight, and gross or histopathology in either sex were noted. Body weight gain was depressed in females at 183 mg/kg/day. Although there was some evidence of liver toxicity during the first year of exposure, but phospholipid, cholesterol, and AP levels in high dose animals returned to control levels and relative liver weight was not statistically significant at study termination. The female systemic NOAEL is 35.1 mg/kg/day. The female systemic LOAEL is 183 mg/kg/day based on depressed body weight and body weight gain throughout the exposure period. No adverse effects were observed in males up to 138 mg/kg/day. Therefore, the male systemic NOAEL is 138.0 mg/kg/day (highest dose tested) and the male systemic LOAEL could not be established. There was no evidence of a treatment-related increase in the incidence of neoplastic lesions up to the highest dose tested in this study (USEPA, 2017).

In a bioassay study, 50/sex/dose male and female CD-1 mice were treated at levels of 0,120, 600, and 3000 ppm (16.8, 84.0, and 420 mg/kg/day in males and 21.0, 109.5, and 547 mg/kg/day in females) for 78 weeks. There was no evidence of carcinogenicity in either sex (USEPA, 2017).

In a chronic dog toxicity study, Pyriproxyfen was administered in gelatin capsules to 4 beagle/sex at doses of 0, 30, 100, 300, and 1000 mg/kg/day for approximately one year. At 1000 mg/kg, death was reported in two of the four males and was attributed to hepatic failure. In the remaining males, there was a significant increase in prothrombin time, and in both sexes there were increases in hepatic enzyme levels and gross and microscopic hepatic lesions. Decreases in body weight gain were also reported for both sexes and there was an increase in relative and absolute liver weights at the high dose level. The NOAEL

was 100 mg/kg/day. The LOAEL was 300 mg/kg/day based on statistically and biologically significant decreases in body weight gain and increases in relative liver weights in both sexes (USEPA, 2017).

- **Developmental Toxicity**

In a developmental toxicity study, Pyriproxyfen technical (97.2% purity) was administered to groups of 36 pregnant Sprague-Dawley rats by gavage at dose levels of 0, 100, 300, or 1000 mg/kg/day from days 7 through 17 of gestation in a volume of 5ml/kg of corn oil. The maternal NOAEL was 100 mg/kg/day based on decreased body weight, body weight gain, and food consumption, and increased water consumption at the LOAEL of 300 mg/kg/day. At 1000 mg/kg/day, increased incidences of mortality and clinical signs were also observed. The developmental NOAEL was 300 mg/kg/day, based upon an increased incidence of fetuses with poorly ossified sternebrae, skeletal variations and renal pelvis dilation at the developmental LOAEL of 1000 mg/kg/day (USEPA, 2017).

In a developmental toxicity study in rabbits, Pyriproxyfen was administered by gavage at doses of 100, 300, or 1000 mg/kg/day in distilled water on gestation days 6-18. The maternal NOAEL was 100 mg/kg/day. The maternal LOAEL was 300 mg/kg/day, based on occurrence of premature delivery/abortions, soft stools, emaciation, lusterless fur, decreased activity, and bradypnea/deep breathing. The developmental NOAEL was 300 mg/kg/day, based on decreased viable litters available for examinations at the LOAEL of 1000 mg/kg/day (USEPA, 2017).

In summary, there is no evidence to suggest that Pyriproxyfen is a selective developmental toxicant since developmental toxic effects occur only in the presence of maternal toxicity in both species.

- **Reproductive Toxicity**

In a two-generation reproduction study, Pyriproxyfen was administered to Sprague-Dawley rats/sex/dose at dietary levels of 0, 200, 1000, or 5000 ppm (18, 87, or 453 mg/kg/day for males and 20, 96, or 498 mg/kg/day for females) for one litter per generation. The parental systemic NOAEL was 1000 ppm (87 mg/kg/day in males, 96 mg/kg/day in females). The parental systemic LOAEL was 5000 (453 mg/kg/day in males, 498 mg/kg/day in females), based on decreased body weight, body weight gain, and food consumption (both sexes) and increased liver weight in the F1 males and females and histopathological lesions of liver and kidneys of F1 males. The reproductive NOAEL was 5000 ppm (453 mg/kg/day in males, 498 mg/kg/day in females). The reproductive LOAEL was not established. The offspring NOAEL was 1000 ppm (87 mg/kg/day in males, 96 mg/kg/day in females). The offspring LOAEL was 5000 ppm (453 mg/kg/day in males, 498 mg/kg/day in females) based on decreased body weight on lactation days 14 and 21 in F1 and F2 pups (USEPA, 2017).

- **Neurotoxicity**

Decreases in motor activity were only observed at 2000 mg/kg in the acute neurotoxicity study. No symptoms of neurotoxicity were observed in the subchronic neurotoxicity study in rats up to the limit dose (1000 mg/kg/day). Based on information available in the database, the concern for neurotoxicity resulting from Pyriproxyfen exposure is low (USEPA, 2017).

- **Genotoxicity**

The genotoxic potential of Pyriproxyfen has been investigated in numerous in-vivo and in-vitro studies using bacterial and mammalian cells for all endpoints of mutagenicity. There is no evidence to suggest that Pyriproxyfen is mutagenic or clastogenic (USEPA, 2017)

- **Immunotoxicity**

In an immunotoxicity study, Pyriproxyfen was given to four groups of female CD-I mice in the diet at dose levels of 0, 1000, 2000, or 5000 ppm (0, 228, 449, 1139 mg/kg bw/day) for at least 28 days. An additional positive group of 8 female mice were administered cyclophosphamide by gavage once daily at a dose of 20 mg/kg/day for 5 consecutive days (study days 22-26). There were no test substance-related clinical signs of toxicity, morbidity, or mortality during the treatment period. The LOAEL for immunotoxicity is not observed. The NOAEL for immunotoxicity is 5000 ppm (1139 mg/kg/day) (USEPA, 2017).

- **Absorption/Distribution/Metabolism/Elimination**

Based on urine concentration Pyriproxyfen was not easily absorbed (<12%); Pyriproxyfen was excreted rapidly (88.9 – 92.9% within 2 days) and occurred primarily through the feces (over 95%). Release of Pyriproxyfen in expired air was not significant (USEPA, 2017).

- **Dermal Absorption**

No information on the dermal penetration of Pyriproxyfen was available. The lack of systemic toxicity following dermal exposure up to dermal doses of 1000 mg/kg/day suggests dermal penetration is limited. However, a dermal absorption factor (DAF) was required for the long-term dermal assessment because an oral POD was selected for that exposure scenario. Consequently, an upper bound DAF of 30% was calculated based on a comparison of the maternal LOAEL from the rat developmental study to the NOAEL from the rat dermal toxicity study (USEPA, 2017).

- **Cancer Classification**

Pyriproxyfen has been classified as a "Group E" chemical (no evidence for carcinogenicity to humans) based on the absence of carcinogenicity in mice and rats (USEPA, 2017).

4.4 Development of the Risk Assessment

A risk assessment for Royal Guard was conducted according to the "A Generic Risk Assessment Model for Insecticide Treated Nets, 2nd edition, 2018". Risk assessment involves three steps: Hazard assessment, Exposure assessment and Risk characterization.

1. **Hazard assessment** is the identification of the possible toxic effects of a substance, the dose/exposure levels at which those effects occur, and the dose/exposure levels below which no adverse effects are observed. Authoritative evaluations may be used as starting points for the risk assessment of vector control products. Examples of authoritative evaluations are: Joint Meeting on Pesticide Residues (JMPR) – monographs and Evaluations; International Programme on Chemical Safety (IPCS): Concise International Chemical Assessment Documents, Environmental Health Criteria Documents; International Agency for Research and Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans: United States Environmental Protection Agency (USEPA) – Pesticide Evaluations; European Food Safety Authority (EFSA) – Pesticide Risk Assessments; European Chemicals Agency –

Information on Chemicals. JMPR assessments, if available, will be used by PQTVC for risk assessment unless a more recent authoritative evaluation exists.

2. **Exposure assessment** may concern insecticide operators, applicators, residents of treated dwellings and users of other treated buildings, bystanders, domestic animals, wildlife and the environment. Exposure is assessed in a "guideline scenario" which assumes that the insecticide is used according to the instructions given on the product label and in WHO guideline information. Conservative high-end point estimates of the default distributions are used as defaults. No account is taken of intended misuse. All relevant routes of exposure are covered.
3. In **risk characterization** estimates of exposure are compared with acceptable exposure levels previously defined in hazard assessment in all relevant exposure situations.

4.4.1 Hazard Assessment

The Points of Departure (POD) and toxicological endpoints of concern used for acute and chronic exposure risk assessments are presented below.

4.4.1.1 *Alpha-cypermethrin*

- **Acute Reference Dose (ARfD)**

The Joint Meeting on Pesticide Residues (JMPR, 2006) established an acute Reference Dose (ARfD) of 0.04 mg/kg/day based on a NOAEL of 4 mg/kg/day obtained from an acute neurotoxicity with rats and a 100-fold Uncertainty Factor (10X for inter-species extrapolation and 10X for intra-species variation). The LOAEL of 20 mg/kg/day was based on gait abnormalities, prostration, thrashing, vocalization, piloerection, unkept appearance, limb abnormalities and increased reactivity in rats in an acute neurotoxicity study.

The USEPA, in accordance with its 2000 Bench Mark Dose (BMD) Guidance established a BMD and a Bench Mark Dose Lower Confidence (BMDL) for the findings in the Wolansky *et al* 2006 study. The BMDL value of 7.1 mg/kg/day based on decreased motor activity in rats at the BMD of 11.2 mg/kg/day was established. The POD was 7.1 mg/kg/day (USEPA, 2012; 2017). The USEPA established an acute Reference Dose (ARfD) of 0.07 mg/kg/day based on the BMDL of 7.1 mg/kg/day and a 100-fold Uncertainty Factor (10X for inter-species extrapolation and 10X for intra-species variation) (USEPA, 2012; 2017). Although the USEPA ARfD value is higher than that of the JMPR ARfD (0.07 mg/kg bw vs. 0.04 mg/kg bw), the ARfD established by the USEPA is selected in this human health risk assessment due to the robustness of the dose-response data in the critical study. In the PQT-VC opinion, the USEPA ARfD is the most robust value that is sufficiently protective for human health in the present application scenario.

Alpha-cypermethrin acute RfD = 0.07 mg/kg bw/day

- **Chronic Reference Dose (cRfD)**

The USEPA determined that there is no increase in hazard from repeated exposures to the alpha cypermethrin due to the rapid reversibility of the most sensitive neurotoxicity endpoint used for quantifying risks. Therefore, the acute exposure assessment will be protective of chronic effects since acute exposure levels are higher than chronic exposure levels. Accordingly, chronic risk assessment was not conducted (USEPA, 2012; 2017).

Alpha-cypermethrin chronic RfD = Not Applicable

- **Acceptable Daily Intake (ADI) – Total Systemic Dose (TSD)**

The JMPR (2006) established an Acceptable Daily Intake (ADI) of 0.02 mg/kg/day based on a NOAEL of 2.25 mg/kg/day obtained from a sub-chronic dog study and a 100-fold Uncertainty Factor (10X for inter-species extrapolation and 10X for intra-species variation). The LOAEL of 6.75 mg/kg/day was based on significant clinical signs characterized by tremors, gait abnormalities, ataxia, agitation, head nodding and lip licking in both sexes. The NOAEL in this study was supported by the NOAEL of 1.5 mg/kg/day based on abdominal skin reddening and alopecia seen at 3 mg/kg/day in a dog chronic toxicity study (JMPR, 2006).

Alpha-cypermethrin ADI or TSD = 0.02 mg/kg bw/day

4.4.1.2 *Pyriproxyfen*

- **Acute Reference Dose (ARfD)**

An acute reference dose (ARfD) was not established for females 13-50 years old or the general population, because there were no effects in the toxicity database that could be attributed to a single-dose exposure (USEPA, 2017).

Pyriproxyfen Acute RfD = Not applicable

- **Chronic Reference Dose**

The NOAEL of 35.1 mg/kg/day obtained from two rat dietary studies (subchronic and chronic) was selected as the chronic dietary point of departure (POD). The NOAEL was set based on observations of body weight and body weight gain depression in female rats at 183 mg/kg/day in the chronic study, and hematology (decreased red blood cell count, hemoglobin, and hematocrit) and liver effects (increased liver weight concurrent with elevated cholesterol and phospholipid serum levels and increased incidence of hepatocyte hypertrophy) in male rats at 118 mg/kg/day in the subchronic study. This NOAEL was selected because it represented the highest observed dose without an adverse response to dietary treatment that was also protective of the liver and hematology effects observed in the subchronic. Uncertainty factors for interspecies extrapolation (10x) and intraspecies variation (10x) were applied to the chronic dietary POD to calculate a chronic reference dose (CRfD) of 0.35 mg/kg/day.

Pyriproxyfen Chronic RfD = 0.35 mg/kg bw/day

- **Acceptable Daily Intake (ADI) = Total Systemic Dose (TSD)**

A single incidental oral point of departure (POD) = 35.1 mg/kg/day was selected for all duration of exposure. Uncertainty factors for interspecies extrapolation (10X) and intraspecies variation (10X) were applied to the chronic dietary POD to calculate an ADI or TSD of 0.35 mg/kg/day.

Pyriproxyfen ADI or TSD = 0.35 mg/kg bw/day

4.4.2 Exposure Assessment

The second step in performing a risk assessment is to estimate exposure to the insecticide in the various groups of people potentially at risk. Exposure must take account of various parameters, including the route of exposure, the actual amounts of material involved, the duration of exposure in terms of both daily and annual exposure and seasonality, and whether this exposure is intermittent or continuous.

The exposure assessment (i.e., exposure calculations) was conducted in accordance with the input parameters (default values) mainly taken from the 2018 *A Generic Risk Assessment Model for Insecticide-Treated Nets* guidance and chemical specific data. Exposure assessment includes the population [adults, children (6-11 years), toddlers (1-2 years), infants (<1year)], the routes of exposure (inhalation, dermal, oral, and via breast milk), and the different scenarios (sleeping under, washing, and sleeping and washing treated nets). In the total exposure assessments, all relevant routes and different scenarios were summed up to derive the total systemic dose. Exposure is assessed in a **"**guideline scenario", which assumes that the insecticide is used according to the instructions given on the product label and in WHO guideline information. The concentration of the active ingredient in the net (TC) is derived from the WHO (2018) specification of the net (default variability of the concentration being $\pm$ 25%).

TC = 125% X Nominal concentration of the a.i. mg/kg net X Weight of the net kg/m^2.

Conservative high-end point estimates of the default distributions are used as defaults as well as chemical-specific data (e.g., alpha-cypermethrin released into various simulated media such as artificial saliva and sweat solutions) when deemed appropriate. Instead of using a surface concentration of insecticide on the net (TC) and assuming a default transfer coefficient of 6% for the amount of dislodgeable insecticide from net to skin, as proposed in the generic risk assessment model (WHO 2012; 2018), analytical measurements of active ingredients released from the Royal Guard into artificial sweats (acidic and alkaline) after 8 hours of treatment at 37 °C could be used for exposure assessment. The mean concentration of Alpha-cypermethrin released into each acidic or alkaline buffered sweat solutions was below the limit of detection (LOD) of 0.46 mg/L. The LOD concentration for alpha-cypermethrin, equivalent to an overall release of $\mathbf{R_{SW}}$ = 0.031 mg/m^2 net/day, was assumed as the worst-case scenario for the Royal Guard.

The mean concentration of pyriproxyfen released into each acidic or alkaline buffered sweat solutions was below the limit of detection (LOD). The LOD concentration for Pyriproxyfen equivalent to an overall release rate of $\mathbf{R_{SW}}$ = 1.97 mg/m^2 net/day, was assumed as the worst-case scenario for the Royal Guard.

Alpha-cypermethrin Release Rate R_{SW} = 0.031 mg/m^2/day
Pyriproxyfen Release Rate R_{SW} = 1.97 mg/m^2/day

In this human health risk assessment, exposure values obtained after using TC and translodgeable fraction (6%) are used since they are more conservative than those obtained with overall release rate, hence would represent a worst-case scenario.

4.4.2.1 *Alpha-cypermethrin*

- **Estimation of Systemic Doses from Inhalation, Dermal and Oral Exposures due to Sleeping under Treated Nets.**

The individual and cumulative exposures in adults, children, toddlers, and infants via inhalation, dermal, and oral routes are estimated and converted to total systemic exposures.

Total Concentration (TC) of active ingredient on the net = 125% x Nominal concentration of the a.i. mg/kg net x Weight of the net kg/m^2

TC = 125% X 5.5 g Alpha-cypermethrin/kg net X 0.038 kg/m2 = 261 mg/m^2

- **Inhalation Exposure from Sleeping under Treated Nets.**

Alpha-Cypermethrin has a vapor pressure of 1.7x10 -7 and a molecular weight of 416.3. In agreement with the generic risk assessment model, the inhalation exposure during sleeping under a Royal Guard is considered negligible because of the low vapor pressure (WHO, 2012, 2018). Inhalation exposure to alpha-cypermethrin is therefore not of concern.

- **Dermal Exposure from Sleeping under Treated Nets.**

The estimated TWA systemic dose due to potential dermal exposure from sleeping under the net is calculated with the use of chemical-specific data for dermal absorption and concentration of the active ingredient in the net. Instead of using a 10% default dermal absorption factor as recommended in the model, a data-derived dermal absorption factor of 13.4% established by the USEPA is used for calculating the systemic dermal dose.

Table 6. Alphacypermethrin: Estimated TWA Systemic (Dermal) Dose for All Population due to Dermal Exposure from Sleeping Under the Treated Nets.

Population	Absorption (%)	Transl (%)	ESA (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic Dose (µg/kg/ bw/day)
Adult	13.4	6	0.408	6.5	261	60.0	0.92
Children	13.4	6	0.225	6.5	261	23.9	1.28
Toddlers	13.4	6	0.115	6.5	261	10.0	1.57
Infants	13.4	6	0.100	6.5	261	8.0	1.70

Systemic TWA dose (Dermal) = Absorption (Dermal) x Transl x ESA x SF x TC ÷ BW x 1000
where:
Abs = Dermal absorption from net surface
Transl = Translodgeable fraction
ESA= Exposed skin area
SF = Surface fraction
TC = Concentration of the a.i in the net
BW = Body weight
1000 = conversion of mg to ug

- **Oral Exposure to Toddler and Infants due to Sleeping under Treated Nets.**

Oral exposure may occur from hand-to-mouth transfer and from direct mouthing activity such as mouthing, chewing and sucking in the case of infants and toddlers.

➢ **From Hand-to-Mouth Transfer**

The estimated TWA systemic dose due to oral exposure via hand-to-mouth transfer is calculated as follows:

Table 7. Alphacypermethrin: Estimated TWA Systemic Dose Due to Hand-to-Mouth Transfer for Toddlers and Infants Sleeping Under Treated Nets.

Population	Absorption (%)	SE (%)	Transl (%)	EHA (m^2)	FHM (%)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic Dose (µg/kg bw/day)
Toddlers	100	57	6	0.008	16.4	6.5	261	10.0	0.076
Infants	100	57	6	0.007	16.4	6.5	261	8.0	0.083

Systemic Dose = Absorption (oral) SE x Transl x EHA x FHM x SF x TC ÷ BW x 1000
Where:
SE = Salivary extraction factor
Transl = Translodgeability
EHA = Exposed hand area
FHM = Fraction of hand mouthed
SF = Surface faction of the a.i
TC = Concentration of the a.i on the net
BW = Body weight
1000 = conversion of mg to ug

➢ **From Direct Mouth Contact**

The estimated TWA systemic dose due to oral exposure via mouthing, chewing and sucking is calculated as follows:

Table 8. Alphacypermethrin: Estimated TWA Systemic Dose Due to Direct Oral (mouthing, chewing and sucking) Exposures for Toddlers and Infants Sleeping Under Treated Nets.

Population	Absorption (%)	SE (%)	NM (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic Dose (µg/kg bw/day)
Toddlers	100	57	0.0014	6.5	261	10.0	1.35
Infants	100	57	0.0014	6.5	261	8.0	1.69

Systemic Dose = Absorption (oral) SE x NM x SF x TC ÷ BW x 1000
Where:
SE = Salivary extraction factor
NE = Net mouthed
SF = Surface faction of the a.i
TC = Concentration of the a. i on the net
BW = Body weight
1000 = conversion of mg to ug

- **Alphacypermethrin: Total (Inhalation + Dermal + Oral) Systemic Dose due to SLEEPING under Treated Nets.**

A worst-case, total daily systemic exposure to the insecticide while sleeping under a Royal Guard LLIN was calculated in the following table as the summation of the values for inhalation, dermal, and oral routes of exposure as given above in the following table.

Table 9. Alphacypermethrin: Estimated Total Systemic Dose (µg/kg bw/day) due to SLEEPING under Treated Nets.

Population	Inhalation Exposure	Dermal Exposure	Oral (indirect) Exposure	Oral (direct) Exposure	Total Systemic Dose (µg/kg bw/day)
Adult	Negligible	0.92	Not Applicable	N/A	0.92
Children	Negligible	1.28	Not Applicable	N/A	1.28
Toddler	Negligible	1.57	0.076	1.35	3.00
Infants	Negligible	1.70	0.083	1.69	3.47

- **Estimation of Dermal Exposure During Washing of Treated Nets**

The generic risk assessment model assumes that both adults and children may carry out the washing of nets; therefore, exposures in toddlers and infants were not determined for this exposure scenario. Although this exposure is acute, washing is done by people using the net and thus also contribute to their long-term exposure. Therefore, acute (maximum) and repeated (TWA) systemic doses are calculated from dermal and oral exposure scenarios.

- **Dermal Exposure during Washing of Treated Nets.**

The amount deposited on the skin and absorbed systemically during washing is calculated as follows:

  - **<u>From Acute (Maximum) Exposure</u>**

Table 10. Alphacypermethrin: Estimated Systemic Dose (Maximum) From ACUTE DERMAL Exposure Due to Washing Treaded Nets.

Population	Absorption (%)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)	SN (m2)	VolW (mL)	BW (kg)	Systemic Dose (µg/kg bw/day)
Adults	13.4	5	36.7	6.5	261	16	4000	60	27.8
Children	13.4	5	17.6	6.5	261	16	4000	23.9	33.3

Systemic Dose (Maximum) = Absorption (Dermal) x NoN-x VLS x SF x TC x SN ÷ (VolW × BW) x1000
Where:
NoN = Number of nets washed per day;
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index).
TC = Concentration of the a.i on the net
SN = Maximal actual size of the net.
VolW = Volume of washing water.
BW= Body weight
1000 = conversion of mg to ug

- **From Repeated (TWA) Exposure**

Table 11. Alphacypermethrin: Estimated Systemic Dose (TWA) From REPEATED DERMAL Exposure Due to Washing Treated Nets.

Population	Absorption (%)	NoW (washes)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)
Adults	13.4	20/3 years	5	36.7	6.5	261
Children	13.4	20/3 years	5	17.6	6.5	261
	SN (m2)	VolW (mL)	BW (kg)	AT (days)	Systemic Dose (µg/kg bw/day)	
Adults	16	4000	60.0	365	1.52	
Children	16	4000	23.9	365	1.82	

Systemic Dose (TWA) = Absorption (Dermal) x NoW × NoN × VLS × SF × TC × SN ÷ (VolW × BW × AT) x1000
Where:
NoW= Number of washes per year.
NoN = Number of nets washed per day default.
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index%).
TC = Concentration of the a.i on the net
SN = Maximal actual size of the net.
VolW = Volume of washing water.
BW= Body weight.
AT= Average time.
1000 = conversion of mg to ug

- **Oral Exposure during Washing of Treated Nets.**
  - **From Acute (Maximum) Exposure**

Table 12. Alphacypermethrin: Estimated Systemic Dose (Maximum) From ACUTE ORAL Exposure Due to Washing Treaded Nets.

Population	Absorption (%)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)	FHM (%)	SN (m2)	VolW (mL)	BW (kg)	Systemic Dose (µg/kg bw/day)
Adults	100	5	8.2	6.5	261	16.4	16	4000	60.0	7.60
Children	100	5	4.3	6.5	261	16.4	16	4000	23.9	10.01

Washing of nets may also lead to oral exposure. This exposure is estimated as follows:
Systemic Dose (Maximum)= Absorption (Oral) NoN-x VLH x SF x TC x FHM x SN ÷ (VolW × BW) x1000
Where:
NoN = Number of nets washed per day default.
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index %).
TC = Concentration of the a.i on the net
FHM=Transfer from hand to mouth.
SN = Maximal actual size of the net.
VolW = Volume of washing water.
BW= Body weight
1000 = conversion of mg to ug

- **From Repeated (TWA) Exposure**

Table 13. Alphacypermethrin: Estimated Systemic Dose (TWA) From REPEATED ORAL Exposure Due to Washing Nets.						
Population	Absorption (%)	NoW (washes)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)
Adults	100	20/3 years	5	8.2	6.5	261
Children	100	20/3 years	5	4.3	6.5	261
	SN (m2)	VolW (mL)	BW (kg)	AT (days)	Systemic Dose (µg/kg bw/day)	
Adults	16	4000	60.0	365	2.54	
Children	16	4000	23.9	365	3.33	

Systemic Dose (TWA)= Absorption (Oral)x NoW x NoN-x VLH x SF x TC x FHM x SN ÷ (VolW × BW x AT) x1000
Where:
NoW= Number of washes per year.
NoN = Number of nets washed per day default.
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index%).
TC = Concentration of the a.i on the net
FHM= Transfer from hand to mouth
SN = Maximal actual size of the net.
VolW = Volume of washing water; BW= Body weight; AT= Average time.
1000 = conversion of mg to ug

- **Total (Dermal + Oral) Systemic Exposure due to WASHING Treated Nets.**

Cumulative exposures from dermal and oral routes were estimated and converted to systemic doses.

Table 14. Alphacypermethrin: Estimated Total Systemic Dose from Dermal + Oral Exposures due to WASHING Nets.			
Subpopulation	Dermal Exposure (µg/kg bw/day)	Oral Exposure (µg/kg bw/day)	Total Systemic Dose (µg/kg bw/day)
Acute Exposure			
Adult	27.8	7.60	35.4
Children	33.3	10.01	43.3
Repeated Exposure			
Adult	1.52	2.54	4.06
Children	1.82	3.33	5.15

- **Exposure via Breast Milk**

Table 15. Alphacypermethrin: Estimated Maximum Systemic Dose from Exposure via Breast Milk.							
Population	Absorption (%)	SolC	Dose (mg/kg/day)	T1/2 (days)	IR (kg/day)	BW (kg)	Systemic Dose (µg/kg bw/day)
Newborns	100	0.361	0.036	2.5	0.66	4.2	5.10
Infants	100	0.361	0.036	2.5	0.66	8.0	2.68

Table 16. Alphacypermethrin: Estimated TWA Systemic Dose from Exposure via Breast Milk.							
Population	Absorption (%)	SolC	Dose (mg/kg/day)	T1/2 (days)	IR (kg/day)	BW (kg)	Systemic Dose (µg/kg bw/day)
Newborns	100	0.361	0.005	2.5	0.66	4.2	0.71
Infants	100	0.361	0.005	2.5	0.66	8.0	0.37

Estimated systemic Maximum and TWA doses from exposure via breast milk is calculated as follows:
Systemic Dose = Absorption x Sol C × Dose (Mother) × T½ × IR ÷ BWx1000
Where:
SolC = Solubility constant
Dose = Daily dose to the mother
T1/2 = First-order kinetics half time in the body of the insecticide in days.IR = Ingestion rate of milks.
BW = Body weight.
1000 = conversion of mg to ug

4.4.2.2 *Pyriproxyfen*

- **Estimation of Systemic Doses from Inhalation, Dermal and Oral Exposures due to Sleeping under Treated Nets.**

The individual and cumulative exposures in adults, children, toddlers, and infants via inhalation, dermal, and oral routes are estimated and converted to total systemic exposures.

Surface Concentration (TC) of active ingredient on the net = 125% x Nominal concentration of the a.i. mg/kg net x Weight of the net kg/m2

TC = 125% X 5.5 g Pyriproxyfen/kg net X 0.038 kg/m2 = 261 mg/m2

- **Inhalation Exposure from Sleeping under Treated Nets.**

Pyriproxyfen has a vapor pressure of 1.33 x10 -5. In agreement with the generic risk assessment model, the inhalation exposure to Pyriproxyfen during sleeping under a Royal Guard is considered negligible because of the low vapor pressure (WHO, 2012, 2018). Inhalation exposure to Pyriproxyfen is therefore not of concern.

- **Dermal Exposure from Sleeping under Treated Nets.**

The estimated TWA systemic dose due to potential dermal exposure from sleeping under the net is calculated with the use of chemical-specific data for dermal absorption and concentration of the active

ingredient in the net. Instead of using a 10% default dermal absorption factor as recommended in the model, a dermal absorption factor (DAF) of 30% is required for the long-term dermal assessment based on a comparison of the maternal LOAEL from the rat developmental study to the NOAEL from the rat dermal toxicity study (USEPA 2017).

Table 17. Pyriproxyfen: Estimated TWA Systemic (Dermal) Dose for All Population due to Dermal Exposure from Sleeping Under the Treated Nets.

Population	Absorption (%)	Transl (%)	ESA (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic Dose (µg/kg/ bw/day)
Adult	30	6	0.408	6.5	261	60.0	2.07
Children	30	6	0.225	6.5	261	23.9	2.87
Toddlers	30	6	0.115	6.5	261	10.0	3.51
Infants	30	6	0.100	6.5	261	8.0	3.82

Systemic TWA dose (Dermal) = Absorption (Dermal) x Transl x ESA x SF x TC ÷ BW x 1000
where:
Abs = Dermal absorption from net surface
Transl = Translodgeable fraction
ESA= Exposed skin area
SF = Surface fraction
TC = Concentration of the a.i on the net
BW = Body weight
1000 = conversion of mg to ug

- **Oral Exposure to Toddler and Infants due to Sleeping under Treated Nets.**

Oral exposure may occur from hand-to-mouth transfer and from direct mouthing activity such as mouthing, chewing and sucking in the case of infants and toddlers.

- **From Hand-to-Mouth Transfer**

The estimated TWA systemic dose due to oral exposure via hand-to-mouth transfer is calculated as follows:

Table 18. Pyriproxyfen: Estimated TWA Systemic Dose Due to Hand-to-Mouth Transfer for Toddlers and Infants Sleeping Under Treated Nets.

Population	Absorption (%)	SE (%)	Transl (%)	EHA (m^2)	FHM (%)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic Dose (µg/kg bw/day)
Toddlers	100	57	6	0.008	16.4	6.5	261	10.0	0.076
Infants	100	57	6	0.007	16.4	6.5	261	8.0	0.083

Systemic Dose = Absorption (oral) SE x Transl x EHA x FHM x SF x TC ÷ BW x 1000
Where:
SE = Salivary extraction factor
Transl = Translodgeability
EHA = Exposed hand area
FHM = Fraction of hand mouthed
SF = Surface faction of the a.i
TC = Concentration of the a.i on the net
BW = Body weight
1000 = conversion of mg to ug

- **From Direct Mouth Contact**

The estimated TWA systemic dose due to oral exposure via mouthing, chewing and sucking is calculated as follows:

Table 19. Pyriproxyfen: Estimated TWA Systemic Dose Due to Direct Oral (mouthing, chewing and sucking) Exposures for Toddlers and Infants Sleeping Under Treated Nets.

Population	Absorption (%)	SE (%)	NM (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic Dose (µg/kg bw/day)
Toddlers	100	57	0.0014	6.5	261	10.0	1.35
Infants	100	57	0.0014	6.5	261	8.0	1.69

Systemic Dose = Absorption (oral) SE x NM x SF x TC ÷ BW x 1000
Where:
SE = Salivary extraction factor
NE = Net mouthed
SF = Surface faction of the a.i
TC = Concentration of the a.i on the net
BW = Body weight
1000 = conversion of mg to ug

- **Total (Inhalation + Dermal + Oral) Systemic Dose due to SLEEPING under Treated Nets.**

A worst-case, total daily systemic exposure to the insecticide while sleeping under a Royal Guard LLIN was calculated in the following table as the summation of the values for inhalation, dermal, and oral routes of exposure as given above in the following table.

Table 20. Pyriproxyfen: Estimated Total Systemic Dose (µg/kg bw/day) due to SLEEPING under Treated Nets.

Population	Inhalation Exposure	Dermal Exposure	Oral (indirect) Exposure	Oral (direct) Exposure	Total Systemic Dose (µg/kg bw/day)
Adult	Negligible	2.07	Not Applicable	N/A	2.07
Children	Negligible	2.87	Not Applicable	N/A	2.87
Toddler	Negligible	3.51	0.076	1.35	4.94
Infants	Negligible	3.82	0.083	1.69	5.59

- **Estimation of Dermal Exposure During Washing of Treated Nets**

The generic risk assessment model assumes that both adults and children may carry out the washing of nets; therefore, exposures in toddlers and infants were not determined for this exposure scenario. Although this exposure is acute, washing is done by people using the net and thus also contribute to their long-term exposure. Therefore, acute (maximum) and repeated (TWA) systemic doses are calculated from dermal and oral exposure scenarios.

- **Dermal Exposure during Washing of Treated Nets.**

The amount deposited on the skin and absorbed systemically during washing is calculated as follows:

- **From Acute (Maximum) Exposure**

Table 21. Pyriproxyfen: Estimated Systemic Dose (Maximum) From ACUTE DERMAL Exposure Due to Washing Treaded Nets.									
Population	Absorption (%)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)	SN (m2)	VolW (mL)	BW (kg)	Systemic Dose (µg/kg bw/day)
Adults	30	5	36.7	6.5	261	16	4000	60	62.2
Children	30	5	17.6	6.5	261	16	4000	23.9	75.0

Systemic Dose (Maximum) = Absorption (Dermal) x NoN-x VLS x SF x TC x SN ÷ (VolW × BW) x1000
Where:
NoN = Number of nets washed per day;
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index).
TC = Concentration of the a.i on the net
SN = Maximal actual size of the net.
VolW = Volume of washing water.
BW= Body weight
1000 = conversion of mg to ug

From Repeated (TWA) Exposure

Table 22. Pyriproxyfen: Estimated Systemic Dose (TWA) From REPEATED DERMAL Exposure Due to Washing Treated Nets.						
Population	Absorption (%)	NoW (washes)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)
Adults	30	20/3 years	5	36.7	6.5	261
Children	30	20/3 years	5	17.6	6.5	261
	SN (m2)	VolW (mL)	BW (kg)	AT (days)	Systemic Dose (µg/kg bw/day)	
Adults	16	4000	60.0	365	3.40	
Children	16	4000	23.9	365	4.10	

Systemic Dose (TWA) = Absorption (Dermal) x NoW × NoN × VLS × SF × TC × SN ÷ (VolW × BW × AT) x1000
Where:
NoW= Number of washes per year.
NoN = Number of nets washed per day default.
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index%).
TC = Concentration of the a.i on the net
SN = Maximal actual size of the net.
VolW = Volume of washing water.
BW= Body weight.
AT= Average time.
1000 = conversion of mg to ug

- **Oral Exposure during Washing of Treated Nets.**
  - **From Acute (Maximum) Exposure**

Table 23. Pyriproxyfen: Estimated Systemic Dose (Maximum) From ACUTE ORAL Exposure Due to Washing Treaded Nets.

Population	Absorption (%)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)	FHM (%)	SN (m2)	VolW (mL)	BW (kg)	Systemic Dose (µg/kg bw/day)
Adults	100	5	8.2	6.5	261	16.4	16	4000	60.0	7.60
Children	100	5	4.3	6.5	261	16.4	16	4000	23.9	10.01

Washing of nets may also lead to oral exposure. This exposure is estimated as follows:
Systemic Dose (Maximum)= Absorption (Oral) NoN-x VLH x SF x TC x FHM x SN ÷ (VolW × BW) x1000
Where:
NoN = Number of nets washed per day default.
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index %).
TC = Concentration of the a.i on the net
FHM=Transfer from hand to mouth.
SN = Maximal actual size of the net.
VolW = Volume of washing water.
BW= Body weight
1000 = conversion of mg to ug

  - **From Repeated (TWA) Exposure**

Table 24. Pyriproxyfen: Estimated Systemic Dose (TWA) From REPEATED ORAL Exposure Due to Washing Nets.

Population	Absorption (%)	NoW (washes)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m2)
Adults	100	20/3 years	5	8.2	6.5	261
Children	100	20/3 years	5	4.3	6.5	261
	SN (m2)	VolW (mL)	BW (kg)	AT (days)	Systemic Dose (µg/kg bw/day)	
Adults	16	4000	60.0	365	2.54	
Children	16	4000	23.9	365	3.33	

Systemic Dose (TWA)= Absorption (Oral)x NoW x NoN-x VLH x SF x TC x FHM x SN ÷ (VolW × BW x AT) x1000
Where:
NoW= Number of washes per year.
NoN = Number of nets washed per day default.
VLS = Volume of liquid on skin.
SF = Surface fraction of the insecticide (100–wash resistance index%).
TC = Concentration of the a.i on the net
FHM= Transfer from hand to mouth
SN = Maximal actual size of the net.
VolW = Volume of washing water; BW= Body weight; AT= Average time.
1000 = conversion of mg to ug

- **Total (Dermal + Oral) Systemic Exposure due to WASHING Treated Nets.**

Cumulative exposures from dermal and oral routes were estimated and converted to systemic doses.

Table 25. Pyriproxyfen: Estimated Total Systemic Dose from Dermal + Oral Exposures due to WASHING Nets.

Subpopulation	Dermal Exposure (µg/kg bw/day)	Oral Exposure (µg/kg bw/day)	Total Systemic Dose (µg/kg bw/day)
Acute Exposure			
Adult	62.2	7.60	69.8
Children	74.5	10.01	85.01
Repeated Exposure			
Adult	3.40	2.54	5.94
Children	4.07	3.33	7.40

- **Exposure via Breast Milk**

Table 26. Pyriproxyfen: Estimated Maximum Systemic Dose from Exposure via Breast Milk.

Population	Absorption (%)	SolC	Dose (mg/kg/day)	T1/2 (days)	IR (kg/day)	BW (kg)	Systemic Dose (µg/kg bw/day)
Newborns	100	0.361	0.072	2.5	0.66	4.2	6.13
Infants	100	0.361	0.072	2.5	0.66	8.0	3.22

Table 27. Pyriproxyfen: Estimated TWA Systemic Dose from Exposure via Breast Milk.

Population	Absorption (%)	SolC	Dose (mg/kg/day)	T1/2 (days)	IR (kg/day)	BW (kg)	Systemic Dose (µg/kg bw/day)
Newborns	100	0.361	0.008	2.5	0.66	4.2	0.68
Infants	100	0.361	0.009	2.5	0.66	8.0	0.36

Estimated systemic Maximum and TWA doses from exposure via breast milk is calculated as follows:
Systemic Dose = Absorption x Sol C × Dose (Mother) × T½ × IR ÷ BWx1000
Where:
SolC = Solubility constant
Dose = Daily dose to the mother
T1/2 = First-order kinetics half time in the body of the insecticide in days.IR = Ingestion rate of milks.
BW = Body weight.
1000 = conversion of mg to ug

4.4.3 Risk Characterization

The purpose of risk characterization is to examine the probability of adverse effects occurring during the use of the insecticide under defined exposure conditions. Risk characterization consists of comparing the estimate of total exposure (i.e., Estimated Systemic Dose) with the Tolerable Systemic Dose (TSD) established in hazard assessment. The TSD is same as the ADI or the chronic RfD established for the active ingredients (GRAM, 2018).

$$\text{Ratio} = \frac{\text{Total Systemic Dose (µg kg bw/day)}}{\text{TSD (µg/kg bw/day)}}$$

When the ratios are less than 1, the health risk is acceptable. Ratios are greater than 1 may indicated possible health risks in which case steps may be taken to reduce the risk such as changing the recommended operational conditions or the amount of active ingredient in the technical product. A risk-benefit analysis in which the risks of potential toxicity are compared with potential health benefits (disease prevention), may be needed in some cases (GRAM, 2018).

Presented in the following tables are the ratios for the all populations (adults, children, toddlers and infants) sleeping under or for adults and children washing the long-lasting bed nets treated with alpha-cypermethrin and pyriproxyfen.

4.4.3.1 *Alpha-cypermethrin*

Table 28. Alpha-cypermethrin: Exposure Estimates and Risk Ratios for Sleeping Under Treated Nets

Exposure Scenario	Sub Population	Dermal Exposure µg/kg/day	Oral Exposure (Indirect and Direct) µg/kg/day	Total Exposure µg/kg/day	TSD µg/kg/day	RATIO
Sleeping Under Treated Nets	Adults	0.92	N/A	0.92	20	0.05
	Children	1.28	N/A	1.28	20	0.06
	Toddlers	1.57	1.43	3.00	20	0.15
	Infants	1.70	1.77	3.47	20	0.17

Acute TSD = 70 ug/kg bw/day; Chronic TSD = 20 mg/kg bw/day

Table 29. Alpha-cypermethrin: Exposure Estimates and Risk Ratios for Washing Treated Nets							
Exposure Scenario	Sub Population	Dermal Exposure μg/kg/day	Oral Exposure (Indirect and Direct) μg/kg/day	Total Exposure μg/kg/day	Acute TSD μg/kg/day	TSD μg/kg/day	RATIO
ACUTE MAXIMUM WASHING	Adults	27.8	7.60	35.4	70	N/A	0.51
	Children	33.3	10.01	43.3	70	N/A	0.62
TWA REPEATED WASHING	Adults	1.52	2.54	4.06	N/A	20	0.20
	Children	1.82	3.33	5.15	N/A	20	0.26

Acute TSD = 70 ug/kg bw/day; Chronic TSD = 20 mg/kg bw/day

Table 30. Alpha-cypermethrin: Total Exposure Estimates and Risk Ratios							
	Total Sleeping Under Treated Nets μg/kg/day	Total Washing Treated Nets μg/kg/day **ACUTE**	Total Washing Treated Nets μg/kg/day **REPEATED**	Combined Total Exposure μg/kg/day	Acute RfD μg/kg/day	TSD or ADI μg/kg/day	RATIO
Adults	0.92	35.4	N/A	36.32	70	N/A	0.52
Children	1.28	43.3	N/A	44.58	70	N/A	0.64
Adults	0.92	N/A	4.06	4.98	N/A	20	0.25
Children	1.28	N/A	5.15	6.43	N/A	20	0.32

Acute TSD = 70 ug/kg bw/day; Chronic TSD = 20 mg/kg bw/day

Table 31. Alpha-cypermethrin: Exposure Estimates and Risk Ratios – Breast Milk					
Exposure Scenario	Sub Population	Breast Milk Exposure μg/kg/day	Acute TSD μg/kg/day	TSD μg/kg/day	RATIO
ACUTE EXPOSURE (MAXIMUM)	Newborn	5.10	70	N/A	0.07
	Infants	2.68	70	N/A	0.04
TWA REPEATED EXPOSURE	Newborn	0.71	N/A	20	0.036
	Infants	0.37	N/A	20	0.02

Acute TSD = 70 ug/kg bw/day; Chronic TSD = 20 mg/kg bw/day

In the risk assessment scenario presented above, the health risk is acceptable since the risk ratios are less than 1 for adults, children, toddlers and infants sleeping under the treated nets, for adults and children washing the treated nets, and for the combined scenarios.

4.4.3.2 *Pyriproxyfen*

Table 32. Pyriproxyfen: Exposure Estimates and Risk Ratios for Sleeping Under Treated Nets

Exposure Scenario	Sub Population	Dermal Exposure µg/kg/day	Oral Exposure (Indirect and Direct) µg/kg/day	Total Exposure µg/kg/day	TSD µg/kg/day	RATIO
Sleeping Under Treated Nets	Adults	2.07	N/A	2.07	350	0.006
	Children	2.87	N/A	2.87	350	0.008
	Toddlers	3.51	1.43	4.94	350	0.014
	Infants	3.82	1.77	5.59	350	0.016

Acute TSD = N/A; Chronic TSD = 350 mg/kg bw/day; ADI = 350 mg/kg bw/day

Table 33. Pyriproxyfen: Exposure Estimates and Risk Ratios for Washing Treated Nets

Exposure Scenario	Sub Population	Dermal Exposure µg/kg/day	Oral Exposure (Indirect and Direct) µg/kg/day	Total Exposure µg/kg/day	Acute TSD µg/kg/day	TSD µg/kg/day	RATIO
ACUTE MAXIMUM WASHING	Adults	62.2	7.60	69.8	N/A	350	0.200
	Children	75.0	10.01	85.01	N/A	350	0.243
TWA REPEATED WASHING	Adults	3.40	2.54	5.94	N/A	350	0.017
	Children	4.10	3.33	7.43	N/A	350	0.021

Acute TSD = N/A; Chronic TSD = 350 mg/kg bw/day; ADI = 350 mg/kg bw/day

Table 34. Pyriproxyfen: Total Exposure Estimates and Risk Ratios							
	Total Sleeping Under Treated Nets μg/kg/day	Total Washing Treated Nets μg/kg/day **ACUTE**	Total Washing Treated Nets μg/kg/day **REPEATED**	Combined Total Exposure μg/kg/day	Acute RfD μg/kg/day	TSD or ADI μg/kg/day	RATIO
Adults	2.07	69.8	N/A	71.9	N/A	350	0.205
Children	2.87	85.01	N/A	87.9	N/A	350	0.251
Adults	2.07	N/A	5.94	8.01	N/A	350	0.023
Children	2.87	N/A	7.40	10.3	N/A	350	0.029

Acute TSD = N/A ; Chronic TSD = 350 mg/kg bw/day; ADI = 350 mg/kg bw/day

Table 35. Pyriproxyfen: Exposure Estimates and Risk Ratios – Breast Milk					
Exposure Scenario	Sub Population	Breast Milk Exposure μg/kg/day	Acute TSD μg/kg/day	TSD μg/kg/day	RATIO
ACUTE EXPOSURE (MAXIMUM)	Newborn	6.13	N/A	350	0.02
	Infants	3.22	N/A	350	0.01
TWA REPEATED EXPOSURE	Newborn	0.68	N/A	350	0.001
	Infants	0.36	N/A	350	0.001

Acute TSD = N/A ; Chronic TSD = 350 mg/kg bw/day; ADI = 350 mg/kg bw/day

In the risk assessment scenario presented above, the health risk is acceptable since the risk ratios are less than 1 for adults, children, toddlers and infants sleeping under the treated nets, for adults and children washing the treated nets, and for the combined scenarios.

4.5 Safety Conclusions

The potential health risk is acceptable for all populations (adults, children, infants and children) sleeping under, for adults and children washing as well as for adults and children sleeping under and washing the Royal Guard treated nets. The risk ratios are < 1 for all populations, routes of exposure (inhalation, dermal and oral) and all activities (sleeping under, washing and sleeping under and washing).

Table 36. Summary of Risk Characterization for Royal Guard Net LLIN	
Activity/Population	Risk Acceptable / Not acceptable
Sleeping Under Royal Guard LLIN: Inhalation Exposure	
Adult	Negligible
Children	Negligible
Toddlers	Negligible
Infants	Negligible
Washing Royal Guard LLIN: Acute Conditions	
Adult	Acceptable
Children	Acceptable
Washing Royal Guard LLIN: Repeated Conditions	
Adult	Acceptable
Children	Acceptable
Sleeping Under and Washing Royal Guard LLIN Acute Conditions	
Adult	Acceptable
Children	Acceptable
Sleeping Under and Washing Royal Guard LLIN Repeated Conditions	
Adult	Acceptable
Children	Acceptable
Exposures via Breast Milk from Mothers Exposed to Royal Guard	
Newborn (acute and chronic)	Acceptable
Infants (acute and chronic)	Acceptable

The potential health risk is acceptable for all populations (adults, children, infants and children) sleeping under Royal Guard 120 D treated nets, for adults and children washing as well as for all combined scenarios for both Alpha-cypermethrin and Pyriproxyfen. The potential health risk is acceptable for infants and children exposed to Alpha-cypermethrin and Pyriproxyfen via breast milk from mothers sleeping under and washing Royal Guard 120 D treated nets (worst case scenario).

Since the Total Concentration (TC) of Alpha-cypermethrin and Pyriproxyfen in Royal Guard 150 D treated nets is only slightly higher than Royal Guard 120 D (TC = 281 mg/m^2 vs. 261 mg/m^2), PQT-VC has no concern for human risks even under the worst-case scenario assessment for Royal Guard 150 D.

The safety component of the dossier is complete. The assessment of the submitted information on safety supports the prequalification of the products Royal Guard 120D and 150D.

5 Assessment of Efficacy

The primary intention for the use of a pesticide is for the control of a pest or vector, whether resistant or susceptible, rather than for resistance management. Tools which provide effective management of pests or vectors can be used as part of a resistance management plan. For public health pesticides, this is a component of Integrated Vector Management (IVM) which relies on a suite of diverse interventions and implementation of best practices to manage the vector and chemical/behavioral resistance.

A series of studies were provided in the submitted dossier in laboratory and experimental hut settings. These studies were conducted in multiple locations. All studies were evaluated individually, and a summary of the results are provided in the next section.

5.1 Summary of Efficacy Study Results

Table 37. Efficacy Studies for Royal Guard

CREC/LSHTM 1501	**Phase I Evaluation of Regeneration Time, Wash Resistance and Efficacy of Long-Lasting Insecticidal Mosquito Nets Royal Guard® of Disease Control Technologies, USA Against Susceptible and Resistant Strains of *Anopheles gambiae*-** CREC, Benin
	Laboratory Study (Endpoints: KD60, M24, regeneration time, wash resistance) Royal Guard contains two *AI*s – alpha-cypermethrin and pyriproxyfen (PPF). Two major *Anopheles* strains were tested in Cone Bioassays (Cove N=60, Kisumu N=60) and Tunnel Tests (N=300): 1. Pyrethroid susceptible *An. gambiae* s.s. Kisumu laboratory strain 2. Pyrethroid resistant *An. gambiae* Cove CREC/LSHTM strain (kdr and CYP 6P3) Treatments: • Royal Guard 120D LN • PPF 120 D LN • Positive control, alpha-cypermethrin LN (prequalified product) • Negative control LN: untreated ***Results:*** The study was conducted in accordance with the requirements for prequalification. ***Regeneration Time*** (Cone Bioassay): • The regeneration time of alpha-cypermethrin in Royal Guard was 1 day. • The regeneration time of PPF in Royal Guard was derived indirectly from pyriproxyfen-only treated nets using sterility endpoints (i.e., oviposition, fecundity, offspring). The regeneration time was 3 days • The regeneration time for Royal Guard was therefore assumed as 3 days. ***Wash Resistance*** (Cone Bioassay): Pyrethroid susceptible *An. gambiae* s.s. Kisumu laboratory strain • For blood-fed females, knockdown (1 hr) was 100% from wash 0-25, while and mortality (24hr) was 100% until wash 3, >90% after wash 15, and >80% after wash 20. • The impact on reproduction assessed by PPF LNs alone was 88-100% reduction in reproduction (RR) (% oviposition, fecundity, offspring) of surviving females when PPF samples were washed 3 times. Thereafter, % RR decreased with each successive wash. • Royal Guard reduced oviposition, fecundity, and offspring by 100% up to wash 15. Thereafter, the reduction in reproduction ranged from 0 – 58%. Pyrethroid resistant *An. gambiae* Cove CREC/LSHTM strain • Blood-fed females exhibited variable % knockdown (KD60 min) and mortality (M24 hr) when exposed to Royal Guard. There were a couple of significant differences for the KD60 and M24 results, but as they did not make sense (less kill at unwashed than for washed LN, etc.), these results were likely due to variable sample size.

	• The impact on reproduction assessed by PPF LNs alone was 88-100% reduction in reproduction (RR) (% oviposition, fecundity, offspring) of surviving females when PPF samples were washed 3 times. Thereafter, % RR decreased with each successive wash. • Percent reduction in fecundity on Royal Guard was 100% for unwashed, <80% for wash 1 – 5 and increased for subsequent washes. Variability makes it difficult to draw conclusions about fecundity passed 3 washes in cone bioassays. • Percent reduction in offspring on Royal Guard was >80% for up to 25 washes. The trend for knockdown and mortality for unfed mosquitoes was similar to blood-fed mosquitoes. ***Wash Resistance*** (Tunnel Test): Susceptible *An. gambiae* (Kisumu) strain • Positive control: alpha-cypermethrin samples washed 20 times consecutively and then allowed 1 day for regeneration, demonstrated >95% M24 after 20. • Royal Guard washed 20x demonstrated >95% M24. Feeding inhibition was 100% as there were no surviving, blood-fed females. • Percent reduction in reproduction was >90% for washes 0-20 for Royal Guard. Pyrethroid resistant *An. gambiae* Cove CREC/LSHTM strain • Royal Guard caused mortality rates >80% after 20 washes, which was unexpected since this was a resistant strain. Different hypotheses were presented by the researchers as to why this may have occurred. • Blood feeding inhibition with Royal Guard was >80% after 20 washes (low sample size). • PPF nets reduced reproduction by 100%; however, there is some uncertainty about the robustness of this result. ***Controls:*** The negative (untreated) and positive controls (appropriate prequalified product [alpha-cypermethrin]) results were reported and did not indicate issues with the experimental procedure. However, low fecundity of the resistant Cove colony in the lab environment was reported for the negative control, which makes interpreting results for PPF effects difficult.
BIT020 R3 RG	**Phase II Evaluation of alpha-cypermethrin and pyriproxyfen Royal Guard 120D long-lasting insecticidal nets compared to positive control against natural populations of *An. arabiensis* in experimental huts, Tanzania** Ulanga district, south-eastern Tanzania Start (Experimental hut): 23/01/2018 End (Experimental hut): 24/04/2018
	Cone Bioassays (Endpoints: KN60, M24, RR) ***& Tunnel Tests*** (Endpoints: M24, RR)**:** Two major *Anopheles* strains were used: • Susceptible *An. gambiae* (Ifkara strain) • Pyrethroid resistant *An. arabiensis* (Kingani strain, 25% and 16% mortality for cone and hut, respectively) ***Experimental Hut Study*** (Endpoints: M24, RR, feeding inhibition)**:** This study presented results on the efficacy of Royal Guard from experimental huts in Lupiro village, south-eastern Tanzania. The type of hut used was not specified. The study duration was 81 nights. The *Anopheles sp.* tested was: • Naturally occurring alpha-cypermethrin resistant *An. arabiensis* (16% mortality) The following were product relevant treatment arms in the hut trial. The analysis had the power to detect 5% differences in mortality when comparing Royal Guard to the positive control. • Royal Guard 120D LN: unwashed • Royal Guard 120D LN: 20x washed • PPF 120 D LN: unwashed • PPF 120D LN: 20x washed • Positive control, alpha-cypermethrin LN (prequalified product): unwashed • Positive control, alpha-cypermethrin LN (prequalified product): 20x Washed • Negative control LN: untreated

	Results: This study was conducted in accordance with the requirements for prequalification. <u>Cone bioassays and Tunnel test:</u> Susceptible *An. gambiae* s.s. Ifakara strain • Unwashed and 20x washed Royal Guard resulted in M24 > 80% and KD60 >95% and, therefore, retained biological activity after 20 washes. • Unwashed Royal Guard resulted in 100% reduction of fecundity, while the pyrethroid control LN provided 0% reduction of fecundity. • 20x washed Royal Guard did not reduce fecundity when compared to the negative control (untreated LN). Pyrethroid resistant *An. arabiensis* Kingani strain • Unwashed and 20x washed Royal Guard and the positive pyrethroid control did not meet the threshold for knockdown and mortality; M24 and KD60 were far below <80% and <95%, respectively. • Unwashed Royal Guard provided 100% reduction in fecundity compared to the negative control, while the pyrethroid control LN provided 20.4% reduction in fecundity. • Washed Royal Guard resulted in 39.8% reduction of fecundity and >90% feeding inhibition, while the positive control LN provided 0% reduction. • Only two replicates per treatment were conducted, which introduces some uncertainty about the robustness of reported numbers. <u>Hut trial</u>: The most abundant species collected in the hut trials were *An. arabiensis*, *An. funestus*, and *Culex quinquefasciatus*. Pyrethroid resistant *An. arabiensis* Kingani strain • There was no significant difference in M24 values (<20%) between unwashed and 20x washed Royal Guard and positive pyrethroid control. Royal Guard performed equally well as the positive control. • Likewise, there was no significant difference in feeding inhibition between unwashed Royal Guard and the positive pyrethroid control. Royal Guard performed equally well as the positive control. • However, for 20x washed Royal Guard and positive pyrethroid control, there was a significant difference in feeding inhibition; Royal Guard showed superior inhibition (76.0% vs. 67.9%, respectively).
CREC/LSHTM1702B	**Phase II Experimental Hut Evaluation of The Efficacy and Wash Resistance of Royal Guard Against Wild Pyrethroid Resistant *Anopheles gambiae* sl in Cove, Benin** Start: 11/11/2017 End: 31/03/2018
	Cone Bioassays (Endpoints: M24, KD60)**:** The objective of this experiment was to assess the resistance/susceptibility level in the wild population relative to the susceptible lab strain when exposed to Royal Guard. • Susceptible *An. gambiae* (Kisumu strain) were used • Pyrethroid resistant wild *An. gambiae* Cove strain The treatments were: 1. Royal Guard net 120D: unwashed 2. Royal Guard net 120D: 20x washed 3. Positive control net 150D (alpha-cypermethrin): unwashed 4. Positive control net 150D (alpha-cypermethrin): 20x washed ***Hut Study*** (Endpoints: deterrence, M24, RR, feeding inhibition)***:*** This study presented information on the efficacy and wash resistance of Royal Guard in an experimental hut station in a rice field of Cove, Southern Benin. The trial lasted 52 nights. The local vector population at Cove consists of a mixture of *An. colluzzii* (77%) and *An. gambiae* s.s. with the latter being less frequent (23%). The population shows high resistance to pyrethroids (>90%, kdr and CYP6P3). The Experimental huts were of the West African design. In the study, 7 huts were used through which 7 different net samples were rotated (three replicates each). 1. Untreated net 120D (negative control): unwashed 2. Positive control net 150D (alpha-cypermethrin): unwashed

	3. Positive control net 150D (alpha-cypermethrin): 20x washed 4. PPF net 120D: unwashed 5. PPF net 120D: 20x washed 6. Royal Guard net 120D: unwashed 7. Royal Guard net 120D: 20x washed ***Results:*** The study was conducted in accordance with the requirements for prequalification. <u>Cone bioassays</u>: No mortality was reported for the wild resistant strain of *An. gambiae* confirming that resistance was very high in the local population. The susceptible strain had M24 and KD60 above threshold values. There was no significant decline in efficacy for Royal Guard and the positive control before and after 20 washes and before and after the hut trial. Negative control mortality rates were not reported. <u>Hut study</u> • Mortality of free flying resistant *An. gambiae* was 19.2% and 9.5% when exposed to unwashed and 20x washed Royal Guard, respectively. When exposed to pyrethroid alone, the mortality was 7.8% and 4.5%, respectively (significant difference). • PPF alone, mortality was 4.4% and 3.2% (no difference). • Control treatments incurred 0% mortality. • In summary: the results between Royal Guard and pyrethroid only LNs were statistically significant for unwashed treatments with Royal Guard leading to greater mortality for resistant *Anopheles.* For 20x washed LNs, there was no difference in corrected mortality between the two products. Reproductive effects were assessed based on N= 2,938 surviving females. • Blood feeding inhibition was most effective (significantly) in the following order: Royal Guard > pyrethroid > PPF > control. • The pyrethroid only treatment had no effect on oviposition and number of offspring produced by the resistant strain as the percentage did not differ from the results for the negative control. • Oviposition inhibition for Royal Guard and PPF were >80% when unwashed; when washed 20x, PPF and Royal Guard provided 31% and 24% oviposition inhibition, respectively. • Likewise, offspring inhibition was great for unwashed PPF (94%) and Royal Guard (95%) and reduced but still 50% for both treatments after 20x washes. Other Endpoints: • Mosquito deterrence was low for PPF LNs and Royal Guard LNs (7-10% and 4-10%, respectively) and much higher for positive pyrethroid control LNs (39-48%). • Exiting rates differed significantly for Royal Guard unwashed than for pyrethroid LNs unwashed (58% vs. 53%). It's unclear whether the statistical difference has a biological difference.

5.2 Efficacy Conclusions

Considering the entirety of the submitted efficacy studies from the lab and experimental hut settings, there is sufficient information and evidence to conclude that Royal Guard meets the efficacy requirements for prequalification of a pyrethroid treated LN. While the data imply that pyriproxyfen adds to the efficacy of the alpha-cypermethrin net, it is unclear what the actual increased efficacy would be after 20 washes and for different resistant wild *Anopheles* strains.

The efficacy component of the dossier is complete. The assessment of the submitted information on efficacy supports the prequalification of the product.

6 Labelling

The proposed Declaration of Labelling has been reviewed by PQT-VC and found to be consistent with the supporting information.

7 Post-Prequalification Commitments

As per the existing WHO Guidelines on the testing of LLINs, the applicant is required to submit results from long-term field trials (Phase 3).

8 Pre-Qualification Listing Decision

The review of the dossier submitted for the product Royal Guard LLIN has been completed by PQT-VC. The results of the assessments show the product is safe and effective when used according to the directions for use on the label. The product is allowed inclusion on the list of prequalified vector control products.

9 References

- Quality

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- Safety

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9. WHO, 2012: Generic Risk Assessment Model For Insecticide-Treated Nets, Geneva: World Health Organization.
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- Efficacy

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3. Ngufor, Corine (2018). Phase II Experimental Hut Evaluation of The Efficacy and Wash Resistance of Royal Guard Against Wild Pyrethroid Resistant *Anopheles gambiae* sl in Cove, Benin - CREC/LSHTM Collaborative Research Programme, Centre de Recherche Entomologique de Cotonou (CREC), Benin

Appendix A: Confidential Business Information

Appendix B: Alpha-cypermethrin Toxicity Profile (JMPR, 2006; TRS, 2018; USEPA, 2018)

Table 23. Acute Toxicity		
Study Type	Results	GHS Category
Oral	Rat	>2000 mg/kg; >5000 mg/kg bw) Aqueous suspension; >57 mg/kg bw (Corn oil)
Dermal	Rat	>2000 mg/kg
Inhalation	Rat	>2.79 mg/L
Eye Irritation	Rabbit	Mild irritant
Skin Irritation	Rabbit	Mild irritant

Table 24. Acute Toxicity- Alpha-cypermethrin Toxicity Profile	
Study Type/ Doses Tested	Results
90-Day Oral -Rat M: 0, 1.02, 1.74, 9.3, or 29.6mg/kg/day F: 0,1.2, 3.8, 11.3 or 35 mg/kg/day	NOAEL = 9.3 mg/kg/day LOAEL = 29.6 mg/kg/day Effects: Decreased body weight, body weight gain, decreased food consumption and gait changes
90-Day Oral – Dog 0, 0.75, 2.25 or 6.75 mg/kg/day	NOAEL = 2.245mg/kg/day LOAEL = 6.75 mg/kg/day Effects: Clinical signs (tremors, gait abnormalities, ataxia, agitation, head bobbling, and lip licking)
21- Day Dermal – Rabbit (TRS, 2018)	NOAEL = 2000 mg/kg/day LOAEL = Not Established Effects: No treatment-related effects at any dose.
14- Day Inhalation – Rat (TRS, 2018)	NOAEL = 0.0291 mg/L
Developmental Toxicity – Rat 0, 3, 9 or 15 mg/kg/day	Maternal Toxicity NOAEL = 9 mg/kg/day Maternal Toxicity LOAEL = 15 mg/kg/day Effects: Decreases in body weight and clinical signs (unsteady gait, piloerection, limb splay and hypersensitivity to sound and touch). Developmental Toxicity NOAEL= 9 mg/kg/day Developmental Toxicity LOAEL = 15 mg/kg/day Effects: Decreased fetal body weight
Developmental Toxicity – Rabbit 0, 3, 15 or 30 mg/kg/day	Maternal Toxicity NOAEL = 15 mg/kg/day Maternal Toxicity LOAEL =30 mg/kg/day Effects: Decreases in body weight and food consumption. Developmental Toxicity NOAEL= 30 mg/kg/day Developmental Toxicity LOAEL = Not Established Effects: No treatment-related effects at any dose
Reproductive Toxicity – Rat 0, 0, 2.5, 7.5 or 37.5 mg/kg/day	Parental/Offspring Toxicity NOAEL = 7.5 mg/kg/day Parental/Offspring Toxicity LOAEL = 37.5 mg/kg/day Effects: Decreased body weight gain, clinical signs, and decreased mean litter weight.
Chronic Toxicity – Dog M: 0, 2.02, 4.11 or 7.90 mg/kg/day F: 0, 2.18, 4.29 or 8.45 mg/kg/day	NOAEL = 4.1 mg/kg/day (M) and 4.29 mg/kg/day (F) LOAEL = 7.9 mg/kg/day (M) and 8.45 mg/kg/day (F) Effects: Clinical signs (skin reddening, hair loss and tail irritation)

Table 24. Acute Toxicity- Alpha-cypermethrin Toxicity Profile	
Study Type/ Doses Tested	Results
Combined Chronic Toxicity/Carcinogenicity – Rat 0, 1, 10, 100 or 1000 ppm Equivalent to 0, 0.05, 0.5, 5 or 50 mg/kg/day.	NOAEL = 5 mg/kg/day LOAEL = 50 mg/kg/day Effects: Decreases in body weight and food consumption in both sexes. No evidence of carcinogenicity
Carcinogenicity – Mice 0, 30, 100 or 300 ppm Equivalent to: M: 0, 3, 10.6 or 35.2 mg/kg/day F: 0, 3.5, 11.5 or 37.7 mg/kg/day	NOAEL = 10.6 mg/kg/day LOAEL = 35.2 mg/kg/day Effects: Decreased body weight, body weight gain and food consumption and clinical signs. No evidence of carcinogenicity.
Gene Mutation Ames Reverse Test	Negative
Gene Mutation Mammalian Cell	Negative
Chromosomal aberration	Negative
Micronucleus Assay	Not clastogenic
Unscheduled DNA Synthesis	Negative for induction of UDS
Dominant lethal (mouse)	Not mutagenic
Acute Neurotoxicity – Rat 0, 4, 20 or 40mg/kg	NOAEL = 4 mg/kg LOAEL = 20 mg/kg Effects: Clinical signs, FOB changes and mortality.
Immunotoxicity – Rat 0, 4, 12, or 34 mg/kg/day	NOAEL = 34 mg/kg/day LOAEL = Not Established Effect: No effects at the highest dose tested.
Metabolism and Pharmacokinetics - Rat	Regardless of the dose or number of doses, urinary excretion of free and conjugated cyclopropane carboxylic acid occurred primarily within the first 24-hour period after administration each day (30-75% AD). The average excretion during this period considering all subjects (and each day following administration in the repeated dose study) was 43% AD in the single study and 49% AD in the repeated dose study.
Non-Guideline Acute Oral Toxicity in Rats – FOB (McDaniel and Moser 1993.)	At >20 mg/kg/day, clinical sign (gait abnormalities, ataxia, tip toe walking, limb dragging) decreased grip strength and decreased motor activity
Non-Guideline (Wolansky Study) Acute Oral – Motor Active	BMDL1SD= 7.16 mg/kg BMD= 11.20 mg/kg
Non-Guideline (WIL Study) Acute Oral -Rat - FOB	BMDL20= 55.9 mg/kg BMD= 76.3 mg/kg