Generic risk assessment – Human Health

PIPERONYL BUTOXIDE
(CAS No. 51-03-6)

A synergist in insecticide-treated nets

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

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Contents

Acronym list

Abs	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
CP	Cyclophosphamide
cRfD	Chronic Reference Dose
DMBA	7,12-Dimethylbenz(a)anthracene
DMSO	Dimethyl Sulfoxide
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
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 piperonyl butoxide-treated nets (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).

Piperonyl butoxide (PBO) has been used as a synergist in combination with synthetic pyrethroids (EFSA, 2017). Although PBO is always used in combination in insecticide-treated-net (ITN) products, the risks associated with the uses of PBO are assessed separately from the pyrethroids due to differences in mode of action, exposure levels and hazard characteristics. However, this human health risk assessment of PBO can be used in conjunction with that of the pyrethroid in question to derive the exposure risks for ITN products containing both ingredients.

The product characteristics, including the fabric weight (g/m^2), concentration of piperonyl butoxide (PBO) (g active ingredient/kg net) and wash resistance index (WRI) were selected as representative values which exemplify currently prequalified ITN products.

The assessment assumes that the product is a uniformly treated PBO ITN with the following product characteristic values:

- Fabric weight: 40 g/m^2
- Concentration by weight of net: 25 g PBO /kg net
- Concentration by net area: 1000 mg PBO /m^2
- Wash resistance index: 90%[1]

Note: The selected values are not intended to put a limit on the possible concentration of PBO in an ITN. The selected values do not represent the maximum concentration of PBO 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 PBO was conducted according to *Generic risk assessment model for insecticide-treated nets, 2nd edition* (GRAM) (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.

1 The measured WRI is determined for all products individually. The measured value is used to estimate the exposure as part of the Surface Fraction (SF).

- **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 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 are compared with acceptable exposure levels as defined in the hazard assessment for all relevant exposure scenarios.

The risk assessment for PBO is based on the proposed uses of the ITN products, for example, nets used over sleeping areas.

2.1 Hazard assessment

The complete PBO hazard assessment conducted to support this risk assessment is included in the appendix. This assessment was conducted based on information available at the time of publication. It is necessary for manufacturers who may rely on this assessment to ensure that points of departure and/or reference doses are still appropriate in the preparation of their product dossier.

Oral exposure

PBO has minimal toxicity via the oral route and acutely it is classified as Category 5 based on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS; 2017). Following repeated exposure, the systemic No Observed Adverse Effect Level (NOAEL) in dogs and rats was 600 ppm (15.5 mg/kg bw/day) and 30 mg/kg bw/day, respectively, with the liver as the primary target organ (USEPA, 2017). No adverse reproductive effects and developmental toxicity effects were noted in multiple species. PBO was not mutagenic or clastogenic in a battery of genotoxicity assays.

The USEPA (2006, 2017) indicated that PBO is classified as Group C (possible human carcinogen), but a cancer quantification is not required for PBO uses.

Dermal exposure

Systemic toxicity via the dermal route is not anticipated. No toxicity was observed following acute dermal exposure up to a dose of 2000 mg/kg/day. Dermal exposure for 21 days up to the limit dose of 1000 mg/kg bw/day did not result in systemic toxicity.

Inhalation exposure

Toxicity via the inhalation route is assumed to be equivalent to toxicity via the oral route. Due to the lack of a long-term inhalation study, the oral exposure is used for risk assessment purposes.

Absorption, distribution, metabolism and elimination (ADME)

The oral absorption of PBO was rapid. The majority of the absorbed dose was excreted in the feces and urine within 48 hours reaching up to 99%. The dermal absorption of PBO after application to the forearm skin and scalp was 2.1% and 8.3%, respectively (Wester et al., 1994). The estimated half-life for PBO was 1.5 days (JMPR, 1992, 1995).

2.1.1 Points of departure

Points of departure (PODs), NOAEL and benchmark dose (BMD) are determined from the toxicology database based on the most sensitive endpoints.

Acute oral exposure

USEPA (2017) selected the oral POD of 500 mg/kg bw/day from an acute neurotoxicity study in rats for acute risk assessment.

Acute oral POD = 500 mg/kg bw/day

Chronic oral exposure

USEPA (2017) selected the oral POD of 15.5 mg/kg bw/day from a one-year dietary study in dogs for chronic risk assessment.

Chronic oral POD = 15.5 mg/kg bw/day

2.1.2 Reference doses

Once PODs are developed, safety factors and uncertainty factors (UF) are used in conjunction with the PODs to derive a safe exposure level, generally referred to as a reference dose (RfD), acceptable daily intake (ADI) or a safe margin of exposure (MOE).

Acute reference dose (aRfD)

In 2001, the FAO/WHO JMPR concluded that an aRfD for PBO was unnecessary.

The USEPA (2006; 2017) selected an acute POD of 500 mg/kg/day for the general population, including children and women of childbearing age from an acute neurotoxicity study in rats based on reduced forelimb grip strength in males, decreased ambulation and fine movement on day 1 of treatment in both sexes noted at 1000 mg/kg bw/day (LOAEL). A UF of 100 was applied (10X for interspecies extrapolation and 10X for intraspecies variation) to derive the aRfD (see Table 1).

aRfD = 5 mg/kg bw/day

Table 1. USEPA aRfD - Oral

Population of concern	POD = NOAEL (mg/kg/day)	Uncertainty factor	aRfD (mg/kg bw/day)	Toxicological endpoint of concern	Study selected	Reference
General population	500	100	5	Decreased forelimb grip strength, decreased ambulation and fine movement at 1000 mg/kg bw/day	Acute neurotoxicity – rat	USEPA, 2006, 2017

Chronic reference dose (cRfD)

The USEPA (2006; 2017) selected the NOAEL of 15.5 mg/kg bw/day (600 ppm) from the 1-year dietary dog study with decreased body weight gain, increased alkaline phosphatase activity, decreased relative liver weight and findings of hepatocellular hypertrophy at the LOAEL of 52.8 mg/kg/day (1000 ppm). A UF of 100 was applied (10X for interspecies extrapolation and 10X for intraspecies variation) to obtain the cRfD (see Table 2).

cRfD = 0.16 mg/kg bw/day

Table 2. USEPA cRfD - Oral

Population of concern	POD = NOAEL (mg/kg/day)	Uncertainty factor	aRfD (mg/kg)	Toxicological endpoint of concern	Study selected	Reference
General population	15.5	100	0.16	Decreased body weight gain, increased alkaline phosphatase, hepatocellular hypertrophy at 52.8 mg/kg bw/day	1-year chronic dog study	USEPA, 2006, 2017

Acceptable daily intake (ADI)

PBO was evaluated by JMPR in 1995, and the committee selected the NOAEL of 600 ppm (15.5 mg/kg bw/day) from the 1-year dog dietary study to establish an ADI. A UF of 100 was applied (10X for interspecies extrapolation and 10X for intraspecies variation) to obtain the ADI (see Table 3).

ADI = 0.16 mg/kg bw/day
(rounded off to 0.2 mg/kg bw/day by JMPR,1995)

Table 3. ADI

NOAEL (mg/kg/day)	Uncertainty factor	ADI (mg/kg/day)	Toxicological endpoint of concern	Study selected	Reference
15.5	100	0.16	Decreased body weight gain, increased alkaline phosphatase, hepatocellular hypertrophy at 52.8 mg/kg bw/day	1-year dog study	JMPR, 1995 USEPA (2006, 2017)

2.1.3. Selection of Tolerable Systemic Dose (TSD)

The PQT/VCP selected the aRfD of 5 mg/kg bw/day established by the USEPA as the TSD for acute risk assessment (TSD_{AC}). Findings in the acute neurotoxicity study in rats are the most robust data set for assessing the acute toxicity of PBO exposure and risk.

The PQT/VCP selected the ADI of 0.16 mg/kg bw/day established by JMPR (1995) as the TSD for long-term risk assessment, and the ADI is appropriate for assessing the toxicity of PBO exposure and risk.

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 TSD. 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 of insecticide on the net (TC) and a default transfer coefficient of 6% for the amount of dislodgeable insecticide from net to skin, as proposed in the generic risk assessment (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%).

$$TC = 125\% \text{ X concentration of the active ingredient mg/kg net X weight of the net kg/m}^2$$

Using the selected product design attributes of 25 g PBO/kg net and 40 g/m^2 fabric weight, the TC is calculated as follows:

$$TC = 125\% \text{ x } 25 \text{ g/kg net x } 40 \text{ g/m}^2 = 1250 \text{ mg/m}^2$$

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

Abs = Dermal absorption from net surface (default = 8.3%)
Abs-o = Oral absorption (default = 100%)
AT = Average time (default = 365 days)
BW = Body weight (default = 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])
ESA = Exposed skin area
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)
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)
SE = Salivary extraction factor (default = 57% [75th percentile])
SN = Maximal actual size of the net (default = 15 m^2)
SF = Surface fraction = 100 – WRI % = 10%
Transl = Translodgeable fraction (default = 6%)
TC = Total concentration of active ingredient on net surface (derived value) = 1250 mg/m^2
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 = 4 liters)
WRI = Wash resistance index = 90%
1000 = Conversion of mg to µg
PBO Molecular Mass = 338.4 g/ml
PBO Vapor Pressure = < 1 x 10-7 mm Hg at 25° Celsius (USEPA, 2017)
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 From inhalation exposure

PBO is impregnated into the ITN and has a low vapor pressure; therefore, inhalation exposure is negligible. It is expected that the contribution of inhalation exposure to total body exposure while sleeping under a treated net is so small that, in practice, it can be ignored.

2.2.1.2 From dermal exposure

The estimated time weighted average (TWA) systemic dose due to potential dermal exposure from sleeping under the net is calculated using an 8.3% default dermal absorption factor (Wester et al., 1994). Table 4 reflects the systemic doses for all populations due to dermal exposures from sleeping under treated nets.

$$\text{Systemic TWA dose} = \frac{\text{Absorption (dermal) x Transl x ESA x SF x TC}}{\text{BW}} \text{ x } 1000$$

Table 4. Piperonyl butoxide: estimated TWA systemic dose for all populations due to dermal exposure from sleeping under the treated nets

Population	Absorption (%)	Transl (%)	ESA (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic dose (µg/kg bw/day)
Adults	8.3	6	0.408	10	1250	60.0	4.233
Children	8.3	6	0.225	10	1250	23.9	5.860
Toddlers	8.3	6	0.115	10	1250	10.0	7.158
Infants	8.3	6	0.100	10	1250	8.0	7.781

Transl = default = 6%
ESA = default values
SF = 100 – WRI % = 10%
TC = 1250 mg/m^2
BW = default values
1000 = conversion of mg to µg

2.2.1.3 From 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 toddlers and infants.

From 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 5.

$$\text{Systemic dose} = \frac{\text{Absorption (oral)} \times \text{SE} \times \text{Transl} \times \text{EHA} \times \text{FHM} \times \text{SF} \times \text{TC}}{\text{BW}} \times 1000$$

Table 5. Piperonyl butoxide: estimated systemic dose due to hand-to-mouth transfer sleeping under treated nets

Population	Absorption (%)	SE (%)	Transl (%)	EHA (m^2)	FHM	SF (%)	TC (mg/m^2)	BW (kg)	Systemic dose (µg/kg bw/day)
Toddlers	100	57	6	0.008	0.164	10	1250	10	0.560
Infants	100	57	6	0.007	0.164	10	1250	8	0.613

SE = default =57%
Transl = default =6%
EHA = default values
FHM = default= 0.164
SF = 100 – WRI % = 10%
TC = 1250 mg/m^2
BW = default values
1000 = conversion of mg to µg

From direct mouth contact

Direct mouth contact is only relevant for toddlers and infants. The estimated systemic dose from direct mouth contact is indicated in Table 6.

$$\text{Systemic dose} = \frac{\text{Absorption (oral)} \times \text{SE} \times \text{NM} \times \text{SF} \times \text{TC}}{\text{BW}} \times 1000$$

Table 6. Piperonyl butoxide: estimated systemic dose due to direct mouth contact sleeping under treated nets

Population	Absorption (%)	SE (%)	NM (m^2)	SF (%)	TC (mg/m^2)	BW (kg)	Systemic dose (µg/kg bw/day)
Toddlers	100	57	0.0014	10	1250	10	9.975
Infants	100	57	0.0014	10	1250	8	12.468

SE = default =57%)
NM = default =0.0014 m^2
SF = 100 – WRI % = 10%
TC = 1250 mg/m^2
BW = default values
1000 = conversion of mg to µg

2.2.1.4 Sleeping under treated nets – total exposure

A total exposure to PBO while sleeping under a treated net was calculated in Table 7 as the summation of the values for inhalation, dermal and oral routes of exposure given above.

Table 7. Piperonyl butoxide: 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 exposure (µg/kg)
Adults	Negligible	4.233	N/A	N/A	4.233
Children	Negligible	5.860	N/A	N/A	5.860
Toddlers	Negligible	7.158	0.560	9.975	17.694
Infants	Negligible	7.781	0.613	12.468	20.863

2.2.2 Estimation of systemic dose during washing of nets

There is no risk associated with inhalation exposure from washing of treated nets because 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 adults and child only.

2.2.2.1 Dermal exposure during net washing

The estimated systemic dose (maximum) from acute dermal exposure is depicted in Table 8.

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

Table 8. Piperonyl butoxide: estimated systemic dose (maximum) from acute dermal exposure due to washing treated nets

Population	Absorption (%)	NoN (nets)	VLS (ml)	SF (%)	TC (mg/m²)	SN (m²)	VolW (ml)	BW (kg)	Systemic dose (µg/kg bw/day)
Adults	8.3	5	36.7	10	1250	15	4000	60	118.99
Children	8.3	5	17.6	10	1250	15	4000	23.9	143.25

NoN = default =5
VLS = default =36.7 ml/adult and 17.6 ml/child
SF = 100 – WRI % = 10%
TC = 1250 mg/m²
SN = default=15 m²
VolW = default = 4000 ml
BW = default values
1000 = conversion of mg to µg

From repeated (TWA) exposure

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

Table 9. Piperonyl butoxide: 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)	SN (m²)	VolW (ml)	BW (kg)	AT (days)	Systemic dose (µg/kg bw/day)
Adults	8.3	20/3 years	5	36.7	10	1250	15	4000	60.0	365	2.171
Children	8.3	20/3 years	5	17.6	10	1250	15	4000	23.9	365	2.613

NoW = default=20/3 years
NoN = default =5
VLS = default;= 36.7 ml/adult and 17.6 ml/child
SF = 100 – WRI % = 10%
TC = 1250 mg/m²
SN = default=15 m²
VolW default= 4000 ml
BW = default values
AT = default =365 days
1000 = conversion of mg to µg

2.2.2.2 Oral exposure during net washing

Estimated systemic dose from acute oral (maximum) exposure is shown in Table 10.

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

Table 10. Piperonyl butoxide: estimated systemic dose (maximum) from acute oral exposure due to washing treated nets

Population	Absorption (%)	NoN (nets)	VLH (ml)	SF (%)	TC (mg/m²)	FHM	SN (m²)	VolW (ml)	BW (kg)	Systemic dose (µg/kg bw/day)
Adults	100	5	8.2	10	1250	0.164	15	4000	60	52.531
Children	100	5	4.3	10	1250	0.164	15	4000	23.9	69.155

NoN = default =5
VLH= default=8.2 ml/adult and 4.3 ml/child
SF = 100 – WRI % = 10%
TC = 1250 mg/m²
FHM = default = 0.164)
SN = default=15 m²
Vol W = default=4000 ml
BW = default values
1000 = conversion of mg to µg

From repeated (TWA) exposure

$$\text{Systemic dose (TWA)} = \frac{\text{Absorption (oral) x NoW x NoN x VLH x SF x TC x FHM x SN}}{\text{VolW} \times \text{BW x AT}} \text{ x } 1000$$

Table 11. Piperonyl butoxide: 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)	FHM	SN (m²)	VolW (ml)	BW (kg)	AT (days)	Systemic dose (µg/kg bw/day)
Adults	100	20/3 years	5	8.2	10	1250	0.164	15	4000	60.0	365	0.958
Children	100	20/3 years	5	4.3	10	1250	0.164	15	4000	23.9	365	1.261

NoN = default =5
NoW = default =20 washes/3 years
VLH = default =8.2 ml/adult and 4.3 ml/child
SF = 100 – WRI % = 10%
TC = 1250 mg/m²
FHM = default =0.164)
SN = default=15 m²
Vol W = default=4000 ml
BW = default values
AT = default =365 days
1000 = conversion of mg to µg

2.2.2.3 Total systemic exposure due to washing of treated nets

Table 12 depicts the estimated total exposure from washing of nets.

Table 12. Piperonyl butoxide: estimated total systemic dose from washing of nets

Subpopulation	Dermal exposure (µg/kg bw/day)	Oral exposure (µg/kg bw/day)	Total systemic dose (µg/kg bw/day)
Acute exposure (maximum)			
Adults	118.98	52.531	171.52
Children	143.25	69.155	212.41
Repeated exposure (TWA)			
Adults	2.171	0.958	3.129
Children	2.613	1.261	3.875

2.2.3 Exposure via breast milk

Pregnant and lactating women may sleep under the ITN and likely wash the nets and may even carry out the net dipping. Infants may therefore be exposed through breast milk.

Estimates for systemic maximum and TWA doses from exposure via breast milk are calculated as follows:

$$\text{Systemic dose TWA} = \frac{\text{Absorption (100\%) x Sol C} \times \text{Dose (mother)} \times T½ \times \text{IR}}{\text{BW}} \text{ x } 1000$$

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

Mother dose = dermal exposure/sleeping under net + total exposure/washing of nets

Mother dose maximum = 4.233 + 171.52 µg/kg bw/day = 175.75 µg/kg bw/day

Mother dose (TWA) = 4.233 + 3.129 µg/kg bw/day = 7.362 µg/kg bw/day

The estimated maximum (acute) systemic dose from exposure via breast milk is shown in Table 13.

Table 13. Piperonyl butoxide: estimated maximum (acute) systemic dose from exposure via breast milk

Subpopulation	Absorption (%)	Sol C	Dose (µg/kg/day)	$T_{1/2}$ (days)	IR (kg/day)	BW (kg)	Systemic dose (µg/kg bw/day)
Infants	100	0.361	175.75	1.5	0.66	8	7.851
Newborns	100	0.361	175.75	1.5	0.66	4.2	14.955

Sol C = 0.361
Dose = daily dose to the mother, maximum
T½ = 1.5 days
IR = default =0.66 kg/day
BW = default values

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

Table 14. Piperonyl butoxide: estimated TWA systemic dose from exposure via breast milk

Subpopulation	Absorption (%)	Sol C	Dose (µg/kg/day)	$T_{1/2}$ (days)	IR (kg/day)	BW (kg)	Systemic dose (µg/kg bw/day)
Infants	100	0.361	7.362	1.5	0.66	8	0.328
Newborns	100	0.361	7.362	1.5	0.66	4.2	0.626

Sol C = 0.361
Dose = daily dose to the mother, TWA
T½ = 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 active ingredient has significant acute toxicity, the aRfD is used as the TSD_{AC} to calculate risk ratios as shown below (WHO, 2018).

$$\text{Ratio} = \frac{\text{Estimated maximal daily systemic dose (µg kg bw/day)}}{TSD_{AC}\ (\text{µg/kg bw/day})}$$

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).

Presented in the following tables are the 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 PBO.

2.3.1 Exposure estimates and risk ratios for sleeping under the treated nets

The risk ratios for all populations sleeping under treated nets are depicted in Table 15.

Table 15. Exposure estimates and risk ratios for all populations sleeping under Piperonyl butoxide-treated nets

Subpopulation	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	4.233	N/A	N/A	4.233	160	0.026
Children	5.860	N/A	N/A	5.860	160	0.036
Toddlers	7.158	0.560	9.975	17.649	160	0.110
Infants	7.781	0.613	12.468	20.863	160	0.130

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 16.

Table 16. Exposure estimates and risk ratios for populations washing Piperonyl butoxide-treated nets

Subpopulation	Dermal exposure (µg/kg bw/day)	Oral (µg/kg/day)	Total exposure (µg/kg/day)	TSD acute (µg/kg/day)	TSD (µg/kg/day)	Risk ratio
Acute exposure (maximum)						
Adults	118.99	52.531	171.52	5000	N/A	0.03
Children	143.253	69.155	212.40	5000	N/A	0.04
Repeated exposure (TWA)						
Adults	2.171	0.958	3.129	N/A	160	0.02
Children	2.613	1.261	3.875	N/A	160	0.02

TSD_{AC} = 5000 µg/kg bw/day
TSD = 160 µg/kg bw/day
Risk ratio = Total Exposure - Acute/TSD_{AC} or Total Exposure - TWA/TSD

2.3.3. Combined risk ratios for sleeping under nets and washing of nets

The combined risk ratios for all populations sleeping under and washing of treated nets are depicted in Table 17.

Table 17. Exposure estimates and risk ratios for populations sleeping under and washing piperonyl butoxide-treated nets

Subpopulation	Sleeping under nets (combined) (µg/kg/day)	Washing of nets (combined) (µg/kg/day	Total exposure (µg/kg/day)	TSD_{AC} (µg/kg/day)	TSD (µg/kg/day)	Risk ratio
Acute exposure (maximum)						
Adults	4.233	171.51	175.75	5000	N/A	0.04
Children	5.860	212.40	218.26	5000	N/A	0.04
Repeated exposure (TWA)						
Adults	4.233	3.129	7.362	N/A	160	0.05
Children	5.860	3.875	9.736	N/A	160	0.06

TSD_{AC} = 5000 µg/kg bw/day
TSD = 160 µg/kg bw/day
Risk ratio = Total Exposure - Acute/TSD_{AC} or Total Exposure - TWA/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 18.

Table 18. Exposure estimates and risk ratios for populations sleeping under piperonyl butoxide-treated nets and consuming breast milk

Subpopulation	Total exposure sleeping under nets (dermal + oral indirect + oral direct) (µg/kg/day)	Breast milk exposure (µg/kg/day)	Total exposure (µg/kg/day)	TSD acute (µg/kg/day)	TSD (µg/kg/day)	Risk ratio
Acute exposure (maximum)						
Infants	20.863	7.851	28.715	5000	N/A	0.005
Newborns	N/A	14.955	14.955	5000	N/A	0.003
Repeated exposure (TWA)						
Infants	20.863	0.328	21.192	N/A	160	0.132
Newborns	N/A	0.626	0.626	N/A	160	0.004

TSD_{AC} = 5000 µg/kg bw/day
TSD = 160 µg/kg bw/day
Risk ratio = Total Exposure - Acute/TSD_{AC} or Total Exposure - TWA/TSD

2.4 Risk conclusions

The potential health risk is acceptable (risk ratios < 1; Table 19) for:

- Acute and repeated exposure for all populations (adults, children, toddlers and infants) sleeping under treated nets
- Acute and repeated exposure for adults and children washing of treated nets
- Acute and repeated exposure for adults and children sleeping under and washing of treated nets
- Acute and repeated exposure for infants and newborns exposed via breast milk from mothers sleeping under and washing of treated nets

Table 19. Summary of risk characterization for piperonyl butoxide as a synergist in ITN (up to 25 g/kg or 1000 mg/m^2)

Activity/population	Risk acceptable/not acceptable
Sleeping under net – repeated exposure	
Adults	Acceptable
Children	Acceptable
Toddlers	Acceptable
Infants	Acceptable
Washing of nets – acute exposure	
Adults	Acceptable
Children	Acceptable
Washing of nets – repeated exposure	
Adults	Acceptable
Children	Acceptable
Sleeping under and washing of nets – acute exposure	
Adults	Acceptable
Children	Acceptable
Sleeping under and washing of nets – repeated exposure	
Adults	Acceptable
Children	Acceptable
Exposures via breast milk from mothers exposed to PBO	
Infants (acute and chronic)	Acceptable
Newborns (acute and chronic)	Acceptable
Sleeping under net and exposed via breast milk from mother exposed to PBO	
Infants (acute and chronic)	Acceptable
Newborns (acute and chronic)	Acceptable

3 Conclusion

The assessment of the available information on safety indicates that a PBO-treated ITN with a concentration of 1000 mg/m^2 (fabric weight 40 g/m^2 and 25 g/kg net) and a WRI of 90% can be used safely for its intended use as a vector control product.

Appendix: Piperonyl butoxide health hazard assessment

Product Name: PIPERONYL BUTOXIDE
Empirical formula: $C_{19}H_{30}O_5$
Chemical Abstract Number: 51-03-6

1 Background information

Piperonyl butoxide (PBO) (5-[2-(2-butoxyethoxy)ethoxy-methyl]-6-propyl-1,3-benzodioxole) is a synergist used in combination with pyrethroids as active ingredients in ITN products. The mode of action of PBO is complex. PBO inhibits the oxidative and esterase-based metabolism (detoxification) of the pyrethroids, therefore prolongs the insecticidal property of the pyrethroids.

2 Relevant toxicity information on deltamethrin

(Note: Only information relevant to hazard assessment is discussed in this document. Additional information can be found in the references cited.)

The toxicity database of PBO is adequate for a full hazard evaluation and an assessment of risks associated with ITN products.

Absorption, distribution, metabolism and excretion (ADME) studies show that PBO was rapidly excreted with up to 99% being excreted within 48 hours with the majority (up to 85%) being eliminated in the feces. PBO has low acute toxicity by oral, inhalation and dermal routes. It is minimally irritating to the eyes and skin and is not a skin sensitizer. In an acute neurotoxicity study, PBO produced neurological signs at the limit dose of 1000 mg/kg but without associated histopathologic findings. A summary of acute toxicity information is shown in Table A1.

In a subchronic dermal study in rabbits, no systemic adverse effects were noted. In repeated dose studies, the liver is the primary target organ of PBO toxicity as evidenced by increased liver weight, increased cholesterol level and hypertrophy of hepatocytes. No developmental toxicity effects were noted in studies in rats and rabbits. No evidence of mutagenic potential was identified from a genotoxicity battery of in vitro and in vivo assays. PBO is classified as a Group C carcinogen (possible human carcinogen) based on findings of liver tumors in CD-1 mice. No carcinogenic potential was noted in the rats. The USEPA recommended that no quantification of human carcinogenic risk to be determined for PBO (USEPA, 2017). A summary of subchronic toxicity, genotoxicity, neurotoxicity, reproductive and developmental toxicity and chronic/carcinogenicity is shown in Table A2.

Table A1. Summary of PBO acute toxicity data

Piperonyl butoxide – toxicity profile: acute toxicity		
Study type	**Species; dose levels; guidelines**	**Results**
Acute oral LD50, rats	OECD 423	LD 50 = 4570 mg/kg (males) LD50 = 7220 mg/kg (females) GHS Category 5
Acute dermal LD50, rabbits	OECD 402 Limit test	LD50 > 2000 mg/kg GHS Category 5
Acute inhalation, rats	OECD 403	LC50 > 5.9 mg/L/4 hours GHS Category 5
Primary skin irritation, rabbits	OECD 404	Minimally irritating GHS Category 2B
Primary eye irritation, rabbits	OECD 405	Minimally irritating GHS Category 3
Dermal sensitization, guinea pigs	OECD 406	Not a sensitizer GHS Category: Not classified

Table A2. Summary of PBO repeated exposure and special studies

Piperonyl butoxide – toxicity profile: sub-chronic, chronic and other special studies		
Study type	**Test material; purity; dose levels; design**	**Results**
Sub-chronic toxicity		
21-day dermal, rabbits	New Zealand white rabbits Doses: 0, 100, 300 and 1000 mg/kg bw/day	Systemic NOAEL = 1000 mg/kg/day (highest dose tested) Dermal NOAEL = not established Dermal LOAEL = 100 mg/kg bw/day (erythema, edema, desquamation, fissure)
90-day inhalation, rats	PBO: 90.78% a.i. Groups of Sprague Dawley rats, 15/sex/dose Doses: 0, 0.015, 0.074, 0.155 and 0.512 mg/L given 6 hours/day, 5 days/week for 90 days	Systemic NOAEC = 0.155 mg/L Systemic LOAEC = 0.512 mg/L (increased liver and kidney weights, mucoid discharge) Respiratory NOAEC = not established Respiratory LOAEC = 0.015 mg/L (metaplasia and metaplasia of the larynx)
13-weeks oral dietary, rats	F344 rats, 10/sex/dose Doses: 0, 6000, 12000 and 24000 ppm (equivalent to 0, 600, 1200 and 2400 mg/kg bw/day, respectively	Systemic NOAEL = 600 mg/kg bw/day Systemic LOAEL = 1200 mg/kg bw/day (increased liver weight)
Reproductive and developmental toxicity		
Developmental toxicity, rats	PBO: 90.78% a.i. 25 Crl:CD female rats per dose Doses: PBO dissolved in water at 0, 200, 500 and 1000 mg/kg bw/day – given by gavage from gestational days 6–15	Maternal toxicity NOAEL = 200 mg/kg bw/day Maternal toxicity LOAEL = 500 mg/kg bw/day (decreased weight gain and food consumption) Developmental tox NOAEL = 1000 mg/kg bw/day Developmental tox LOAEL = not established
Developmental toxicity, rabbits	PBO: 100 % a.i. 16 New Zealand rabbits/dose Doses: 0, 50, 100 and 200 mg/kg bw/day – given by gavage from gestational days 7–19.	Maternal toxicity NOAEL = 200 mg/kg bw/day Maternal toxicity LOAEL = not established Developmental tox NOAEL = 200 mg/kg bw/day Developmental tox LOAEL = not established
2-generation reproduction, rats	PBO: 88% a.i. Groups of 26 CD rats/sex/dose given in diet at 0, 300, 1000 and 5000 ppm (equivalent to 0, 27, 89 and 469 mg/kg bw/day in males and 0, 30, 102 and 528 mg/kg bw/day in females, respectively	Parental systemic NOAEL = 1000 ppm (89 mg/kg bw/day) Parental systemic LOAEL = 5000 ppm (469 mg/kg bw/day) based on decreased body weight gains Reproductive NOAEL = 5000 ppm (469 mg/kg bw/day) Reproductive LOAEL = not established Offspring NOAEL = 1000 ppm (89 mg/kg bw/day) Offspring LOAEL = 5000 ppm (469 mg/kg bw/day) based on decreased body weight gain at post-natal day 21
Neurotoxicity		
Acute neurotoxicity, Sprague Dawley rats	PBO: 93.9% a.i. Groups of 10 Crl:CD rats (both sexes) Doses: 0, 100, 500 and 1000 mg/kg bw/day	Neurotoxicity NOAEL = 500 mg/kg bw Neurotoxicity LOAEL = 1000 mg/kg bw (unusual posture, abnormal gait, but no histopathologic findings)

Study type	Test material; purity; dose levels; design	Results
Genotoxicity		
Bacterial reverse mutation assay (Ames assay)	Doses: 100 to 5000 ug/plate *Salmonella typhimurium*	No evidence of mutagenicity up to 5000 ug/plate in the presence and absence of metabolic activation
Bacterial reverse mutation assay (Ames assay)	Doses: 0 to 10 mg/plate *Salmonella typhimurium*	No evidence of mutagenicity up to 10 mg/plate in the presence and absence of metabolic activation
In vitro, mammalian cell gene mutation assay	Chinese hamster ovary cells (CHO) Doses: 10 – 100 ug/ml (without activation) Doses: 25 – 500 ug/ml (with activation)	Equivocal response in 75 ug/ml only under non-activated condition
In vitro, mammalian cell chromosomal aberration assay	Doses: 15.0 – 30.0 ug/ml (non-activated assays) Doses: 12 – 120 ug/ml (activated assays)	No induction of chromosomal aberration in CHO cells
Unscheduled DNA synthesis, rats	Rat primary hepatocytes used Doses: 5, 10, 25 and 50 ug/ml	No evidence of induction of unscheduled DNA synthesis
In vitro, sister chromatid exchange assay	CHO cells	No sister chromatid exchanged in CHO cells with and without metabolic activation
Chronic-carcinogenicity		
1-year chronic oral dietary, dogs	PBO: 90.78% a.i. Beagle dogs 4 sex/group Doses: 0, 100, 600 or 2000 ppm (equivalent to 0, 2.9, 15.5 and 52.8 mg/kg bw/day in males and 0, 2.8, 16.3 and 71 mg/kg bw/day in females, respectively	Systemic NOAEL = 15.5 mg/kg bw/day Systemic LOAEL = 52.8 mg/kg bw/day (decreased body weight gain, decreased liver weight, increased alkaline phosphatase, hepatocellular hypertrophy)
18-month chronic/ carcinogenicity, mice	CD-1 mice, 60/sex/dose PBO: 90.8% a.i. Doses: 0, 30, 100 or 300 mg/kg bw/day	Systemic NOAEL = 30 mg/kg bw/day Systemic LOAEL = 100 mg/kg bw/day (hepatotoxicity) Positive dose-related trend in incidence of hepatocellular adenomas and carcinomas at 100 mg/kg bw/day (males) and 300 mg/kg bw/day (females)
2-year chronic/carcinogenic, rats	Sprague Dawley rats PBO: 94.3% a.i. Doses: 0, 30, 100, 500 mg/kg bw/day	Systemic NOAEL = 30 mg/kg bw/day Systemic LOAEL = 100 mg/kg bw/day (effects noted on liver and kidneys) No evidence of carcinogenic potential
2-year chronic/carcinogenicity, rats	F344 rats PBO: 94.5% a.i. Dietary at 0, 0.6, 1.2 or 2.4% (equivalent to intake of 0, 547, 1052 or 1877 mg/kg bw/day in males and 0, 527, 1061 and 2002 mg/kg bw/day in females, respectively	Systemic NOAEL = not established Systemic LOAEL = 527 mg/kg bw/day based on decreased body weight gain, increased liver weight. Dose-related increase in lymphoreticular neoplasia was noted in females and no tumor findings in males
Metabolism		
Metabolism, rats	Doses: single oral dose of 50 or 500 mg/kg bw	Majority of administered radioactive material excreted in feces and urine within 48 hours and less than 1.5% of radioactivity was present in tissues after 168 hours
Metabolism, rats	Doses: single oral dose of 50 or 500 mg/kg	Majority excreted in feces and urine within 48 hours and less than 0.5% was identified in tissues after 168 hours

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WHO Prequalification of Vector Control Products:
https://extranet.who.int/pqweb/vector-control-products