Prequalification Team Vector Control Decision Document

Royal Sentry 2.0

(Long Lasting Mosquito Net Treated with Alpha-cypermethrin)

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 Sentry 2.0 which provide the basis for the prequalification listing decision.

2 Product Identification

Royal Sentry 2.0 is an alpha-cypermethrin incorporated long lasting insecticide treated bednet (LLIN) made from a proprietary blend of High Density Polyethylene (HDPE). The insecticidal treatment is incorporated into the monofilament yarn during the extrusion process from a proprietary Linear Low-Density Polyethylene (LLDPE) masterbatch containing the active ingredient (alpha-cypermethrin). The finished product contains 5.8 g AI/kg which corresponds to 203 mg AI/m². The denier of the yarn is 120. The fabric construction relies on a warp knit using a 4-lock stitch. The mass or grams per square meter (GSM) of the finished fabric is 35 GSM. The product is available in rectangular and conical shapes in a variety of sizes and colours.

The source active ingredient and the declared minimum content is:

Alpha-cypermethrin, declared minimum content 930 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 9 May 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 Sentry 2.0 were provided. Product specific properties are summarized in Table 1. These data were obtained from studies conducted according to Good Laboratory Practices (GLP) and are complete.

Table 1. Chemical & Physical Properties- Royal Sentry 2.0			
Title	Study Number	Test method ID	Result
Colour	NA	NA	Standard colours include white, blue, light blue, green, dark green. Custom colours such as pink, khaki, yellow are available upon request. All colours are incorporated via masterbatch pigments and have no effect on insecticidal properties.
Active ingredient content before washing (3 batches in 5 replicates were tested) Batch:RS120D2017-500	TÜV SÜD PSB Report 7191164623-CHM17/03CSY	Extension for LN CIPAC 454/TC/(M)2.1 and 454/TC/(M)3	alpha-cypermethrin content (average of five replicates): 5.69 g/kg
Active ingredient content before washing Batch:RS120D2017-702	TÜV SÜD PSB Report 7191164623-CHM17/05CSY	Extension for LN CIPAC 454/TC/(M)2.1 and 454/TC/(M)3	alpha-cypermethrin content (average of five replicates): 6.37 g/kg
Active ingredient content before washing (3 batches in 5 replicates were tested) Batch:RS120D2017-601	TÜV SÜD PSB Report 7191164623-CHM17/04CSY	Extension for LN CIPAC 454/TC/(M)2.1 and 454/TC/(M)3	alpha-cypermethrin content (average of five replicates): 5.59 g/kg
Average			Mean of the batches: 5.88 g/kg Acceptable range: 4.35-7.25 g/kg
Wash resistance index (after 4 washing, 3batches in 3replicates) Batch:RS120D2017-500	TÜV SÜD PSB Report 7191164623-CHM17/06CSY	CIPAC 4827/m-MT195	Average wash resistance index: 99.2%
Wash resistance index Batch:RS120D2017-601	TÜV SÜD PSB Report 7191164623-CHM17/06CSY	CIPAC 4827/m-MT195	Average wash resistance index: 98.0%
Wash resistance index Batch:RS120D2017-702	TÜV SÜD PSB Report 7191164623-CHM17/06CSY	CIPAC 4827/m-MT195	Average wash resistance index: 96.0%
Average			Mean wash resistance index of the batches: 97.7 % Acceptable range: 90-100%
Wash resistance index (after storage at 54 °C for 14 days and after 4 washing, 3batches in 3replicates) Batch:RS120D2017-500	TÜV SÜD PSB Report 7191164623-CHM17/07CSY	CIPAC 4827/m-MT195	Average wash resistance index: 99.3%
Wash resistance index (after storage at 54 °C for 14 days and after 4 washing, 3batches in 3replicates) Batch:RS120D2017-601	TÜV SÜD PSB Report 7191164623-CHM17/07CSY	CIPAC 4827/m-MT195	Average wash resistance index: 95.7%
Wash resistance index (after storage at 54 °C for 14 days and after 4 washing, 3batches in 3replicates) Batch:RS120D2017-702	TÜV SÜD PSB Report 7191164623-CHM17/07CSY	CIPAC 4827/m-MT195	Average wash resistance index: 97.2%
Average			Mean wash resistance index of the batches after storage at 54 °C for 14 days and after 4 washing: 97.4 % Acceptable range: 90-100%

Table 1. Chemical & Physical Properties- Royal Sentry 2.0			
Title	Study Number	Test method ID	Result
Storage stability data Batch:RS120D2017-500	TÜV SÜD PSB Report 7191164623-CHM17/08CSY	Extension for LN CIPAC 454/TC/(M)2.1 and 454/TC/(M)3	Percentage of active ingredient present in the net after 14 days at 54 °C: 95.9%
Storage stability data Batch:RS120D2017-601	TÜV SÜD PSB Report 7191164623-CHM17/08CSY	Extension for LN CIPAC 454/TC/(M)2.1 and 454/TC/(M)3	Percentage of active ingredient present in the net after 14 days at 54 °C: 97.9%
Storage stability data Batch:RS120D2017-702	TÜV SÜD PSB Report 7191164623-CHM17/08CSY	Extension for LN CIPAC 454/TC/(M)2.1 and 454/TC/(M)3	Percentage of active ingredient present in the net after 14 days at 54 °C: 96.2%
Average			Mean percentage of active ingredient present in the net after 14 days at 54 °C: 96.7%
Mass per unit area	TÜV SÜD PSB Report 7191164623-EEC17/01-CSL	ISO 3801:1977	Batch:RS120D2017-500 35.4 g/m^2 Batch:RS120D2017-601 35.1 g/m^2 Batch:RS120D2017-702 35.4 g/m^2
Average			Mean of the batches: 35.3 g/m^2 Acceptable range: 31.5 – 38.5 g/m^2
Mesh size	TÜV SÜD PSB Report 7191164623-EEC17/09-CSL	ISO 7211/2:1984	Batch:RS120D2017-500 20.3 holes/cm^2 Batch:RS120D2017-601 20.3 holes/cm^2 Batch:RS120D2017-702 20.4 holes/cm^2
Average			Mean of the batches: 20.3 holes/cm^2 Acceptable value, minimum 16 holes/cm^2
Bursting strength of fabric (before storage stability)	TÜV SÜD PSB Report 7191164623-EEC17/10-CSL	ISO 13398-2:1999 (pneumatic method)	Batch:RS120D2017-500 380 kPa Batch:RS120D2017-601 393 kPa Batch:RS120D2017-702 413 kPa
Average			Mean of the batches: 395 kPa Acceptable value, minimum 350 kPa
Bursting strength of fabric (after storage stability)	TÜV SÜD PSB Report 7191164623-EEC17/12-CSL	ISO 13398-2:1999 (pneumatic method)	Batch:RS120D2017-500 428 kPa Batch:RS120D2017-601 421 kPa Batch:RS120D2017-702 430 kPa
Average			Mean of the batches: 426 kPa Acceptable value, minimum 350 kPa
Bursting strength of fabric (before storage stability)	TÜV SÜD PSB Report 7191165089-EEC17-CSL	ISO 13398-1:1999 (hydraulic method)	Batch:RS120D2017-500 473 kPa Batch:RS120D2017-601 507 kPa Batch:RS120D2017-702 503 kPa
Average			Mean of the batches: 494 kPa Acceptable value, minimum 350 kPa
Bursting strength of fabric (after storage stability)	TÜV SÜD PSB Report 7191169260-EEC17-CSL	ISO 13398-1:1999 (hydraulic method)	Batch:RS120D2017-500 517 kPa Batch:RS120D2017-601 514 kPa Batch:RS120D2017-702 510 kPa
Average			Mean of the batches: 514 kPa Acceptable value, minimum 350 kPa
Dimensional stability (before storage stability)	TÜV SÜD PSB Report 7191164623-EEC17/11-CSL	ISO 3759:2012 ISO 6330:2012 ISO 5077:2007	shrinkage values: -3.0-0.0 % extension values: 0-0.5 %

Table 1. Chemical & Physical Properties- Royal Sentry 2.0			
Title	Study Number	Test method ID	Result
Dimensional stability (after storage stability at 54±2 °C for 2 weeks)	TÜV SÜD PSB Report 7191164623-EEC17/13-CSL	ISO 3759:2012 ISO 6330:2012 ISO 5077:2007	shrinkage values: -3.2-0.0 % extension values: no extension was observed
Dimensional stability			Acceptable value: Extension no more than 5%. Shrinkage no more than 10%
Flammability	TÜV SÜD PSB Report 7191164623-EEC17/02-CSL	EN 1102:2016 EN ISO 6941:2003	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 has been provided and are adequate. A summary is presented in Table 2. Detailed information on the manufacturing process and product formulation is considered Confidential Business Information (CBI) and is presented in Appendix A.

Table 2. Data Submitted for Royal Sentry 2.0		
Description of Starting Material	Confidential Information of Disease Control Technologies, LLC, Royal Sentry 2.0 Statement of Formulation	The manufacturing process starts with mixing HDPE granules with a proprietary mixture of alpha-cypermethrin masterbatch.
Production / Formulation Process	Confidential Information of Disease Control Technologies, LLC, Royal Sentry 2.0 Manufacturing Process	Included in the Confidential Business Information. Appendix A (Internal use only).
Discussion of Impurities	Confidential Information of Disease Control Technologies, LLC, Royal Sentry 2.0 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 Dossier	Included in the Confidential Business Information. Appendix A (Internal use only).
Certification of Limits	TÜV SÜD PSB Reports: - 7191164623-CHM17/03CSY - 7191164623-CHM17/04CSY - 7191164623-CHM17/05CSY	Mean of the batches: 5.88 g/kg Acceptable range: 4.35-7.25 g/kg
Enforcement Analytical Method	TÜV SÜD PSB Reports: - 7191164623-CHM17/03CSY - 7191164623-CHM17/04CSY - 7191164623-CHM17/05CSY	Extension for LN CIPAC 454/TC/(M)2.1 and 454/TC/(M)3

3.3 Enforcement Analytical Method

Table 3. Details of the analytical method used to determine alpha-cypermethrin in Royal Sentry 2.0	
Method ID	**Alpha-cypermethrin 454 CIPAC method extension for LN – 454/TC/M2.1 – Identification in LN** **Alpha-cypermethrin 454 CIPAC method extension for LN – 454/TC/3.2 – Quantification in LN**

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 1%.

3.4 Specifications

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

The proposed specification for the formulated product will be 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

Alpha-cypermethrin is a broad-spectrum insecticide, effective against target pests through contact and ingestion. Alpha-cypermethrin is a type II synthetic pyrethroid chemical. Pyrethroids disrupt the voltage-gated sodium channels in the nervous system, resulting in neurotoxicity.

In 2012, the World Health Organization (WHO) published a revised edition of a *"Generic Risk Assessment Model for Insecticide Treated Nets (GRAM)"*. Using the 2012 GRAM, Toxicology Regulatory Services, Inc, (TRS), Charlottesville, Virginia conducted a risk assessment for Royal Sentry 2.0 (TRS, 2018).

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.

The existing toxicology database is adequate to support the proposed labelled use of Royal Sentry 2.0.

4.1 Product Specific Toxicity Data

Acute toxicity data for Royal Sentry 2.0 were not submitted by the applicant. The acute toxicity profile of the two components of the product; High Density Polyethylene (HDPE) and alpha-cypermethrin are shown below:

Table 4. Acute Toxicity Data for the Components in Royal Sentry 2.0		
	High Density Polyethylene	Alpha-Cypermethrin
CAS No.	9002-88-4	67375-30-80
Oral LD50	Not toxic	>5000 mg/kg bw (Aqueous suspension) >57 mg/kg bw (Corn oil)
Dermal LD50	Not toxic	> 2000 mg/kg bw (Rat)
Inhalation LC50	Not toxic	>1.2 mg/L (Rat)
Primary Dermal Irritation	Non-irritant	Non-irritant
Primary Eye Irritation	Non-irritant	Non-irritant
Skin Sensitization	Non-sensitizing	Non-sensitizing

The applicant, in lieu of conducting the acute toxicity studies, requested a waiver for the product specific data based on the following rationale:

The formulated product Royal Sentry 2.0 LLIN has two components, HDPE and alpha-cypermethrin. The applicant contends that HDPE is a nontoxic, non-hazard material that can be safe for human contact. Alpha-cypermethrin, an insecticide, has low toxicity for humans.

The hazard profile of the formulated product results from the release of the insecticide, which is limited due to the incorporation of the rather low amount of the active substance in the HDPE monofilament and the slow diffusion of the active ingredient to the surface of the fiber. The release of active ingredient by a similar net with alpha-cypermethrin was in the range of 1 mg/L water per net/day.

- In accordance with the Global Harmonizing Schedule (GHS), Alpha-Cypermethrin is placed in Category 3;
- For the ATE mix, an acute toxicity value of >2000 mg/kg is estimated due to the low concentration (0.58%) in the net;
- Due to the limited availability/release estimate of the active ingredient from the net, no acute oral and/or contact toxicity is expected for the net; and
- Acute toxicity to humans is not expected as no component is regarded as highly toxic.

Based on the above rationale, the applicant concluded that acute toxicity testing is not required for the formulated product (Royal Sentry 2.0). Additionally, the applicant cited the OECD 237 Guidance for waiving or bridging of mammalian acute tests (OECD 2016).

Data waivers were granted for Royal Sentry 2.0 based on the following factors:

- High density polyethylene is practically non-toxic via the oral route (LD50 > 2000 mg/kg in rats), non-irritating to the skin and eyes and is not a skin sensitizer. Polyethylene is safely used for a variety of purposes in cosmetics. It is also used in food packaging materials and medical products, including prosthetics.
- The acute toxicity data for the active ingredient alpha-cypermethrin is completed. It is classified as non-toxic by the oral and dermal route of exposure. It is a mild eye and skin irritant and is not a skin sensitizer.
- Although alpha-cypermethrin has a relatively low acute inhalation LC_{50} (greater than 1.2 mg/L in rats), the inhalation route of exposure is not of concern since Royal Sentry 2.0 has low vapor pressure.

- The concentration of alpha-cypermethrin in the product is minor (0.58%).
- It is not expected that the toxicity of Royal Sentry 2.0 would be different from that of alpha-cypermethrin and/or polyethylene.

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.

The existing toxicology database is adequate to support the proposed use of Royal Sentry 2.0.

4.2.1 Mammalian Toxicity

- **Acute Toxicity**

Table 5. Acute Toxicity of Alpha-cypermethrin

Route of Exposure	Species	Toxicity	GHS Category	Reference
Oral	Rat	>2000 mg/kg	5	USEPA, 2017
		>5000 mg/kg bw (Aqueous suspension) >57 mg/kg bw (Corn oil)	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 Development of the Risk Assessment

A risk assessment for Royal Sentry 2.0 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 insecticides for space spraying. 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.

4.3.1 Hazard Assessment

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

- **Acute Reference Dose (ARfD)**

The Joint Meeting on Pesticide Residues (JMPR) established an acute Reference Dose (ARfD) of 0.04 mg/kg/day based on a NOAEL of 4 mg/kg/day 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 (JMPR, 2006).

The USEPA selected the POD based on the results of a study by Wolansky *et al.* 2006. The authors measured motor activity at the time of peak effect after exposure to 11 pyrethroids, including alpha-cypermethrin. Dose-response relationships were determined using 6 doses and 8 animals/group minimizing variability and increasing the confidence in the study. Moreover, each pyrethroid was evaluated by the same scientist thus decreasing some of the variability associated with neurobehavioral measures (USEPA, 2016).

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; 2016; 2017).

The USEPA established an acute Reference Dose (ARFD) of 0.07 mg/kg/day based on the BMDL of 7.6 mg/kg/day and a 100 -fold Uncertainty Factor which includes 10X for inter-species extrapolation and 10X for intra-species variation (USEPA, 2012; 2017).

Table 6. Acute Reference Dose Established by U.S. EPA

POD= BMDL (mg/kg/day)	Uncertainty Factor	Acute RfD (mg/kg)	Toxicological Endpoint of Concern	Study Selected	Reference
7.1	100	0.07	Decreased motor activity	Wolansky, 2006	USEPA, 2012; 2016

The PQT-VC selected the ARfD established by the USEPA. One of the key factors in the POD selected for deriving the ARfD was the robustness of the dose-response data in the critical study. Therefore, this ARfD is the most robust value that is sufficiently protective for human health in the present application scenario.

- **Chronic Reference Dose (CRfD) - USEPA**

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, a chronic risk assessment was not conducted (USEPA, 2012; 2017).

- **Acceptable Daily Intake (ADI) - JMPR**

The JMPR established an Acceptable Daily Intake (ADI) of 0.02 mg/kg/day based on a NOAEL of 2.25 mg/kg/day 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 based on significant clinical signs characterized by tremors, gait abnormalities, ataxia, agitation, head nodding and lip licking in both sexes' dogs in a subchronic toxicity study. 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 dogs in a chronic toxicity study (JMPR, 2006).

Table 7. Acceptable Daily Intake - JMPR

NOAEL (mg/kg/day)	Uncertainty Factor	ADI (mg/kg/day)	Toxicological Endpoint of Concern	Study Selected	Reference
2.25	100	0.02	Neurotoxic clinical signs (tremors, gait changes, ataxia, agitation, head nodding and lip licking)	Subchronic and chronic Toxicity- Dog.	JMPR, 2006

Based on these assessments (JMPR, 2006 and USEPA, 2012; 2016), the Tolerable Systemic Dose (TSD) for acute is 0.07 mg/kg and the for chronic is 0.02 mg/kg/day.

4.3.2 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. Conservative high-end point estimates of the default distributions are used as defaults.

- **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 5.8 g Alpha-cypermethrin/kg net x 0.035 kg/m2 x 1000 = 253.75 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. The worst-case systemic dose after inhalation exposure is 0.328 x 416.3 x $1.7x10^{-7}$ = 0.000023 mg/kg bw. This is 0.1% of the Tolerable Systemic Dose of 0.02 mg/kg/day. Thus, inhalation exposure is negligible and can be ignored.

- **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.

Dermal absorption: 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 8. Estimated TWA Systemic (Dermal) Dose for All Population due to Dermal Exposure from Sleeping Under the Treated Nets.							
Population	Absorption (%)	Transl (%)	ESA (m2)	SF (%)	TC (mg/m2)	BW (kg)	Systemic Dose (µg/kg bw/day)
Adult	13.4	6	0.408	6.5	253.75	60.0	0.10
Children	13.4	6	0.225	6.5	253.75	23.9	1.25
Toddlers	13.4	6	0.115	6.5	253.75	10.0	1.53
Infants	13.4	6	0.100	6.5	253.75	8.0	1.66
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-t-mouth transfer is calculated as follows:

Table 9. Estimated TWA Systemic Dose Due to Hand-to-Mouth Transfer for Toddlers and Infants Sleeping Under Treated Nets.									
Population	Absorption (%)	SE (%)	Transl (%)	EHA (m2)	FHM (%)	SF (%)	TC (mg/m2)	BW (kg)	Systemic Dose (µg/kg bw/day)
Toddlers	100	57	6	0.008	16.4	6.5	253.75	10.0	0.074
Infants	100	57	6	0.007	16.4	6.5	253.75	8.0	0.081
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 10. 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(m2)	SF (%)	TC(mg/m^2)	BW(kg)	Systemic Dose (µg/kg bw/day)
Toddlers	100	57	0.0014	6.5	253.75	10.0	1.31
Infants	100	57	0.0014	6.5	253.75	8.0	1.65

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 (data derived value)
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 Sentry 2.0 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 11. 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	0.10	Not Applicable	Not Applicable	0.10
Children	Negligible	1.25	Not Applicable	Not Applicable	1.25
Toddler	Negligible	1.53	0.074	1.31	2.91
Infants	Negligible	1.66	0.081	1.65	3.39

- **Estimation of Dermal and Oral 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 12. 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	253.75	15	4000	60.0	25.35
Children	13.4	5	17.6	6.5	253.75	15	4000	23.9	30.51

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 = Target concentration in 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 13. 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	253.75
Children	13.4	20/3 years	5	17.6	6.5	253.75
	SN (m2)	VolW (mL)	BW (kg)	AT (days)	Systemic Dose (µg/kg bw/day)	
Adults	15	4000	60.0	365	1.39	
Children	15	4000	23.9	365	1.67	

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 = Target concentration in 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 14. 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	253.75	16.4	15	4000	60.0	6.93
Children	100	5	4.3	6.5	253.75	16.4	15	4000	23.9	9.12

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 = Target concentration in 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 15. 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	253.75
Children	100	20/3 years	5	4.3	6.5	253.75
	SN (m2)	VolW (mL)	BW (kg)	AT (days)	Systemic Dose (µg/kg bw/day)	
Adults	15	4000	60.0	365	2.31	
Children	15	4000	23.9	365	3.04	

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 = Target concentration in 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 16. 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	25.35	6.93	32.28
Children	30.51	9.12	39.63
Repeated Exposure			
Adult	1.39	2.31	3.70
Children	1.67	3.04	4.71

- **Exposure via Breast Milk**

Table 17. 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	0.0083	2.5	0.66	4.2	1.18
Infants	100	0.361	0.0083	2.5	0.66	8.0	0.62

Table 18. 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	0.001	2.5	0.66	4.2	0.142
Infants	100	0.361	0.001	2.5	0.66	8.0	0.074

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

4.3.3 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).

Ratio = Total Systemic Dose (µg kg bw/day)

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 Table 19 and Table 20 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.

Table 19. Exposure Estimates and Ratios for Sleeping Under OR Washing Treated Nets

Exposure Scenario	Sub Population	Estimated Systemic Dose (µg/kg bw/day)				Total Dose (µg/kg bw/day)	TSD (µg/kg bw/day)	Ratio
		Inhalation	Dermal	Oral				
				Indirect[a]	Direct[b]			
Sleeping Under Treated Nets	Adult	Negligible	0.10	Not Applicable		0.10	20	0.005
	Children	Negligible	1.25	Not Applicable		1.25	20	0.063
	Toddlers	Negligible	1.53	0.074	1.31	2.91	20	0.146
	Infants	Negligible	1.66	0.081	1.65	3.39	20	0.170
Washing Treated Nets (Acute, Maximum)	Adult	Not Applicable	25.35	6.93	Not Applicable	32.28	70	0.461
	Children	Not Applicable	30.51	9.12	Not Applicable	39.63	70	0.566
Washing Treated Nets (Repeated, TWA)	Adult	Not Applicable	1.39	2.31	Not Applicable	3.70	20	0.185
	Children	Not Applicable	1.67	3.04	Not Applicable	4.71	20	0.236

a= Hand-to-mouth transfer; b= Mouthing, chewing, sucking
TSD = For acute exposures = 0.07 mg/kg/day and for repeated exposures = 0.02 mg/kg/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 and for adults and children washing the treated nets.

Presented in the following table are the ratios for adults and children sleeping under and washing the long-lasting bed nets treated with alpha-cypermethrin.

Table 20. Total (Dermal + Oral) Exposure Estimates and Ratios for Sleeping Under AND Washing Treated Nets.							
Sub Population	Sleeping Under Net		ACUTE Washing Net		Total Systemic Dose (µg/kg/day)	TSD µg/kg /day)	Ratio
	Inhalation[a]	Dermal	Dermal	Oral			
	Estimated Systemic Maximum Dose (µg/kg bw/day)						
Adult	Negligible	0.10	25.35	6.93	32.38	70	0.463
Children	Negligible	1.25	30.51	9.12	40.88	70	0.584
	Sleeping Under Net		REPEATED Washing Net				
	Estimated Systemic TWA Dose (µg/kg bw/day)						
Adult	Negligible	0.10	1.39	2.31	3.8	20	0.190
Children	Negligible	1.25	1.67	3.04	5.96	20	0.298
TSD = For acute exposures = 0.07 mg/kg/day and for repeated exposures = 0.02 mg/kg/day							

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

Table 21. Exposure Estimates and Risk Ratios – Breast Milk					
Exposure Scenario	Sub Population	Breast Milk Exposure (µg/kg/day)	Acute TSD (µg/kg/day)	Chronic TSD (µg/kg/day)	RATIO
ACUTE EXPOSURE (MAXIMUM)	Newborn	1.18	70	N/A	0.017
	Infants	0.62	70	N/A	0.009
TWA REPEATED EXPOSURE	Newborn	0.142	N/A	20	0.007
	Infants	0.074	N/A	20	0.004

4.4 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 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 22. Summary of Risk Characterization for Royal Sentry 2.0.

Activity/Population	Risk Acceptable / Not acceptable
Sleeping Under LLIN: Inhalation Exposure	
Adult	Negligible
Children	Negligible
Toddlers	Negligible
Infants	Negligible
Washing LLIN: Acute Conditions	
Adult	Acceptable
Children	Acceptable
Washing LLIN: Repeated Conditions	
Adult	Acceptable
Children	Acceptable
Sleeping Under AND Washing LLIN Acute Conditions	
Adult	Acceptable
Children	Acceptable
Sleeping Under AND Washing LLIN Repeated Conditions	
Adult	Acceptable
Children	Acceptable
Sleeping Under Net and Breast Milk	
New Born	Acceptable
Infants	Acceptable

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

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 23. Efficacy studies for Royal Sentry 2.0	
BIT020 Phase I	**Phase I evaluation of alpha-cypermethrin (Royal Sentry 2.0) long-lasting insecticidal nets compared to Positive Control against susceptible *Anopheles gambiae* s.s. and pyrethroid-resistant *Anopheles arabiensis* strains -** Ifakara Health Institute, Bagamoyo, Tanzania
	Laboratory Study: This study presented information on the regeneration time, induced knockdown/mortality, and wash resistance of Royal Sentry 2.0. Three different *Anopheles* strains were used: 1. Pyrethroid susceptible *An. gambiae* s.s. Ifakara strain 2. *An. arabiensis* Kingani RS strain 3. Moderately resistant *An. arabiensis* Mbita strain. ***Results:*** The study was conducted in accordance with the requirements for prequalification. **Regeneration Time** The regeneration time of Royal Sentry 2.0 is 1 day. The regeneration time was determined as the time required to reach the plateau of mortality in standard cone bioassays. **Knockdown and Mortality** Royal Sentry 2.0 samples washed 3 times consecutively and then allowed 1 day for regeneration, demonstrated 100% knockdown (KD60 min) and mortality (24h) in the susceptible *An. gambiae* s.s. (Ifakara) strain. Royal Sentry 2.0 samples washed 3 times consecutively and then allowed 1 day for regeneration, demonstrated 86% knockdown (KD60 min) and 89% mortality (24h) in the resistant *An. arabiensis* Kingani RS strain. **Wash Resistance** Wash resistance was reported based on standard cone tests for knockdown and mortality. For the pyrethroid susceptible *An. gambiae* Ifakara strain, 24h mortality rate of 100% was observed at 0, 1, 3, 5 and 10 washes. The 24h mortality declined to 81.3% at 20 washes and 55.0% at 25 washes. Up to 25 washes, Royal Sentry 2.0 showed 100% 60 minutes knockdown. For pyrethroid resistant *An. arabiensis* Kingani RS strain, 24h mortality rates of 74%, 46% and 60% (0, 1 and 3 washes) were observed. Knockdown rates of 99%, 96%, 90%, 81% (0, 1, 3 and 5 washes) were observed. For the intermediate pyrethroid resistance strain, *An. arabiensis* Mbita, 24h mortality rates of 90%, 91%, 81% (0, 1 and 3 washes) were observed. The knockdown rates were 90%, 98%, 81%, 98% and 90% (0, 1, 3, 5 and 10 washes) were observed. **Controls** The negative (untreated) and positive controls (Appropriate prequalified product [alpha-cypermethrin]) results were reported and did not indicate issues with the experimental procedure.
BIT020 RS P2	**Phase II evaluation of alpha-cypermethrin Royal Sentry 2.0** **long-lasting insecticidal nets compared to Positive Control against natural populations of *Anopheles arabiensis* in experimental huts, Tanzania** Ulanga district, south-eastern Tanzania Start (Experimental hut): 23/01/2018 End (Experimental hut): 24/04/2018
	Experimental Hut Study: This study presented information on the efficacy of Royal Sentry 2.0 (unwashed and washed) in experimental huts. The experimental hut study was conducted in Lupiro village, Ulanga district, south-eastern Tanzania. Local malaria vectors are *An. gambiae* s.l. with >99.9% *An. arabiensis* and *An. funestus* s.l. with >80% *An. funestus* s.s.

	Wild *An. arabiensis* are resistant to alpha-cypermethrin (i.e. 24 hours mortality in WHO susceptibility test is 16%). The study investigated Royal Sentry 2.0 and other LLINs which are under development. Here, the results from the following trials are presented: 1. Unwashed Positive Control (Appropriate prequalified product [alpha-cypermethrin]) 2. 20x Washed Positive Control (Appropriate prequalified product [alpha-cypermethrin]) 3. Royal Sentry 2.0 unwashed 4. Royal Sentry 2.0 20x washed 5. Untreated net (Negative Control) ***Results:*** The study was conducted in accordance with the requirements for prequalification. **Threshold Endpoints** Cone bioassays with Royal Sentry 2.0 unwashed and washed 20x, using fully susceptible *An. gambiae* s.s. Ifakara strain, resulted in 24h mortality rates above 80% and 60 minutes knockdown was above 95%. In the negative controls, the 24h mortality was 0.63%. Tunnel tests were conducted with the pyrethroid resistant *An. arabiensis* Kingani strain. All net samples (Royal Sentry 2.0 and positive control) exceeded the feeding inhibition threshold of 90%. **Additional Reported Endpoints** For the unwashed nets, the geometric mean control corrected mortality of wild free-flying mosquitoes at 24h was 14.6% for Royal Sentry 2.0 and 12.2% for the positive control. For the 20x washed net samples control corrected mortality rates were 12.4% for Royal Sentry 2.0 and 12.3% for the positive control. According to the authors of the study the low rates are normal for the area where the trial was conducted due to more exophilic behavior of local mosquito populations. Blood feeding inhibition was 66.9% for unwashed Royal Sentry 2.0 and 67.9% for the unwashed positive control. For the 20x washed net samples, blood feeding inhibition was 61.7% for Royal Sentry 2.0 and 58.6% for the positive control.
CREC/LSHTM1702	**Phase 2 experimental hut evaluation of the efficacy and wash resistance of Royal Sentry 2.0 LN against pyrethroid resistant *Anopheles gambiae* sl in Cove, Benin** Start: 11/11/2017 End: 31/03/2018
	Experimental Hut Study: This study presented information on the efficacy and wash resistance of Royal Sentry 2.0 in experimental huts. The local vector population at Cove consists of a mixture of *An. colluzzii* and *An. gambiae* s.s. with the latter being less frequent (approx. 23%). The population shows high phenotypic resistance to pyrethroids. The Experimental huts at Cove are of the West African design. In the study, 5 huts were used through which 5 different net samples were rotated: 6. Unwashed Positive Control (Appropriate prequalified product [alpha-cypermethrin]) 7. 20x Washed Positive Control (Appropriate prequalified product [alpha-cypermethrin]) 8. Royal Sentry 2.0 unwashed 9. Royal Sentry 2.0 20x washed 10. Untreated net (Negative Control) ***Results:*** The study was conducted in accordance with the requirements for prequalification. **Threshold Endpoints** Cone Bioassays using Susceptible *An. gambiae* Kisumu strain: Knockdown and mortality rates were >95% for all samples that were tested. There was no decline in efficacy for Royal Sentry 2.0 and the positive control before and after 20 washes and before and after the hut trial. Negative control mortality rates were not reported.

	Additional Reported Endpoints Mosquito entry and exiting rate: Royal Sentry 2.0 did not exhibit any deterrence when compared to the negative control. The positive control exhibited deterrence of 47.6% (unwashed) and 38.7% (20x washed) when compared to the negative control. Exit rates in Royal Sentry 2.0 trials were 60% with unwashed and 56% with 20x washed samples. Exit rates in the positive control were 53% with unwashed and 47% with 20x washed samples. Blood feeding inhibition: The blood-feeding rate in the negative control was 50%. Blood feeding inhibition rates for Royal Sentry 2.0 were 68% when unwashed and 29% after 20 washes. For the positive control, blood feeding inhibition with the unwashed samples was 34%. This effect declined to 7% when using 20x washed samples. Mortality in wild free-flying resistant *An. gambiae sl* mosquitoes: The mortality in the negative control was 2.4%. For Royal Sentry 2.0 trials, mortality was 26.5% for unwashed samples and 13.9% for 20x washed samples. Mortality in the positive control was 19.4% for unwashed samples and 11.2% for 20x washed samples.

5.2 Efficacy Conclusions

Taking into account the entirety of the submitted efficacy studies in lab and experimental hut settings, there is sufficient information to demonstrate that Royal Sentry 2.0 meets the efficacy requirements for prequalification.

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 Sentry 2.0 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

1. 2018. Royal Sentry 2.0 Dossier submitted to WHO

- Safety

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- Efficacy

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Appendix A: Confidential Business Information

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

Table 24. 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 25. 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 25. 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