Generic risk assessment – Human Health

PYRIPROXYFEN
(CAS No. 95737-68-1)

An active ingredient in insecticide-treated nets

Prequalification Unit – Vector Control Products Assessment
Regulation and Prequalification Department
Access to Medicines and Health Products

October 2024

World Health Organization

Contents

Acronym list

Abs-D	Dermal Absorption from Net Surface
Abs-O	Oral Absorption
ADI	Acceptable Daily Intake
aRfD	Acute Reference Dose
AT	Average Time
BMD	Benchmark Dose
$BMDL_{1SD}$	Benchmark Dose Lower Bound 1 Standard Deviation
BW	Body Weight
CHO	Chinese Hamster Ovary
cRfD	Chronic Reference Dose
DNA	Deoxyribonucleic Acid
DNT	Developmental Neurotoxicity
EC	European Commission
EFSA	European Food Safety Authority
EHA	Exposed Hand Area
ESA	Exposed Skin Area
FAO	Food and Agriculture Organization
FHM	Fraction Transferred from Hand to Mouth
FOB	Functional Observational Battery
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
GRAM	Generic Risk Assessment Model
IARC	International Agency for Research and Cancer
IPCS	International Programme on Chemical Safety
IR	Ingestion Rate
ITN	Insecticide-Treated Net
JMPR	Joint Meeting on Pesticide Residues
LOAEL	Lowest Observed Adverse Effect Level
MOE	Margin of Exposure
NM	Net Mouthed
NOAEL	No Observed Adverse Effect Level
NoN	Number of Nets
NoW	Number of Washes
PCE	Polychromatic Erythrocytes
PND	Postnatal Day
PODs	Points of Departure
PQT/VCP	Prequalification Unit – Vector Control Products Assessment
RfD	Reference Dose
RSW	Release Rate
SE	Salivary Extraction Factor
SF	Surface Fraction

SN	Size of Net
TC	Total Concentration
TEM	Triethylenemelanine
Transl	Translodgeable Fraction
TSD	Tolerable Systemic Dose
TSD_{AC}	Tolerable Systemic Dose – acute
TWA	Time-Weighted Average
UF	Uncertainty Factor
USEPA	United States Environmental Protection Agency
VLH	Volume Liquid on Hand
VLS	Volume of Liquid on Skin
VolW	Volume of Washing Water
WHO	World Health Organization
WRI	Wash Resistance Index

1 Purpose

The purpose of this document is to present a human health hazard and risk assessment for Pyriproxyfen-treated bednets (factory coated or incorporated). It is intended to be used by applicants, regulatory authorities and other stakeholders as an example of the implementation of the *Generic risk assessment model for insecticide-treated nets, 2nd edition (GRAM)* (WHO, 2018).

The product characteristics, including the fabric weight (40 g/m^2) concentration of Pyriproxyfen (6.5 g active ingredient/kg net), and the wash resistance index (90%) were selected as representative values which exemplify currently prequalified insecticide-treated net (ITN) products.

The assessment assumes that the product is a uniformly treated Pyriproxyfen ITN (coated or incorporated) with the following product characteristic values:

- Fabric weight: 40 g/m^2
- Concentration by weight of the net: 6.5 g Pyriproxyfen/kg net
- Concentration by net area: 260 mg Pyriproxyfen/m2
- Wash Resistance Index: 90%

Note: The selected values are not intended to put a limit on the possible concentration of Pyriproxyfen in an ITN.

The selected values do not represent the maximum concentration of Pyriproxyfen at which the assessed risks may become unacceptable.

In support of new product applications or change applications submitted to the World Health Organization (WHO) Prequalification Unit – Vector Control Products Assessment (PQT/VCP), applicants may include reference to this document as part of the product dossier; however, applicants must determine if the design of their product or certain characteristics would require altering the presented risk assessment to properly reflect and assess the proposed product.

2 Development of the risk assessment

The present human health risk assessment for Pyriproxyfen was conducted according to the *Generic risk assessment model for insecticide-treated nets, 2nd edition* (WHO, 2018). Risk assessment involves three steps: hazard assessment, exposure assessment and risk characterization.

- **Hazard assessment** is the identification of the possible toxic effects of a substance, the dose/exposure levels at which those effects may 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. 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, are used by WHO for risk assessment unless a more recent authoritative evaluation exists.
- **Exposure assessment** may concern insecticide operators, applicators, residents of treated dwellingsand 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 accordingto the instructions given on the product label and in WHO guideline information. Conservative high endpoint estimates of the default distributions are used as defaults. No account is taken of intended misuse. All relevant routes of exposure are considered.
- **Risk characterization** is the final step in a risk assessment whereby estimates of exposure arecompared with acceptable exposure levels as defined in the hazard assessment for all relevantexposure scenarios.

The risk assessment for Pyriproxyfen is based on the proposed uses of the ITN products, i.e., net used oversleeping areas.

2.1 Hazard assessment

The complete Pyriproxifen Pyriproxyfen hazard assessment conducted to support this generic risk assessment is included in Appendix A. In summary, after acute exposure, Pyriproxyfen is placed in GHS Category 5 for oral and dermal toxicities and in GHS Category 4 for inhalation toxicity. It is a mild eye irritant (GHS Category 3B) and is neither a dermal irritant nor a skin sensitizer. Following repeated oral exposure to mice, rats and dogs, body weight effects and hematological alterations were the common effects, and the liver and kidney were the principal targets for Pyriproxyfen. Following repeated exposures, there was no evidence of dermal or inhalation toxicity concerns. There was no evidence of neuro-, immuno-, developmental, reproductive, carcinogenic, or genotoxic concerns.

Oral exposure

Several studies with laboratory animals are available to describe the metabolism in mammals. Oral absorption of pyriproxyfen appears to be slow (50%) in rats. Elimination from the body was rapid and occurred primarily through the faeces. Neither sex nor dose affected absorption or elimination; however, the extent of metabolism and prevalence of metabolites was sex dependent.

Dermal exposure

Following dermal application, no dermal or systemic toxicity was seen following repeated dermal application at doses up to 1000 mg/kg/day. 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 suggested dermal penetration was limited. However, a dermal absorption factor (Abs-D) was required for the long-term dermal assessment because an oral POD was selected for that exposure scenario. Consequently, an upper bound Abs-D of 30% was calculated based on a comparison of the maternal LOAEL from the rat developmental study to the NOAEL (upper bound estimate) from the rat dermal toxicity study (USEPA, 2017).

Inhalation exposure

Absorption via the inhalation route is assumed to be 100% of that via the oral route. Due to the lack of a long-term inhalation study, oral equivalent is used for risk assessment purposes.

2.1.1 Points of departure

Points of departure (PODs) (No Observed Adverse Effect Level [NOAEL]; Benchmark Dose [BMD]) are determined from the toxicological database based on the most sensitive endpoints. Once PODs are developed, safety factors (SF) and uncertainty factors (UF) are used in conjunction with the PODs to derive a safe exposure level, generally referred to as reference dose (RfD) or acceptable daily intake (ADI).

Acute oral exposure

According to the risk assessment model, authoritative sources may be used as starting points for the risk assessment of insecticides used in long-lasting mosquito nets.

JMPR (1999 and 2001) did not select a POD for acute exposure since the meeting concluded that this value was unnecessary due to hazard following a single dose.

USEPA (2017) did not select a POD for acute exposure to Pyriproxyfen since a toxicity endpoint attributable to a single exposure was not identified in the toxicology database including the acute neurotoxicity and the developmental toxicity studies.

Chronic oral exposure

JMPR (1999) selected the NOAEL of 10.0 mg/kg bw/day from two chronic dog studies as the POD for the establishment of the ADI (acceptable daily intake). The NOAEL is based on the absence of treatment-related toxicity at 10 mg/kg bw/day in the second study and changes in lipid metabolism and increased liver weight at 30 mg/kg bw per day in the first study.
USEPA (2017) established the POD for chronic oral exposure based on a NOAEL of 35.1 mg/kg/day using subchronic and chronic toxicity studies in rats as co-critical studies. At the LOAEL of 118 mg/kg/day, decreased mean red blood cell count, hemoglobin, and hematocrit, and elevated cholesterol and phospholipid levels

accompanied by increased liver weight and incidence of hepatocyte hypertrophy in the subchronic study with rats and decreased body weight and body weight gain were noted in the chronic toxicity study.

2.1.2 Reference doses

A reference dose (RfD) is an estimate of daily oral exposure (acute, aRfD) for a duration of 24 hours or less or chronic oral exposure (cRfD) for a duration of several months to a lifetime that is likely to be without an appreciable risk of deleterious effects during a lifetime. Both an aRfD and a cRfD can be derived from a NOAEL, LOAEL, or a benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used.

Acute reference dose (aRfD)

Both JMPR (1999 and 2001) and USEPA (2017) did not establish an acute RfD for pyriproxyfen since a toxicity endpoint attributable to a single exposure was not identified in the toxicology database including the acute neurotoxicity and the developmental toxicity studies.

aRfD = Not established

Chronic reference dose (cRfD)

USEPA established an cRfD of 0.35 mg/kg/day based on a NOAEL of 35.1 mg/kg/day using subchronic and chronic toxicity studies in rats as co-critical studies and an uncertainty factor of 100 to account for interspecies extrapolation(10X) and intraspecies variation (10X). The LOAEL of 118 mg/kg/day is based on decreased mean red blood cell count, hemoglobin, and hematocrit, and elevated cholesterol and phospholipid levels accompanied by increased liver weight and incidence of hepatocyte hypertrophy in the subchronic study with rats and decreased body weight and body weight gain at 183 mg/kg/day in the chronic toxicity study.

cRfD = -0.35 mg/kg bw

Table 1. USEPA cRfD

Population of concern	POD = NOAEL (mg/kg/day)	Uncertainty factor	LOAEL (mg/kg/bw)	Toxicological endpointof concern	Study selected	Reference
General population	35.1	100	118	Decreased mean red blood cell count, hemoglobin, and hematocrit, and elevated cholesterol and phospholipid levels accompanied by increased liver weight and incidence of hepatocyte hypertrophy	Subchronic- rat	USEPA, 2017

Acceptable daily intake (ADI)

The JMPR (1999) established an acceptable daily intake (ADI) of 0.1 mg/kg bw/day for Pyriproxyfen based on the NOAEL of 10.0 mg/kg bw/day from the results of two chronic toxicity studies in dogs and an uncertainty factor of 100 to account for interspecies extrapolation(10X) and intraspecies variation (10X). The NOAEL is on the basis of the absence of treatment-related toxicity at 10 mg/kg bw/day in the second study and changes in lipid metabolism and increased liver weight at 30 mg/kg bw per day in the first study.

ADI = 0.1 mg/kg bw

Table 2. JMPR – ADI

NOAEL (mg/kg/day)	Uncertainty factor	ADI (mg/kg/day)	Toxicological endpoint of concern	Study selected	Reference
10.0	100	0.1	Changes in lipid metabolism and increased liver weight	Chronic toxicity - dogs	JMPR, 1999

2.1.3 Selection of tolerable systemic dose (TSD)

Tolerable systemic doses (TSDs) are established for acute and long-term for risk assessments. Guidance values for acute exposure (TSDAC) and long-term (TSD) have already been set by international or national bodies such as JMPR, EU and/or USEPA; these include acute reference dose (aRfD) for assessing risk from acute exposure and acceptable daily intake (ADI) or chronic reference dose (cRfD) for assessing risk from long-term exposure.

The PQT/VCP did not select a TSD for acute exposure (TSD_{AC}) and no risk ratio is calculated for acute exposure since an aRfD was not established either by JMPR or the USEPA due to the lack of toxicity endpoint of concern attributable to a single exposure (dose).

The PQT/VCP selected the lower ADI of 0.1 mg/kg bw/day established by JMPR as the TSD for long-term risk assessments for Pyriproxyfen.

TSD = 0.1 mg/kg bw/day

2.2 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 various parameters into consideration, 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.

Conservative, high-end point estimates of the default distributions are used as defaults in the exposure assessment. The default values may be modified by users of the models on a case-by-case basis and replaced with appropriate measured or otherwise improved point estimates or distributions, when applicable. Similarly, application of anthropometric and physiological datasets derived from the true target population, when available, is likely to yield more accurate exposure predictions.

The exposure assessment (i.e., exposure calculations) was conducted in accordance with the input parameters (default values) mainly taken from generic risk assessment model for insecticide-treated nets (WHO, 2018) 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 under 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.

For exposure assessment, the total concentration (TC) of insecticide on the net and a default transfer coefficient of 6% for dislodgeable insecticide from net to skin, as proposed in the generic risk assessment model (WHO, 2018), are used.

The concentration of the active ingredient in the net (TC) is derived from the WHO GRAM (2018) specification of the net (default variability of the concentration being +/- 25%).

TC = 125% X Nominal concentration of the active ingredient g/kg net X weight of the net kg/m^2

Using the selected product design attributes of 6.5g Pyriproxyfen/kg net and 40 g/m^2 fabric weight, the TC is calculated as follows:

TC = 125% x 6.5 g/kg net x 0.040 kg/m2 x 1000 = 325 mg Pyriproxyfen/m^2

The following abbreviations and default values are used throughout the exposure assessment.

Abs-D = Dermal from net surface (data derived = 30%)

Abs-O = Oral absorption (default) = 100%

AT = Average time (default = 365 days)

BW = Body weight (default = adult = 60 kg adult; 23.9 kg/child; 10 kg/toddler; 8 kg infant [less than 12 months]; 4.2 kg/newborn [birth to 1 month]

$Dose_{Mbw}$ = 7.798 µg/kg bw/day

ESA = Exposed skin area (default = adult, 0.408; child, 0.225; toddler, 0.115; infant, 0.100 m^2)

EHA = Exposed hand area (default = 0.008 m^2/toddler; 0.007 m^2/infant)

FHM = Fraction transferred from hand to mouth (default = 0.164 [75th percentile])

H = Average time spent under net each day in hours (default= adult, 9 hours; child, 10 hours)

IR = Ingestion rate of milk (default = 0.66 kg/day)

NM = Net mouthed (default = 0.0014 m^2)

NoN = Number of nets washed per day (default = 5)

NoW = Number of washes per year (default = 20 washes/3 years)

RSW = Release rate (analytical data)

SE = Salivary extraction factor (default = 57%)

SolC = Solubility constant (default = 2.2 for water soluble and 0.361 for lipid-soluble insecticides)

SN = Maximal actual size of the net (default = 15 m^2)

SF = Surface fraction (100 – wash resistance index %) = 10%

Transl = Translodgeable fraction (default = 6%)

T ½ = First-order kinetics half time in the body of the insecticide (derived value = 1.5 days)

TC = Total concentration of active ingredient on net surface (derived value = 260 mg /m^2 AI)

VLH = Volume liquid on hand (default = 8.2 ml/adult; 4.3 ml/child)

VLS = Volume of liquid on skin (default = 36.7 ml/adult; 17.6 ml/child)

VolW = Volume of washing water (default = 4000 mL)

WRI = Wash resistance index = 90%

2.2.1 Exposure from 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.

2.2.1.1 Inhalation exposure

Inhalation exposure from impregnated materials is expected to be negligible, since many pesticides that are used in impregnated materials have relatively low vapor pressure. Pyriproxyfen has a vapor pressure of 1.7 $X10^{-7}$. As a result, inhalation exposure is not expected to result in appreciable exposure when compared with dermal or non-dietary ingestion exposure (USEPA, 2017; GRAM, 2018).

Therefore, for Pyriproxyfen, while sleeping under a net the contribution of inhalation to total body exposure is so small that, in practice, it can be ignored.

2.2.1.2 Dermal exposure

The estimated time-weighted average (TWA) systemic dose due to potential dermal exposure from sleeping under the net is calculated using a data-derived Abs-D of 30 % established by the USEPA. Table 5 reflects the systemic doses for all populations due to dermal exposures from sleeping under treated nets.

$$\text{Systemic TWA dose} = \frac{\text{Abs-D x Transl x ESA x SF x TC}}{\text{BW}} \times 1000$$

Table 3. Estimated systemic dose (TWA) from dermal exposure from sleeping under treated nets

Population	Abs-D (%)	Transl (%)	ESA (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic dose (µg/kg/ bw/day)
Adult	30	6	0.408	10	325	60.0	3.978
Children	30	6	0.225	10	325	23.9	5.507
Toddlers	30	6	0.115	10	325	10.0	6.728
Infants	30	6	0.100	10	325	8.0	7.313

Translodgeable = default = 6%
ESA = default value
SF= 100 – wash resistance index % = 10%
TC = 325 mg/m^2
BW = default values
1000 = conversion of mg to µg

2.2.1.3 Oral exposure to toddlers and infants

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.

2.2.1.3.1 Indirect oral exposure – Hand-to-mouth transfer

It is assumed that hand-to-mouth transfer is significant only for toddlers and infants. The estimated daily systemic dose from hand-to-mouth transfer is calculated as indicated in Table 6.

$$\text{Systemic Dose} = \frac{\text{Abs-O x SE x Transl x EHA x FHM x SF x TC}}{\text{BW}} \times 1000$$

Table 4. Estimated systemic dose (TWA) from indirect oral exposure – Hand-to-mouth transfer

Population	Abs-O (%)	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	0.164	10	325	10.0	0.146
Infants	100	57	6	0.007	0.164	10	325	8.0	0.160

SE = default value = 57%
Transl = default value = 6%
EHA = default values
FHM = default value = 0.164
SF= 100 – wash resistance index % = 10%
TC = 325 mg/m^2
BW = default values
1000 = conversion of mg to µg

2.2.1.3.2 Direct oral exposure

Direct mouth contact is only relevant for toddlers and infants. The estimated systemic dose from direct mouth contact is indicated in Table 7.

$$\text{Systemic Dose} = \frac{\text{Abs-O x SE x NM x SF x TC}}{\text{BW}} \times 1000$$

Table 5. Estimated systemic dose (TWA) from direct oral exposure – Sleeping and mouthing, chewing and sucking

Population	Abs-O (%)	SE (%)	NM (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic dose (µg/kg bw/day)
Toddlers	100	57	0.0014	10	325	10.0	2.60
Infants	100	57	0.0014	10	325	8.0	3.24

SE = default value = 57%
NM = default = 0.0014 m^2
SF= 100 – wash resistance index % = 10%
TC = 325 mg/m^2
BW = default values
1000 = conversion of mg to µg

2.2.1.4 Combined (inhalation + dermal + oral) exposure from sleeping under treated nets

A total daily systemic exposure to Pyriproxyfen while sleeping under a treated net was calculated in Table 8 as the summation of the values for inhalation, dermal and oral routes of exposure given above.

Table 6. Estimated total systemic dose (µg/kg bw/day) from sleeping under treated nets

Population	Inhalation exposure	Dermal exposure	Oral Exposure		Total systemic dose (µg/kg bw/day)
			Indirect	Direct	
Adult	Negligible	3.978	Not Applicable	Not Applicable	3.978
Children	Negligible	5.507	Not Applicable	Not Applicable	5.507
Toddler	Negligible	6.728	0.146	2.60	9.47
Infants	Negligible	7.313	0.160	3.24	10.71

2.2.2 Washing treated nets

There is no risk associated with inhalation exposure from washing of treated nets since the inhalation route of exposure is not of concern. However, exposure due to dermal absorption and oral transfer from hands may occur during washing of nets. This exposure is estimated for adult and child only.

2.2.2.1 Dermal exposure during net washing

2.2.2.1.1 Acute (maximum) exposure

The estimated acute (maximum) systemic dose due to potential dermal exposure during washing of treated nets is presented in Table 9.

$$\text{Systemic dose (maximum)} = \frac{\text{Abs-D x NoN x VLS x SF x TC x SN}}{\text{VolW x BW}} \times 1000$$

Table 7. Estimated systemic dose (maximum) from dermal exposure - Washing treated nets

Population	Abs-D (%)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m^2)	SN (m^2)	VolW (mL)	BW (kg)	Systemic dose (µg/kg bw/day)
Adults	30	5	36.7	10	325	15	4000	60.0	111.82
Children	30	5	17.6	10	325	15	4000	23.9	134.62

NoN = default = 5
VLS = default = 36.7 ml/adult and 17.6 ml/child)
SF= 100 – wash resistance index % = 10%
TC = 325 mg/m^2
SN = default = 15 m^2
VolW = default=4000 ml
BW = default values
1000 = conversion of mg to µg

2.2.2.1.2 Repeated (long-term) exposure

The estimated systemic dose (TWA) due to potential repeated dermal exposure during washing of treated nets is presented in Table 10.

$$\text{Systemic dose (TWA)} = \frac{\text{Abs-D x NoW x NoN x VLS x SF x TC x SN}}{\text{VolW x BW x AT}} \times 1000$$

Table 8. Estimated systemic dose (TWA) from repeated dermal exposure - Washing treated nets

Population	Abs-D (%)	NoW (washes)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m^2)	SN (m^2)	VolW (mL)	BW (kg)	AT (days)	Systemic dose (µg/kg bw/day

Adults	30	20/3 years	5	36.7	10	325	15	4000	60.0	365	2.040
Children	30	20/3 years	5	17.6	10	325	15	4000	23.9	365	2.456

NoW= default =20/3 years
NoN = default=5
VLS = default = 36.7 ml/adult and 17.6 ml/child
SF= 100 – wash resistance index % = 10%
TC = 325 mg/m^2
SN = default =15 m^2
VolW = default=4000 ml
BW =default values
AT=default=365 days
1000 = conversion of mg to µg

2.2.2.2 Oral exposure – Hand-to-Mouth

2.2.2.2.1 Acute exposure

The estimated acute (maximum) systemic dose due to potential oral exposure via hand-to-mouth activity during washing of treated nets is presented in Table 11.

$$\text{Systemic dose (maximum)} = \frac{\text{Abs-O x NoN x VLH x SF x TC x FHM x SN}}{\text{VolW x BW}} \times 1000$$

Table 9. Estimated systemic dose (maximum) from acute oral exposure - Washing treated nets

Population	Abs-O (%)	NoN (Nets)	VLH (mL)	SF (%)	TC (mg/m^2)	FHM	SN (m^2)	VolW (mL)	BW (kg)	Systemic dose (µg/kg bw/day)
Adults	100	5	8.2	10	325	0.164	15	4000	60.0	13.65
Children	100	5	4.3	10	325	0.164	15	4000	23.9	17.98

NoN = default = 5
VLH = default = 8.2 ml/adult and 4.3 ml/child
SF= 100 – wash resistance index % = 10%
TC = 325 mg/m^2
FHM = default = 0.164
VolW = default = 4000 ml
BW = default values
1000 = conversion of mg to µg

2.2.2.2.2 Repeated (long-term) exposure

The estimated systemic dose (TWA) due to potential repeated oral exposure via hand-to-mouth activity during washing of treated nets is presented in Table 12.

$$\text{Systemic dose (TWA)} = \frac{\text{Abs-O x NoW x NoN x VLS x SF x TC x FHM x SN}}{\text{VolW x BW x AT}} \times 1000$$

Table 12. Estimated systemic dose (TWA) from repeated oral exposure - Washing treated nets

Population	Dermal absorption (%)	NoW (washes)	NoN (Nets)	VLS (mL)	SF (%)	TC (mg/m^2)	FHM	SN (m^2)	VolW (mL)	BW (kg)	AT (days)	Systemic dose (µg/kg bw/day
Adults	100	20/3 years	5	8.2	10	325	0.164	15	4000	60.0	365	0.249
Children	100	20/3 years	5	4.3	10	325	0.164	15	4000	23.9	365	0.328

NoN = default = 5
NoW = default = 20 washes/3 years
VLH = default= 8.2 ml/adult and 4.3 ml/child
SF= 100 – wash resistance index % = 10%
TC = 325 mg/m^2
FHM = default=0.164
SN =default = 15 m^2
Vol W = default=4000 ml
BW = default values
AT = default = 365 days
1000 = conversion of mg to µg

2.2.3 Combined (dermal + oral) exposure from washing treated nets

A total daily systemic exposure to Pyriproxyfen while washing treated net was calculated in Table 13 as the summation of the values for dermal and oral routes of exposure given above.

Table 10. Estimated total exposure from dermal and oral routes due to washing treated nets

Subpopulation	Dermal Exposure (µg/kg bw/day)	Oral Exposure (µg/kg bw/day)	Total Systemic Dose (µg/kg bw/day)
Acute exposure (maximum)			
Adult	111.82	13.658	125.478
Children	134.62	17.980	152.60
Repeated exposure (TWA)			
Adult	2.040	0.249	2.289
Children	2.456	0.328	2.784

2.2.4 Exposure via breast milk

Newborns might be exposed to Pyriproxyfen through breast milk of lactating mothers who sleep under the treated nets and/or wash the nets. Since data on the actual excretion in milk are not available, an upper bound of the exposure from the mother's milk can be roughly estimated from the physicochemical characteristics and kinetics of the pesticide (WHO, 2018). The estimated systemic dose to the newborn is calculated based on the estimated systemic dose to the mother. Estimates for systemic maximal and TWA doses from exposure via breast milk are calculated as follows:

$$\text{Systemic dose} = \frac{\text{Abs-O} \times \text{Sol C} \times \text{dose (mother)} \times T½ \times \text{IR}}{\text{BW}}$$

Since inhalation exposure to the mother sleeping under the net is negligible, the maximum systemic doses and TWA doses are derived from dermal exposure sleeping under the net and oral exposure through washing of nets.

Dose_{Mbw} (maternal dose) = Total exposure from sleeping under net + Total exposure from washing of net

Dose_{Mbw} (maximum) = 3.978 + 125.478 µg/kg bw/day = 129.456 µg/kg bw/day

Dose_{Mbw} (TWA) = 3.978 + 2.289 µg/kg bw/day = 6.267 µg/kg bw/day

The estimated maximum systemic dose from exposure via breast milk is shown in Table 14.

Table 11. Estimated maximum systemic dose from exposure via breast milk

Subpopulation	Abs-O (%)	SolC	Dose (ug/kg/day)	$T_{1/2}$ (days)	IR (kg/day)	BW (kg)	Systemic dose (µg/kg bw/day)
Newborns	100	0.361	129.456	1.5	0.66	4.2	11.11
Infants	100	0.361	129.456	1.5	0.66	8.0	5.78

The estimated TWA systemic dose from exposure via breast milk is shown in Table 15.

Table 12. Estimated systemic dose (TWA) from repeated exposure via breast milk

Subpopulation	Abs-O (%)	SolC	Dose (ug/kg/day)	$T_{1/2}$ (days)	IR (kg/day)	BW (kg)	Systemic dose (µg/kg bw/day)
Newborns	100	0.361	6.267	1.5	0.66	4.2	0.533

Infants	100	0.361	6.267	1.5	0.66	8.0	0.279

SolC = Solubility constant, default value
$T_{1/2}$ = First-order kinetics half time of Pyriproxyfen in days, 1.5 days
IR = default = 0.66 kg/day
BW = default values

2.3 Risk characterization

The purpose of risk characterization is to examine the probability of adverse effects occurring resulting from 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 TSD established in the hazard assessment. The TSD is the same as the ADI or the cRfD established for the active ingredients (WHO, 2018).

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

When the ratios are less than 1, the health risk is deemed acceptable. Ratios greater than 1 may indicate 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 (WHO, 2018).

The risk ratios for all the populations (adults, children, toddlers and infants) sleeping under treated nets and for adults and children washing the long-lasting bed nets treated with Pyriproxyfen are presented in Tables 16–18.

2.3.1 Exposure estimates and risk ratios for sleeping under treated nets

The risk ratios for all populations sleeping under treated nets are presented in Table 16.

Table 13. Exposure estimates and risk ratios for all populations sleeping under Pyriproxyfen-treated nets

Subpopulation	Inhalation exposure	Dermal exposure (µg/kg/day)	Oral indirect exposure (µg/kg/day)	Oral direct exposure (µg/kg/day)	Total exposure (µg/kg/day)	TSD (µg/kg/day)	Risk ratio
Adults	Negligible	3.978	Not applicable	Not applicable	3.978	100	0.039
Children	Negligible	5.507	Not applicable	Not applicable	5.507	100	0.055
Toddlers	Negligible	6.728	0.146	2.60	9.47	100	0.095
Infants	Negligible	7.313	0.160	3.24	10.71	100	0.107

Acute and repeated exposure TSD = 100 µg/kg bw/day
Risk ratio = Total exposure/TSD

2.3.2 Exposure estimates and risk ratios for washing treated nets

The risk ratios for all populations washing treated nets are depicted in Table 17.

Table 14. Exposure estimates and risk ratios for populations washing Pyriproxyfen-treated nets

Subpopulation	Dermal exposure (µg/kg bw/day)	Oral exposure (µg/kg bw/day)	Total exposure (µg/kg bw/day)	Acute RfD (µg/kg bw)	TSD (µg/kg bw/day)	Risk ratio
Repeated exposure (TWA)						
Adult	2.040	0.249	2.289	Not applicable	100	0.02
Children	2.456	0.328	2.784	Not applicable	100	0.03

Acute and repeated exposure TSD= 100 µg/kg bw/day
Risk ratio = Total Exposure/TSD

2.3.3 Combined for sleeping under nets and washing nets

The combined risk ratios for all populations sleeping under and washing Pyriproxyfen-treated nets are presented in Table 18.

Table 15. Exposure estimates and risk ratios for populations sleeping under and washing Pyriproxyfen-treated nets

Sub population	Sleeping under nets (combined) (µg/kg/day)	Washing of nets combined (µg/kg/day)	Total exposure (µg/kg/day)	Acute RfD (µg/kg bw)	TSD (µg/kg/day)	Risk ratio
Repeated exposure (TWA)						
Adults	3.978	2.289	6.267	N/A	100	0.06
Children	5.507	2.784	8.291	N/A	100	0.08

Acute and repeated exposure TSD= 100 µg/kg bw/day
Risk ratio = Total Exposure/TSD

2.3.4 Combined exposure estimates for infants and newborns

The combined risk ratios for infants and newborns sleeping under treated nets and consuming breast milk are shown in Table 19.

Table 16. Exposure estimates and risk ratios for populations sleeping under Pyriproxyfen-treated nets and consuming breast milk

Sub population	Total exposure sleeping under nets (dermal + oral indirect + oral direct) (µg/kg/day)	Breast milk exposure (µg/kg/day)	Total exposure (µg/kg/day)	Acute RfD (µg/kg /day)	TSD (µg/kg/day)	Risk ratio
Repeated exposure (TWA)						
Newborns	N/A	0.533	0.533	N/A	100	0.005
Infants	10.713	0.279	10.992	N/A	100	0.11

Acute and repeated exposure TSD= 100 µg/kg bw/day
Risk ratio = Total Exposure/TSD

2.4 Risk conclusion

The risk ratios are < 1 and the potential health risk is acceptable for all routes of exposure (inhalation, dermal and oral) for:

- Acute and repeated exposure for all populations sleeping under treated nets
- Acute and repeated exposure for all adults and children washing treated nets
- Acute and repeated exposure for adults and children sleeping under and washing the treated nets
- Acute and repeated exposure for infants and newborns exposed via breast milk and sleeping under nets

Table 17. Summary of risk characterization for Pyriproxyfen-treated nets (up to 6.5 g/kg or 260 mg/m^2)

Activity/population	Risk acceptable/not acceptable
Sleeping under net – dermal exposure	
Adult	Acceptable
Children	Acceptable
Toddlers	Acceptable
Infants	Acceptable
Washing of nets – acute	
Adult	Acceptable
Children	Acceptable
Washing of nets – repeated conditions	
Adult	Acceptable
Children	Acceptable
Sleeping under and washing of nets – acute condition	
Adult	Acceptable
Children	Acceptable
Sleeping under and washing of nets – repeated conditions	
Adult	Acceptable
Children	Acceptable
Exposures via breast milk from mothers	
Infants (acute and chronic)	Acceptable
Newborn (acute and chronic)	Acceptable
Combined: sleeping under net and breast milk	
Infants (acute and chronic)	Acceptable
Newborn (acute and chronic)	Acceptable

3 Conclusion

Considering the selected product characteristics identified in section 1.0, the use of Pyriproxyfen formulated as an ITN in the course of vector control does not present any unacceptable risk for adults, children, toddlers, and infants sleeping under the treated nets, or for adults and children both sleeping under and washing of treated nets, or for infants and newborns sleeping under the treated nets and exposed through breast milk. The calculated exposure levels were in all cases below the long-term tolerable systemic doses of Pyriproxyfen and can be used safety for its intended use as a VCP ITN.

Appendix. Pyriproxyfen health hazard assessment

Introduction to Pyriproxyfen

The use of vector control products (VCPs) is critical in the protection of global human health by combatting and preventing the transmission of major vector-borne diseases. Through the procedures of Prequalification Unit Vector Control Product Assessment Team (PQT/VCP), VCPs and public health pesticide active ingredients are assessed to determine that they can be used safely and effectively and are manufactured to a high-quality standard. The intent of the hazard assessment of each active ingredient (AI) is to consolidate the relevant toxicological information and authoritative evaluations from national and/or international organizations and characterize the chemical as it relates to uses of VCPs. PQT/VCP relies on these authoritative evaluations, focusing on the studies and endpoints applicable to the risk assessment of the use patterns of VCPs. As such, this hazard assessment is not exhaustive in its summary or assessment of publicly available information characterizing the hazard of the AI.

Pyriproxyfen [2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine], CAS No. 95737-68-1, is a broad spectrum pyridine based insect growth regulator used to control a variety of insects. It acts by suppressing embryogenesis within the insect egg and inhibiting metamorphosis and adult emergence of target insects. It has numerous agriculture and non-agricultural uses.

There is sufficient information on the toxicity of Pyriproxyfen to conduct a human health hazard assessment. Pyriproxyfen was evaluated by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) (JMPR) most recently in 2006. A Human Health Risk Assessment was most recently conducted by the US Environmental Protection Agency (USEPA) in 2017. The toxicology database is adequate for purposes of PQT/VCP.

Hazard characterization

Pyriproxyfen is a broad-spectrum pyridine-based insect growth regulator used to control a variety of insects. It acts by suppressing embryogenesis within the insect egg and inhibiting metamorphosis and adult emergence of target insects.

The toxicology database for pyriproxyfen is complete and adequate for human health hazard assessment and risk assessment purposes.

Pyriproxyfen is placed in GHS Category 5 for oral and dermal toxicities and in GHS Category 4 for inhalation toxicity. It is a mild eye irritant (GHS Category 3B) and is neither a dermal irritant nor a skin sensitizer.

Following repeated oral exposure to mice, rats and dogs, body weight effects and haematological alterations were the common effects, and the liver and kidney were the principal targets for pyriproxyfen.
No dermal or systemic toxicity was seen following repeated dermal application at doses up to 1000 mg/kg/day and following repeated inhalation exposure at concentration up to 1 mg/L. There was no evidence of increased sensitivity in the young. Developmental and offspring toxicity were observed only at doses that also elicited maternal or parental toxicity. There was no evidence of neurotoxicity or immunotoxicity. Pyriproxyfen is classified as a Group E Carcinogen (no evidence for carcinogenicity to humans) based on the lack of evidence of carcinogenicity in male and female mice or rats. There was no evidence of mutagenicity/genotoxicity in a battery of *in vivo* and *in vitro* assays.

Oral absorption of pyriproxyfen appears to be slow (50%) in rats. Elimination from the body was rapid and occurred primarily through the feces. Neither sex nor dose affected absorption or elimination; however, the extent of metabolism and prevalence of metabolites was sex dependent.

Acute toxicity

Pyriproxyfen is classified as GHS Category 5 for acute oral and dermal toxicity; GHS Category 4 for acute inhalation; and as GHS Category 3B for eye irritation. It is neither a dermal irritant nor a skin sensitizer. The acute toxicity profile for pyriproxyfen is summarized in Appendix 1.

Subchronic Toxicity

21-day dermal study – rat:
Pyriproxyfen (97.2%, purity) suspended in corn oil was applied to the skin of male and female Sprague-Dawley rats at 0, 100, 300 and 1000 mg/kg bw/day for 6 hours/day for 21 consecutive days. No dermal effects nor systemic toxicity were reported at any dose level tested. For dermal and systemic toxicity, the NOAEL was 1000 mg/kg bw/day, the highest dose tested; a LOAEL was not established (USEPA, 2017).
In a dermal toxicity study, a formulation product (0.83% Emulsified Concentrate containing 11.1% pyriproxyfen) was applied to the shaved skin of Sprague Dawley rats (5/sex/dose) at dose levels of 0, 100, 300, or 1000 mg/kg/day for 6 hours/day, 5 days/week, for 3 weeks. Rats in the 1000 mg/kg/day treatment group exhibited higher frequencies and more severe irritation of the treated skin than the control group. Symptoms of dermal irritation included moderate/marked epidermal acanthosis, slight dermal lymphoid cell infiltrate, and dermal haemorrhage. Dermal irritation observed in the rats in the 100 or 300 mg/kg/day treatment groups were comparable to that observed in the controls. No systemic toxicity was seen. For dermal toxicity, the NOAEL was 300 mg/kg/day bw and the LOAEL was 1000 mg/kg/day bw based on the presence of treatment-related dermal irritation. For systemic toxicity, the NOAEL was 1000 mg/kg/day bw; the highest dose tested; a LOAEL was not established (USEPA, 2017).

28-day inhalation study – rats:
Groups of Sprague-Dawley rats (10 rats/sex/concentration) were exposed whole body to pyriproxyfen (97%, purity) at concentrations at 0, 269, 482, or 1000 mg/m^3, 4 hours/day, 7 days/week for 28 days. No treatment-related effects were observed on survival, clinical signs of toxicity, body weight, gross and histopathology. The NOAEC was 1000 mg/ m^3 or 1 mg/L; a LOAEC was not established (EPA, 2017).
90-day feeding – rats:
Groups of Crl:CD BR rats (10 rats/sex/dose) were fed diets containing pyriproxyfen (95.3%, purity) 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, clinical signs of toxicity, or substantial changes in food consumption or urinalysis. Body weight was significantly depressed in males and females exposed to 642 and 784 mg/kg/day, respectively. Mild decreases in red blood cell count, hemoglobin, and hematocrit were observed in males at doses >118 mg/kg/day and in females at doses >356 mg/kg/day. Females, but not males, also exhibited a decrease in blood cell volume that was statistically significant at 141 and 784 mg/kg/day, but not at 356 mg/kg/day. Total cholesterol and phospholipid levels were elevated in males at doses >118 mg/kg/day and in females >356 mg/kg/day and were accompanied by an increase in absolute and relative liver weight. Total protein and albumin levels were also significantly elevated in both sexes at 642/784 mg/kg/day. Males exposed to 784 mg/kg/day further exhibited an increased incidence of livers with abnormal appearance including enlargement, pale, dark or red areas, and/or a mottled pattern in the gross pathology. A similar trend in the gross liver findings was not observed in females. Microscopic lesions were limited to slight hepatocyte hypertrophy and were concordant with increases in liver weight. The NOAEL is 24 mg/kg/day in males and 141 mg/kg/day in females and the LOAEL is 118 mg/kg/day in males and 356 mg/kg/day in females based on alterations in hematology and clinical chemistry parameters accompanied by increased liver weight and increased incidence of hepatocyte hypertrophy in both sexes (USEPA, 2017).

90-Day feeding – mice:
Groups of CD-1 mice (10 mice/sex/dose) were fed diets containing pyriproxyfen (technical) at dose levels of 0, 200, 1000, 5000, or 10,000 ppm (equivalent to 0, 28.2, 149.4, 838.1 and 2034.5 mg/kg bw/day for males and 0, 37.9, 196.5, 963.9 nd 2345.4 mg/kg bw/day for females) for 90 consecutive days. There was an increase in mortality in both sexes at the highest dose tested. In animals of both sexes that received the two highest doses, kidney lesions were present. Macroscopically, there was dilation of the renal pelvises, fluid filled renal pelvises, unequal kidney sizes, cysts, and paleness. Microscopically, there was nephrosis with renal tubular dilation and dilation and mineralization of the renal pelvises. In addition to these effects, there was a decrease in body weight gain in males at the two highest dose levels, significant decrease in red blood cells, hemoglobin, hematocrit, MCV and MCH, and increased liver to body weight ratios (USEPA, 2017). Females at the 5000-ppm dose level had significant decreases in red blood cells, hemoglobin, and hematocrit, significantly increased liver weights, and significantly increased liver to body weight ratios. At 10000 ppm there was only one surviving female; therefore, the numbers were not sufficient for evaluation. The kidney pathology was not associated with any clinical findings. Urinalysis was unremarkable. Regarding serum chemistry, of the parameters that could be correlated with renal disease, only the blood urea nitrogen (BUN) was elevated in both sexes at 5000 ppm. No

similar elevations in BUN were reported in the three surviving males and in the one surviving female at 10000 ppm. The NOAEL was 149.4 mg/kg/day in males and 196.5 mg/kg/day in females. The LOAEL was 838.1 mg/kg/day in males and 963.9 mg/kg/day in females 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).

90-Day Oral (capsule) – dogs:
Beagle dogs (four of each sex per group) were fed pyriproxyfen (97.2%, purity) in gelatine capsules daily at dose levels of 0, 30, 100, 300 or 1000 mg/kg body weight for 90 days. The NOAEL was 100 mg/kg/day, and 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).

Chronic Toxicity and Carcinogenicity

1-year Oral (capsule) – dogs:
In a chronic toxicity study, S-31183 (Pyriproxyfen 94.4%, purity) was administered in Beagle dogs (4/sex/dose) at doses of 0, 30, 100, 300, and 1000 mg/kg/day in gelatine capsule for approximately one year. In males receiving 300 mg/kg/day there was also significant increases in cholesterol levels throughout the study and increases in triglyceride levels at week 50. Mild anemia was also present in males at this dose level and was characterized by significant decrease in hemoglobin and red cell count when compared to controls. At 1000 mg/kg, 2 of 4 male dogs died due 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, and the LOAEL was 300 mg/kg/day based on significant decreases in body weight gain and increases in relative liver weights (USEPA, 2017).

In a complementary study, Beagle dogs (4/sex/dose) were given gelatine capsules containing pyriproxyfen (purity, 95.3%) at doses of 0, 3, or 10 mg/kg bw per day for 52 weeks. The observations included clinical signs, deaths, food consumption, body weight, and ophthalmic, clinical chemical, hematological, urinary, and histological examinations. Blood samples were collected after 12, 24, 36, and 50 weeks of treatment. There were no deaths, signs of clinical toxicity, or changes in body weight, body-weight gain, or food consumption. Prothrombin time was not prolonged in males at any dose. Females at 10 mg/kg bw per day also showed significantly increased platelet counts in weeks 36 and 50 (by 8% at 3 mg/kg bw per day and 10% at 10 mg/kg bw per day), and prothrombin time was slightly but significantly prolonged at these doses at the end of study. There were no other treatment-related changes in hematological parameters. The total cholesterol concentration was unchanged; slight but significant increases in total triglyceride concentrations in males at 10 mg/kg bw per day were seen in weeks 12 and 36 of treatment. There were no treatment-related changes in urinary parameters. The absolute weight of the liver was slightly increased in females at 10 mg/kg bw per day (110% of control), but this was not significant. No histopathological changes were found in any organ, including the liver and kidney. The increased numbers of platelets and the prolonged prothrombin time were therefore treatment-related changes although no significant increase in platelet counts was observed in animals at 30 mg/kg bw per day in the study discussed above (JMPR, 1999).
The NOAEL for the two 52-week studies in dogs was 10 mg/kg bw per day, on the basis of the absence of treatment-related toxicity at 10 mg/kg bw per day in the second study and changes in lipid metabolism and increased liver weight at 30 mg/kg bw per day in the first study (JMPR, 1999).

Chronic-carcinogenicity study – rats:
In a combined chronic/carcinogenicity study, S-31183 (95.3%, purity) 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. There were no treatment-related adverse effects on survival, clinical signs of toxicity or changes in clinical chemistry, hematology, urinalysis, organ weight, and gross or histopathology in either sex (USEPA, 2017),

Body weight gain was depressed in females at 183 mg/kg/day relative to controls, particularly over the first 52 weeks of exposure, and translated to reduced body weight in this treatment group relative to controls throughout the exposure period. Food consumption was generally lower in high dose females for the first year of exposure, achieving statistical significance on Weeks 13, 26 and 50; however, it was not considered the sole cause of body weight depression because the difference was slight compared to controls (5-7%). High dose males body weight was also lower than controls; however, the difference between treated and control animals was slight and not biologically significant. Slight to moderate increases in liver weight (significant in females only) and significant increases in total cholesterol and phospholipids levels were observed in high dose males and females during the first 26-52 weeks of treatment. Males also exhibited a dose dependent increase in alkaline phosphatase (AP) levels that was significant in low dose animals on Weeks 52 and 78 and in the mid and high dose animals on weeks 26, 52, and 78. Females did not exhibit an increase in AP levels at any time point over the course of the exposure. Many of these findings were consistent with liver toxicity (increased liver weight, increase total cholesterol and phospholipids) noted after 13 weeks of treatment in the rat subchronic dietary study in males and females at doses of >118 and >356 mg/kg/day, respectively. Although there was clear evidence of liver toxicity during the first year of exposure, the liver effects were less prominent by the end of the treatment period. Phospholipid, cholesterol, and AP levels in high dose animals returned to control levels and relative liver weight was still elevated in high dose females but was not statistically significant. Surviving high dose females did, however, exhibit a unique gross anomaly, an increased incidence of dark areas on the liver (1/21 in controls v. 11/34), that was not observed in high dose females during the first year nor in the subchronic study. A similar gross finding was not present in males. There were no findings of note in the histopathology of either sex for any tissue examined at Week 52 or Week 104. In the absence of correlating microscopic lesions, the liver findings were not considered adverse. There was no evidence of carcinogenicity in either sex. For chronic toxicity, in males, the NOAEL was 138.0 mg/kg/day, the highest dose tested; a LOAEL could not be established. For females, the NOAEL was 35.1 mg/kg/day, and the LOAEL was 183 mg/kg/day based on depressed body weight and body weight gain throughout the exposure period (USEPA, 2017).

Carcinogenicity study – mice

In a carcinogenicity study, technical pyriproxyfen (95.3%, purity) was administered by diet to randomized groups of 50/sex/dose male and female CD-1 mice at levels of 0,120, 600, and 3000 ppm (equivalent to 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**.** The NOAEL for systemic toxicity was 84.0 mg/kg bw/day in males and 109.5 mg/kg/day in females (USEPA, 2017).

Developmental Toxicity

Developmental toxicity – rats:

Pyriproxyfen (97.2%, purity) in corn oil was administered by gavage to groups of 36 pregnant Sprague Dawley rats at dose levels of 0, 100, 300 or 1000 mg/kg bw/day from days 7 through 17 of gestation. The study was conducted in two segments. In one, the dams were killed on gestation day 21 and fetuses were evaluated. In the other, the dams delivered naturally, and pups were weaned at postnatal day 21. Pups were killed serially at postnatal day 21 (after assessment of reflexes and sensory response), at 8 weeks of age (following open field testing, rotor testing, and examination of learning ability in a water maze), or after assessment of reproductive performance. For maternal toxicity, the NOAEL was 100 mg/kg/day, and the LOAEL was 300 mg/kg/day based on decreased body weight, body weight gain, and food consumption, and increased water consumption. For developmental toxicity, the NOAEL was 300 mg/kg/day, and the LOAEL was 1000 mg/kg/day based upon an increased incidence of fetuses with poorly ossified sternebrae, opening of foramen transversarium of the 7th cervical vertebrae and total number of skeletal variations on gestation day 21 and renal pelvis dilation on postnatal day 56 (USEPA, 2017).

In a modified developmental toxicity study, pyriproxyfen (S-31183, 97.2%, purity) was administered by gavage in corn oil once daily to Slc:SD rats (24 rats/sex/dose) at dose levels of 0, 100, 300, 500, or 1000 mg/kg/day. In the parental animals, males were administered the test substance beginning at 6 weeks of age, were dosed for 9 weeks prior to mating, and for an additional 3 weeks until the end of the mating period; females were administered the test substance beginning at 9 weeks of age, for 2 weeks prior to the start of the mating, and continued to be dosed throughout mating and until gestation day (GD) 7.

All live fetuses were weighed, sexed, and examined for external abnormalities. Approximately one-third of the fetuses per litter were subjected to a visceral examination, while all remaining fetuses were examined for skeletal anomalies. There were no differences of toxicological concern observed in the reproductive performance or Caesarean section parameters. For parental toxicity, the NOAEL was 100 mg/kg/day, and the

LOAEL was 300 mg/kg/day based on clinical signs, decreased body weight gains, and increased water consumption in both sexes, and increased food consumption, changes in organ weights, and gross pathological findings in males only. No developmental toxicity was observed at any dose level tested. For developmental toxicity, the NOAEL was 1000 mg/kg/day, the highest dose tested; a LOAEL was not established (USEPA, 2017).

Developmental toxicity – rabbit:
Groups of pregnant JW-NIBS rabbits received pyriproxyfen (97.2%, purity) in distilled water at 0, 100, 300 or 1000 mg/kg bw/day by gavage during gestation days 6–18. All does were killed, and fetal examination was carried out on day 28 of gestation. For maternal toxicity, the NOAEL was 100 mg/kg/day, and the LOAEL was 300 mg/kg/day based on occurrence of premature delivery/abortions, soft stools, emaciation, lustreless fur, decreased activity, and bradypnea/deep breathing. At 1000 mg/kg/day, these signs increased in incidence and frequency. For developmental toxicity, NOAEL was 300 mg/kg/day, and the LOAEL was 1000 mg/kg bw/day based on decreased viable litters available for examinations (USEPA, 2017).

Reproduction Toxicity

In a two-generation reproduction study, pyriproxyfen (95.3% purity) was administered to 26 Sprague-Dawley rats/sex/dose at dietary levels of 0, 200, 1000, or 5000 ppm (equivalent to 18, 87, or 453 mg/kg/day for males and 20, 96, or 498 mg/kg/day for females) for one litter per generation. For parental toxicity, the NOAEL was 87 mg/kg/day in males and 96 mg/kg/day in females and the LOAEL was 453 mg/kg/day in males and 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. For reproductive toxicity, the NOAEL was 453 mg/kg/day in males and 498 mg/kg/day in females; the highest dose tested; a LOAEL was not stablished. For offspring toxicity, the NOAEL was 87 mg/kg/day in males and 96 mg/kg/day in females and the LOAEL was 453 mg/kg/day in males and 498 mg/kg/day in females based on decreased body weight on lactation days 14 and 21 in F1 and F2 pups (USEPA, 2017).

In a modified reproductive toxicity study, pyriproxyfen (S-31183, 97.2%, purity) in corn oil was administered to pregnant Slc:SD rats (23-24/dose) at doses of 0, 30, 100, 300, or 500 mg/kg/day on gestation day (GD) 17 to lactation day (LD) 20. On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter with 4/sex/litter, as nearly as possible; excess pups were sacrificed and examined for skeletal anomalies. Physical and sexual development of all F1 pups was monitored and on postnatal day (PND) 20, all pups were subjected to a sensory function test. At 3 weeks of age, all F1 pups (except for pups retained for learning tests and reproductive ability) were necropsied, the organs were examined and weighed, and the pups were examined for visceral and skeletal anomalies. One F1 pup/sex/litter was subjected to an open field test, motor coordination test, learning ability test, and necropsied at 8 weeks of age. One F1 pup/sex/litter was raised to 11 weeks of age and then paired for mating within the same dose group for a reproductive performance assessment (USEPA, 2017). For parental toxicity, the NOAEL was 100 mg/kg bw/day, and the LOAEL was 300 mg/kg/day based on clinical signs, deceased body weight gains, and decreased food consumption. No treatment-related findings were observed in the caesarean section parameters for the F1 reproductive performance. No differences were noted in the physical or sexual development, behavioural aspects, locomotor activity, rotarod performance, or learning ability of the pups. No changes in viability were noted in the pups selected for learning or reproductive phases; additionally, no differences in body weights were observed in the reproductive animals. There were no differences of toxicological concern in organ weights, external examination, or skeletal examinations at 3 weeks, 8 weeks, or following the reproductive performance test. For reproductive toxicity, the NOAEL was 100 mg/kg bw/day, and the LOAEL was 300 mg/kg/day based on decreased body weight and increased incidence of dilation of the renal pelvis (USEPA, 2017).

Genotoxicity

In a preincubation Ames assay, pyriproxyfen (97.2%), purity) was non-mutagenic at doses ranging from 10 to 5000 µg/plate with or without S9 activation in Salmonella typhimurium strains TA1535, TA 1537, TA 1538 or TA 100 or in *Escherichia coli* strain WP2 uvrA (USEPA, 2017).

In a gene mutation assay with cultured Chinese hamster v79 cells, S-31183 was tested at doses ranging from 10 to 300 µg/mL (without S-9 metabolic activation) and at doses ranging from 3 to 100 µg/mL (with S-9 metabolic activation). There was no evidence of mutagenicity (USEPA, 2017).

S-31183 was tested at doses of 10, 30 and 100 µg/mL (with S-9 metabolic activation) and at doses of 30, 100 and 300 µg/mL (without S-9 activation) for its potential to induce chromosomal aberrations in Chinese Hamster

Ovary cells. There was no evidence of mutagenicity USEPA, 2017).

In an *in vitro* unscheduled DNA synthesis assay in HeLa cells, S-31183 was tested at doses ranging from 0.1 to 204.8 µg/mL with or without S-9 metabolic activation for the potential to induce unscheduled DNA synthesis. There was no evidence for mutagenicity (USEPA, 2017).

In an i*n vivo* micronucleus assay, female CD-1 mice received a single intravenous injection at 5000 mg/kg bw at 24, 48 and 72 hours. There was no evidence of mutagenicity (JMPR, 1999).

Neurotoxicity

Acute neurotoxicity study – rats:

In an acute neurotoxicity study, groups of 6 weeks old Crl:CD(SD) rats, 12/sex/dose were given a single oral dose of pyriproxyfen (99.5%, purity) in corn oil at doses of 0, 300, 1000 or 2000 mg/kg bw and observed for 14 days. Neurobehavioral assessment, functional observational battery and motor activity testing were performed on all animals at 8 hours postdosing. At study termination, 6/sex/group from both controls and 2000 mg/kg were subjected to histopathological evaluation of brain and peripheral nervous system tissues. There were no treatment related effects on mortality, bodyweight, brain weight or gross and histologic pathology, neuropathology, or FOB measurements. Motor activity testing revealed test substance-related, lower total and ambulatory counts at 1000 and 2000 mg/kg group males, compared with the control group on study Day 0 only. Compared to the control group, mean motor activity counts were lower (total and (ambulatory) at 1000 and 2000 mg/kg group in both sexes. By study Days 7-14, mean total and ambulatory activity counts for males of both groups were unaffected by test substance. No histopathological lesions supportive of clinical signs or changes in motor activity were observed, indicating that these effects could be due to either general malaise or transient changes in neural activity. The NOAEL was 1000 mg/kg bw and the LOAEL was 2000 mg/kg bw/day based on deceases in motor activity on Day 0 in males and unkempt appearance in females (USEPA, 2017).

13-week neurotoxicity study – rats:

In a subchronic neurotoxicity study pyriproxyfen (99.5%, purity) was administered to 12 Crl:CD(SD) rats/sex/group at dose levels of 0,1500, 5000, or 15,000 ppm (equivalent to 0, 108, 359, 1111 mg/kg bw/day for males and 0, 102, 407, 1212 mg/kg bw/day for females) for 13 weeks. Neurobehavioral assessment (functional observational battery and motor activity testing) was performed in 12 animals/sex/group at pretest (study week -1), study weeks 1, 3, 7, and 12, respectively. At study termination, 6 animals/sex/group were euthanized and perfused in situ for neuropathological examination. Of the perfused animals, 6/sex/group from both control and 15,000 ppm group were subjected to histopathological evaluation of brain and peripheral nervous system tissues. There were no treatment-related effects on survival and clinical signs of neurotoxicity. At 15,000 ppm group, significantly ($p<0.01$) lower mean body weight gains were noted during study Days 0-14 in males and 0-7 in females. Mean body weight gains and mean food consumption for both sexes at the high dose were significantly lower than the control group during the study. There was no evidence of neurotoxicity; FOB, motor activity, gross and histopathology, and weights were not affected by treatment. For neurotoxicity, the NOAEL was 1111 mg/kg/day in males and 1212 mg/kg/day in females; a LOAEL was not established., For systemic toxicity, the NOAEL was 359 mg/kg/day in males and 407 mg/kg/day in females and the LOAEL was 1111 mg/kg/day in males and 1212 mg/kg/day in females based on lower mean body weights, body weight gains, and food consumption for both sexes (USEPA, 2017).

Absorption, Distribution, Metabolism, and Excretion (ADME)

Oral route studies

In a metabolism study rat were dosed with ^{14}C labelled Sumilarv at oral doses of 2 or 1000 mg/kg, and at repeated oral doses (14 daily doses) of unlabelled Sumilarv at 2 mg/kg followed by administration of a single oral dose of labelled Sumilarv at 2 mg/kg. The major routes of excretion of radioactivity were via the urine and feces. The excretion of radioactivity into urine and feces was rapid and over a 7-day period, most (92-97%) of the test compound administered was excreted in the urine (5-12%) and feces (81-92%) from the animals. Most (63-83%) of the dose was eliminated in the urine and feces within 24 hours after dosing. Expired air was not detected. Radioactivity in tissue residues was very low in all tissues expect the fat. Recoveries of the administered doses in the tissues including carcass were not more than 0.3% indicating that the potential for bioaccumulation of Sumilarv is minimal even after high dose or repeated low dose exposures. In the urine, a total of 11 metabolites were detected and two of them (4'-OH-31183 sulfate and

4'-OH-POP sulfate) were identified (USEPA, 2017).

In the feces, an unmetabolized Sumilarv (7-37% of the dose) and a total of up to 17 metabolites were detected and 10 of them (6 unconjugated and 4 sulfate conjugates) were identified. The four major fecal metabolites were 4'-OH-31183, 4'-OH-31183 sulfate, 5, 4'-OH-31183 sulfate, and 4'-OH-POPA. The metabolites in the liver, kidney, blood, bile, and fat of rats were also examined. Based on the metabolites identified by TLC chromatography with synthetic standards the major biotransformation reactions of Sumilarv include: 1) Oxidation at 4'-postion, of the terminal phenyl group; 2) Oxidation at 5-psition of pyridine; 3) Cleavage of the ether linkage and conjugation of the resultant phenols with sulfuric acid. No significant sex-or dose-related differences exist in the absorption, distribution, or metabolism of Sumilarv for all dosing regimens (USEPA, 2017).

In another metabolism study, male and female Sprague-Dawley rats (7-week-old) were given. a single oral gavage dose of pyriproxyfen, which was labelled at the 2 and 6 positions of the pyridyl ring with ^{14}C ([pyridyl-2,6-14C] pyriproxyfen), at a rate of 2 mg/kg (low-does) or 1000 mg/kg (high-dose). Radiocarbon was rapidly eliminated from the body within 7 days after administration in both groups. From 88.9% to 92.9% of the dosed ^{14}C was excreted into urine, feces, and expired air in the first two days, and 92.3-98.5% of the dosed ^{14}C was excreted within 7 days after administration. ^{14}C-Excretion into expired air was less than 0.5% for all groups. Fecal excretion was predominant, being 84.7-93.2% of the dose. ^{14}C -Residues in tissues on the 7th day after administration were less than 0.3% of the dosed ^{14}C, being the highest in fat. ^{14}C-Residues levels in blood, blood cell, kidney, and liver were 0.002 – 0.009 ppm for the low dose group, and 1.2 – 4.5 ppm for the high dose group. ^{14}C -Residue levels in other tissues were low. There were no significant sex-or dose-related differences in ^{14}C -excretion and ^{14}C-distribution. Thirteen or more metabolites were found by HPLC and TLC analyses of feces and urine collected in the first two days. Ten of them were identified, accounting for 70-83% of the dosed ^{14}C. The metabolic pathways of pyriproxyfen in rats were proposed based on identified metabolites (USEPA, 2017).

The major fecal metabolite was 4-(4'-hydroxyphenosyphenyl)-2-(2-pyridyloxy) propyl ether, which had one hydroxyl group at the 4'-position of the terminal phenyl ring and accounted for 23-48% of the dosed ^{14}C. Each of the other metabolic pathways, such as hydroxylation at the 2'-position of the terminal phenyl ring or at the 5'-postion of the pyridyl ring, dephenylation, cleavage of ether linkages, and conjugations of the resulting phenols with sulfuric acid or glucuronic acid, accounted for less than 10% of the dosed ^{14}C. Pyriproxyfen was detected mainly in feces and its amount was 21-35% of the dosed ^{14}C. The major urinary metabolite was 2-(2-pyridyloxy) propionic acid, accounting for 1-5% of the dose. Comparison of metabolites in feces and urine, between male rats given a single oral low dose of pyriproxyfen labelled uniformly with ^{14}C at the phenoxy phenyl ring [phenoxyphenyl-14C] pyriproxyfen and those given the same dose of [pyridyl-2,6-14C] pyriproxyfen, showed that metabolites resulting from cleavage of ether linkages accounted for 5.2% of the dosed radioactivity (USEPA, 2017).

Points of Departure (POD), Reference Doses (RfD), and Cancer Classification

Points of Departure

Points of departure (PODs) (No Observed Adverse Effect Level [NOAEL]; Lowest Observed Adverse Effect Level (LOAEL); Benchmark Dose [BMD]) are determined from the toxicological database based on the most sensitive endpoints. Once PODs are developed, safety factors (SF) and uncertainty factors (UF) are used in conjunction with the PODs to derive a safe exposure level, generally referred to as reference dose (RfD) or acceptable daily intake (ADI).

Reference Doses

Acute Reference Dose (aRfD)

JMPR (1999 and 2001) did not establish an acute RfD for pyriproxyfen since the meeting concluded that this value is unnecessary due to lack of hazard following a single dose.

USEPA (2017) did not establish an acute RfD for pyriproxyfen since a toxicity endpoint attributable to a single exposure was not identified in the toxicology database including the acute neurotoxicity and the developmental toxicity studies (USEPA, 2017).

aRfD = Not Established

Chronic Reference Dose (cRfD)

The USEPA (2017) established a cRfD of 0.35 mg/kg/day based on a NOAEL of 35.1 mg/kg/day using subchronic and chronic toxicity studies in rats as co-critical studies and an uncertainty factor of 100 to account for interspecies extrapolation(10X) and intraspecies variation (10X). The LOAEL of 118 mg/kg/day is based on the based on decreased mean red blood cell count, hemoglobin, and hematocrit, and elevated cholesterol and phospholipid levels accompanied by increased liver weight and incidence of hepatocyte hypertrophy in the subchronic study with rats and decreased body weight and body weight gain at 183 mg/kg/day in the chronic toxicity study.

cRfD = 0.35 mg/kg bw

Acceptable Daily Intake (ADI)

The JMPR (1999) established an acceptable daily intake (ADI) of 0.1 mg/kg bw/day for pyriproxyfen based on the NOAEL of 10.0 mg/kg bw/day based on the results of two chronic toxicity studies in dogs and an uncertainty factor of 100 to account for interspecies extrapolation(10X) and intraspecies variation (10X). The NOAEL is on the basis of the absence of treatment-related toxicity at 10 mg/kg bw/day in the second study and changes in lipid metabolism and increased liver weight at 30 mg/kg bw per day in the first study.

ADI = 0.1 mg/kg bw

Cancer Classification

The USEPA classified pyriproxyfen as a Group E Chemical (no evidence of carcinogenicity to humans) based on the lack of evidence for carcinogenicity in both sexes of mice and rats (USEPA, 2017).

Toxicity profile tables

Acute toxicity

Table A1. Acute toxicity of Pyriproxyfen technical

Route of exposure	Species	Toxicity	GHS category	Reference
Acute oral toxicity	Rat	LD50 > 5000 mg/kg	5	USEPA, 2017
Acute dermal toxicity	Rat	LD50 > 2000 mg/kg	5	USEPA, 2017
Acute Inhalation	Rat	LC50 > 1.3 mg/L/4 hours	4	USEPA, 2017
Dermal irritation	Rabbit	Non-irritant	Not classified	USEPA, 2017
Eye irritation	Rabbit	Mild-irritant	3B	USEPA, 2017
Skin sensitization	Guinea Pig	Non-sensitizer	Not classified	USEPA, 2017

Subchronic, chronic, and carcinogenicity studies

Table A2. Subchronic, chronic, carcinogenicity, and other toxicity studies with Pyriproxyfen

Pyriproxyfen – Toxicity Profile – Sub-Chronic, Chronic, and Other Studies		
Study type	**Dose levels; design**	**Results**
Subchronic- toxicity		

Pyriproxyfen – Toxicity Profile – Sub-Chronic, Chronic, and Other Studies		
Study type	**Dose levels; design**	**Results**
21-day dermal - Rat	Doses: 0, 100, 300 and 1000 mg/kg bw/day	Systemic NOAEL = 1000 mg/kg/day Systemic LOAEL = Not established Dermal NOAEL = 1000 mg/kg/day Dermal LOAEL = not established
28-day inhalation - Rats	0, 269, 482 or 1000 mg/m^3, 4 hours/day, 7 days/week for 4 weeks	NOAEC = 1 mg/L (1000 mgm^3) LOAEC = Not established
90-day oral dietary – Rats	Doses: 0, 400, 2000, 5000 or 10000 ppm Equivalent to 0, 24, 118, 309 or 642 mg/kg/day for males and respectively 0, 28, 141, 356 or 784 mg/kg/day for females	NOAEL = 24 mg/kg/day (males) and 141 mg/kg/day (females) LOAEL = 118 mg/kg/day (males) based on changes in hematology and clinical chemistry; increased liver weight and increased hepatocyte hypertrophy.
90-day oral dietary - Mice	0, 200, 1000, 5000, or 10000 ppm Equivalent to 0, 28.2, 149.4, 838.1 and 2034.5 mg/kg bw/day for males and 0, 37.9, 196.5, 963.9 and 2345.4 mg/kg bw/day for females	NOAEL =149.4 mg/kg/day in males and 196.5 mg/kg/day in females. LOAEL was 838.1 mg/kg/day in males and 963.9 mg/kg/day in females based on renal pathology, statistically significant increases in liver weights and statistically significant decreases in red blood cell parameters and in body weight gain.
90-day oral - Dogs	Gelatine capsules daily at dose levels of 0, 30, 100, 300 or 1000 mg/kg body weight for 90 days.	NOAEL = 100 mg/kg/day, LOAEL = 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
1-year oral - Dogs	Doses of 0, 30, 100, 300, and 1000 mg/kg/day in gelatine capsule	NOAEL < 30 mg/kg/day LOAEL = 30 mg/kg/day based on significant decreases in body weight gain and increases in relative liver weights and cholesterol levels.
1-year oral - Dogs	Doses of 0, 3, or 10 mg/kg bw given in gelatine capsule per day for 52 weeks.	NOAEL = 10 mg/kg/day LOAEL = not established.
104 week - Combined chronic/carcinogenicity – Rats	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.	Systemic NOAEL = 35.1 mg/kg/day (females) and 138 mg/kg/day (males) Systemic LOAEL = 183 mg/kg/day (females) based on depressed body weight and body weight gain throughout the exposure period No evidence of carcinogenicity
78 week – carcinogenicity – oral dietary - Rats	Dose levels of 0,120, 600, and 3000 ppm (equivalent to 0, 16.8, 84.0, and 420 mg/kg/day in males and 0, 21.0, 109.5, and 547 mg/kg/day in females) for 78 weeks.	Systemic NOAEL = 84.0 mg/kg/day (males) Systemic LOAEL = 420 mg/kg/day (males) based on increased liver weight, increased severity of systemic amyloidosis and histopathology. Changes in kidneys noted No evidence of carcinogenicity
Developmental and reproductive toxicity		
Oral gavage in corn oil - Pregnant Sprague-Dawley Rats	Doses: 0, 100, 300 or 1000 mg/kg/day dissolved in corn oil given by gavage during gestation Days 7–17, inclusive. The study was conducted in two segments. In one, the dams were	Maternal Toxicity NOAEL = 100 mg/kg/day Maternal Toxicity LOAEL = 300 mg/kg/day (based on decreases in body weight and water and food consumption)

Pyriproxyfen – Toxicity Profile – Sub-Chronic, Chronic, and Other Studies		
Study type	**Dose levels; design**	**Results**
	killed on gestation day 21 and fetuses were evaluated. In the other, the dams delivered naturally, and pups were weaned at postnatal day 21. Pups were killed serially at postnatal day 21 (after assessment of reflexes and sensory response), at 8 weeks of age (following open field testing, rotor testing, and examination of learning ability in a water maze), or after assessment of reproductive performance	Developmental Toxicity NOAEL= 100 mg/kg/day Developmental Toxicity LOAEL = 300 mg/kg/day based on increased litter incidence of opening of foramen transversarium of 7th cervical centra. Offspring NOAEL = 300 mg/kg/day Offspring LOAEL = 1000 mg/kg/day based onpoorly ossified sternebrae, increased number of skeletal variations on gestation day 21 and renal pelvis dilation on postnatal day 56.
Oral – gavage in water - New Zealand White Rabbit	Doses: 0, 100, 300 or 1000 mg/kg bw/day by gavage during gestation days 6–18. All does were killed, and fetal examination was carried out on day 28 of gestation.	Maternal Toxicity NOAEL = 100 mg/kg/day Maternal Toxicity LOAEL =300 mg/kg/day based on premature delivery/abortions, soft stools, emaciation, lustreless fur, and decreased activity. Developmental Toxicity NOAEL= 300 mg/kg/day Developmental Toxicity LOAEL = 1000 mg/kg/day based on decreased viable litters for examination.
Two generation reproduction study, oral dietary - Sprague Dawley Rats	26 Sprague-Dawley rats/sex/dose at dietary levels of 0, 200, 1000, or 5000 ppm Equivalent to 18, 87, or 453 mg/kg/day for males and 20, 96, or 498 mg/kg/day for females	Parental systemic NOAEL = 87 mg/kg/day Parental systemic LOAEL = 453 mg/kg/day (males) and 498 mg/kg/day (females) based on decreased body weight gain and food consumption, increased liver weight and lesions in liver and kidneys of F1 males. Reproductive toxicity NOAEL = 453 mg/kg/day (males) and 498 mg/kg/day (females). Reproductive tox LOAEL = not established. Offspring toxicity NOAEL = 87 mg/kg/day (males) and 96 mg/kg/day (females) Offspring toxicity LOAEL = 453 mg/kg/day (males) and 498 mg/kg/day (females) based on decreased body weight.
Neurotoxicity		
Acute oral – in corn oil rats	A single gavage dose at 0, 300, 1000 or 2000 mg/kg bw in corn oil and observed for 14 days	NOAEL = 1000 mg/kg bw LOAEL = 2000 mg/kg bw/day based on deceases in motor activity on Day 0 in males and unkempt appearance in females
13-week oral dietary, CD rats	Dose levels of 0,1500, 5000, or 15,000 ppm Equivalent to 0, 108, 359, 1111 mg/kg bw/day for males and 0, 102, 407, 1212 mg/kg bw/day for females) for 13 weeks. FOB and motor activity testing) were performed in 12 animals/sex/group at pretest, study weeks 1, 3, 7, and 12. At study termination, 6 animals/sex/group were euthanized and perfused in situ for neuropathological examination.	Neurotoxicity NOAEL = 1111 mg/kg/day in males and 1212 mg/kg/day in females. Neurotoxicity LOAEL = not established. Systemic toxicity NOAEL = 359 mg/kg/day in males and 407 mg/kg/day in females Systemic LOAEL was 1111 mg/kg/day in males and 1212 mg/kg/day in females based on lower mean body weights, body weight gains, and food consumption for both sexes
Immunotoxicity		
28-day oral dietary, mice	Female CD-1 mice (10/dose) Dose levels of 0, 1000, 2000, or 5000 ppm (0, 228, 449, 1139 mg/kg bw/day)	NOAEL = 449 mg/kg/day LOAEL = 1139 mg/kg/day based on lower bodyweight gain and increase of absolute and relative body weight to liver weight. There were no statistically significant changes in the numbers of cells/spleen, PFC/106 viable cells or PFC/spleen for CD-I mice in any of the dosed groups.

Pyriproxyfen – Toxicity Profile – Sub-Chronic, Chronic, and Other Studies		
Study type	**Dose levels; design**	**Results**
Genotoxicity		
Bacterial reverse mutation (Ames assay)	*Salmonella typhimurium & E Coli* Doses ranging from 0 to 5000 µg/plate	Negative in both presence and absence of metabolic activation
Gene mutation assay-Chinese hamster lung cells	Doses ranging from 10 to 100 µg/mL (no metabolic activation) and ranging from 3 to 100 µg/mL (with metabolic activation)	Negative in both presence and absence of metabolic activation
Chromosome aberration assay – Chinese hamster ovary cells	Doses ranging from 10 to 100 µg/mL (no metabolic activation) and ranging from 10 to 300 µg/mL (with metabolic activation)	No evidence of structural chromosomal aberrations
In vitro Unscheduled DNA synthesis-HeLa cell	Doses ranging from 0.1 to 204.8 µg/mL with or without S-9 metabolic activation for the potential to induce unscheduled DNA synthesis.	Negative
In vivo micronucleus assay – CD1 mice	Female CD-1 mice received a single intravenous injection at 5000 mg/kg bw at 24, 48 and 72 hours	Negative
Dermal absorption		
Dermal Absorption study	No information on the dermal penetration of pyriproxyfen was available at the time of this Hazard Assessment to estimate dermal absorption.	Following dermal application, no dermal or systemic toxicity was seen following repeated dermal application at doses up to 1000 mg/kg/day. 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 suggested dermal penetration was 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; JMPR, 2006)

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