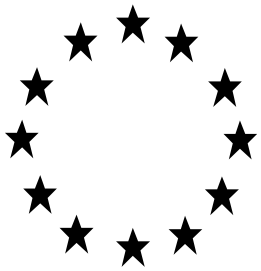
Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products

Evaluation of active substances

Assessment Report



Piperonyl Butoxide

Product-type 18
(insecticides, acaricides and products to control other arthropods)

January 2017

Greece

CONTENTS

1. STATEMENT OF SUBJECT MATTER AND PURPOSE

1.1. Procedure followed

This assessment report has been established as a result of the evaluation of the active substance Piperonyl Butoxide as product-type 18 (Insecticide), carried out in the context of the work programme for the review of existing active substances provided for in Article 89 of Regulation (EU) No 528/2012, with a view to the possible approval of this substance.

Piperonyl Butoxide (CAS no. 51-03-6) was notified as an existing active substance, by Endura S.p.A., hereafter referred to as the applicant, in product-type 18.

Commission Regulation (EC) No 1451/2007 of 4 December 2007[1] lays down the detailed rules for the evaluation of dossiers and for the decision-making process.

In accordance with the provisions of Article 7(1) of that Regulation, Greece was designated as Rapporteur Member State to carry out the assessment on the basis of the dossier submitted by the applicant. The deadline for submission of a complete dossier for Piperonyl Butoxide as an active substance in Product Type 18 was 30 October 2008, in accordance with Annex V of Regulation (EC) No 1451/2007.

On 17 October 2006 competent authorities received a dossier from the applicant. The Rapporteur Member State accepted the dossier as complete for the purpose of the evaluation on 26 October 2007.

On 29 May 2015, the Rapporteur Member State submitted to the Agency and the applicant a copy of the evaluation report, hereafter referred to as the competent authority report.

In order to review the competent authority report and the comments received on it, consultations of technical experts from all Member States (peer review) were organised by the Agency. Revisions agreed upon were presented at the Biocidal Products Committee and its Working Groups meetings and the competent authority report was amended accordingly.

1.2. Purpose of the assessment report

The aim of the assessment report is to support the opinion of the Biocidal Products Committee and a decision on the approval of Piperonyl Butoxide for product-type 18, and, should it be approved, to facilitate the authorisation of individual biocidal products. In the evaluation of applications for product-authorisation, the provisions of Regulation (EU) No 528/2012 shall be applied, in particular the provisions of Chapter IV, as well as the common principles laid down in Annex VI.

For the implementation of the common principles of Annex VI, the content and conclusions of this assessment report, which is available from the Agency web-site shall be taken into account.

However, where conclusions of this assessment report are based on data protected under the provisions of Regulation (EU) No 528/2012, such conclusions may not be used to the benefit of another applicant, unless access to these data for that purpose has been granted to that applicant.

[1] Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market. OJ L 325, 11.12.2007, p. 3

2. OVERALL SUMMARY AND CONCLUSIONS

2.1. Presentation of the Active Substance

2.1.1. Identity, Physico-Chemical Properties & Methods of Analysis

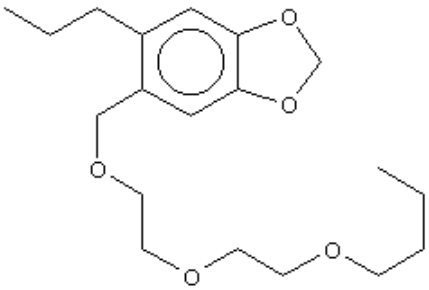
2.1.1.1. Names/addresses of Applicant/manufacturer of the active substance

Applicant	Name: **Endura S.p.A.** Address: Viale Pietramellara, 5, 40121 Bologna, Italy Telephone: +39 051 5281711 Fax number: +39 051 557255 E-mail address: endura@endura.it
Manufacturer of active substance (if different)	Name: **ENDURA S.p.A.** Address: Via Baiona, 107-111 48100 Ravenna Italy

2.1.1.2. Identity of the active substance

Table 1: Identity of Piperonyl Butoxide

CAS-No.	51-03-6
EINECS-No.	200-076-7
Other No. (CIPAC, ELINCS)	CIPAC no. 33
IUPAC Name	5-[2-(2-butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole
Common name, synonyma	Piperonyl Butoxide
Minimum purity of the active substance as manufactured (g/kg or g/l)	94.0 %w/w
Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg)	Safrole: max. content <0.004% w/w Dihydrosafrole: max. content <0.0085% w/w Dipiperonyl methane: max. content 1.95% w/w*** Dipiperonyl ether: max. content 0.9%w/w*** Isosafrole: max. content <0.004% w/w** Methyl dihydrosafrole: max. content 0.5%w/w Piperonyl Butoxide-x (Piperonyl Butoxide

	homologue): max. content 0.47 % w/w ortho-Piperonyl Butoxide (Piperonyl Butoxide homologue): max. content 0.51 % w/w N.N-dimethylformamide: max. content <0.04% w/w* Dichloromethane: max. content <0.05% w/w*
Molecular formula	$C_{19}H_{30}O_5$
Structural formula	
Molecular weight (g/mol)	338.43 g/mol

* In the tox ad-hoc (April-May 2016) N.N-dimethylformamide and Dichloromethane were considered as relevant impurities. The proposed limit was accepted at APCP WG III (May 2016).
** The limit was proposed in the tox ad-hoc (April-May 2016). The proposed limit was accepted at APCP WG III (May 2016).
*** In the enviroment ad-hoc (April-May 2016) Dipiperonyl methane and Dipiperonyl ether were considered as relevant impurities.

2.1.1.3. Physico-chemical properties

Piperonyl Butoxide (PBO) is a pale yellow transparent oily liquid at 20°C with a mildly aromatic odour. Its boiling point is 203°C at 2.78 mbar. The relative density of Piperonyl Butoxide technical material is about 1.058 g/mL at 20°C and its vapour pressure is less than 1.33×10^{-5} Pa at 25°C. Piperonyl Butoxide is not expected to have oxidising or explosive properties and shows no re-activity towards its container material.

One representative formulation has been evaluated in support of Piperonyl Butoxide.

AquaPy (UVP 06477402) containing 30 g/L pyrethrins and 135 g/L Piperonyl butoxide, is formulated as an emulsion, oil in water (EW). It is an opaque white homogenous liquid with chemical odour. Its pH value is 4.82 (1% aqueous dilution) and its density is approximately 0.9895 g/cm^3 at 20^{o}C. AquaPy forms foam in the acceptable limits. The dynamic viscosity of the formulation varies with shear rate indicating non-Newtonian behaviour and its surface tension is 25.8 mN/m at 25^{o}C indicating that it is a surface active product. AquaPy is considered non-oxidizing, non-explosive, non-flammable and exhibits an auto-ignition temperature of 300 ^{o}C. No tank mixing recommendation is included in the label of AquaPy proposed by the applicant.

AquaPy (UVP 06477402) has been proven to be chemically and physically stable after the accelerated storage stability test (storage for 14 days at 54 ^{o}C) in PE/PA and HDPE/EVOH bottles, after the shelf life test (2 years at ambient temperature) in plastic jerry cans and after storage at low temperatures.

Nevertheless, it is commonly known that prolonged storage at high temperatures can lead to degradation of pyrethrins (the a.s. in AquaPy), hence its label should indicate that: "This product

should not be stored at temperatures above 35°C". Additionally, in preparing the spray liquid the following phrase should be added in the product label: "The spray liquid should not be exposed to sunlight."

However the following points need to be clarified by the applicant:

- Concerning the 9.5% increase in the content of PBO in the shelf life test (2 years at ambient temperature) in plastic jerry cans the applicant has stated, that either a justification or a new study will be provided for product authorisation.
- According to the WG II (March 2016) conclusion: Information on the pH, and acidity/alkalinity where relevant, of the neat oil in water formulation must be submitted. Data can be submitted at product authorisation stage.

2.1.1.4. Methods of Analysis

A fully validated GC/FID analytical method has been submitted for the determination of pure Piperonyl Butoxide and its impurities (significant and relevant) in Piperonyl Butoxide technical material. Piperonyl Butoxide and its ten impurities were identified by GC/MS. Representative chromatograms have been submitted and are acceptable.

For residue analysis, fully validated analytical methods with acceptable data for linearity, specificity and recovery and with appropriate LOQ (where applicable) were submitted for the determination of Piperonyl Butoxide in soil, air, and surface and drinking water.

Piperonyl Butoxide is not indicated to be toxic or highly toxic. Therefore, analytical methods for the determination of Piperonyl Butoxide in animal and human body fluids and tissues are not required.

Piperonyl Butoxide is an active substance in PT 18 (insecticides) used in public and private areas, as well as in areas where foodstuffs and other goods are stored, prepared and packaged. Therefore, residues are possible and potential risks have to be assessed at product authorization level. However, based on the updated dietary risk assessment at product authorization level an analytical method might be required.

In case of setting a MRL for Piperonyl Butoxide, analytical methods for the determination in potentially (directly or indirectly) exposed food and feedstuffs should be provided.

The CIPAC method (Pyrethrum + Piperonyl Butoxide + MGK 264 Technical Concentrates 32+33+345/TK/(M)/-, CIPAC Handbook, volume H: pages 239-242) is proposed for the analysis of Pyrethrum, Piperonyl Butoxide and MGK 264 in AquaPy and is acceptable.

In the WG III (May 2016) it was decided that a justification or storage stability data must be submitted to prove that relevant impurity methyl dihydrosafrole is not formed during storage in the formulation.

Since the relevant impurities (exept methyl dihydrosafrole) are not formed during storage, the WG members concluded that the methods for monitoring the relevant impuritiesin the biocidal product are not required under the BPR.

2.1.2. Intended Uses and Efficacy

Piperonyl Butoxide (PBO) has been used in insecticidal formulations for over 50 years and always in combination with other insecticides mainly belonging to pyrethrins and synthetic pyrethroids.

Piperonyl Butoxide is currently used in the market in various formulations and application methods. In combination with other insecticides, it is used in pest control for hygiene and health purposes in public places. With this dossier professional use indoor and outdoor has been considered.

The applicant supported the approval of Piperonyl Butoxide as an active substance in the Union list through the representative product AquaPy. AquaPy is an EW formulation containing 3 % w/v pyrethrins and 13.5% w/v Piperonyl Butoxide and it is intended for professional indoor use in public and domestic premises and outdoor use in amenity areas and woodlands to control flying insects such as houseflies and mosquitoes. The representative product is not to be used where food, feed and livestock animals will be exposed. AquaPy is applied outdoors as a ground ULV (Ultra Low Volume) space spray application against adult mosquitoes at 0.0125-0.02 ml product /m^2 (0.0017-0.0027 g Piperonyl Butoxide/ m^2) and indoors as a space spray application against adult mosquitoes and houseflies at 0.033 ml product/ m^3 (0.0045 g Piperonyl Butoxide/ m^3).

The mode of action of Piperonyl Butoxide is complex. According to the literature, Piperonyl Butoxide stabilises the co-applied insecticide inside the insect body and potentiates more toxins to reach their target molecules. This results in an increased mortality of the target organism, and likewise, the same effect may be observed by using decreased amounts of insecticide, i.e. synergism. There is strong evidence from the literature, that Piperonyl Butoxide inhibits the oxidative and esterase-based metabolism (detoxyfication) of the co-applied insecticide. Therefore, Piperonyl Butoxide delays the degradation of co-applied insecticidal substances and thereby prolongs the potential action of the compounds.

According to the literature Piperonyl Butoxide is usually applied at a dose that on its own is sublethal to the target species. When Piperonyl Butoxide is applied in combination with a known toxicant, the performance of the latter is enhanced at a rate that becomes lethal when on its own would be sublethal. Nevertheless, Piperonyl Butoxide on its own can exhibit some toxic effects, and hence at sublethal doses is likely to exert some stress on the insect.

According to the results of the submitted laboratory efficacy studies and a publication, Piperonyl Butoxide exerts innate lethal effect against houseflies, mosquitoes, cockroaches and house dust mites.

Efficacy studies evaluated in Doc-IIIB5 indicated that the representative product AquaPy is effective against the claimed target organisms at the dose rates as indicated in the List of Intended Uses. Human and environmental risk assessments have been performed considering Piperonyl Butoxide concentrations of the efficacious dose of the representative product AquaPy. The representative product AquaPy is a mixture of Piperonyl Butoxide with another active substance (pyrethrins), and hence the efficacy of AquaPy could not entail the innate toxic effect of Piperonyl Butoxide. In order to demonstrate insecticidal activity of Piperonyl Butoxide, seven (7) efficacy laboratory studies (Doc-IIIA5.10.2/06, 07, 15, 18-21), in which Piperonyl Butoxide was formulated in simple formulations alone ("dummy products"), were evaluated. In two of these studies (A5.10.2/15 & 21) the application method was similar to the intended uses of the reference product AquaPy, namely indoor space spray application.
The "dummy products" applied as indoor space spray treatment contained high doses of Piperonyl Butoxide (30-65 mg Piperonyl Butoxide /m^3), which are not indicated in the List of Intended Uses. Therefore, these doses were not considered further in other Sections of the CAR.

It is noted that only a basic efficacy of Piperonyl Butoxide was demonstrated at the active substance approval stage and for product authorisation studies representative for the intended use have to be provided.
Also, according to the submitted efficacy studies, Piperonyl Butoxide is effective against mosquitoes, houseflies and cockroaches as a synergist, formulated in combination with insecticides, particularly natural pyrethrins and synthetic pyrethroids.

Piperonyl Butoxide has been evaluated for its intended use as an insecticide (PT 18); In addition, in order to facilitate the work of Member States in granting or reviewing authorisations, the

intended uses of the substance, as identified during the evaluation process, are listed in Appendix II.

2.1.3. Classification and Labelling

Classification and labelling of the active substance

There is no current harmonized classification and labelling for Piperonyl Butoxide. The eCA proposes the following classification of the active substance according to the (EC) Regulation 1272/2008:

Classification of the active substance according to the (EC) Regulation 1272/2008

Classification	STOT SE 3 Carc. 2 Aquatic Acute 1; Acute M-factor: 1 Aquatic Chronic 1; Chronic M-factor: 1
GHS Pictograms	
Signal Word	Warning
Hazard Statements	H335: May cause respiratory irritation H351: Suspected of causing cancer H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects
Precautionary Statements	P261: Avoid breathing fume P271: Use only outdoors or in well-ventilated area P273: Avoid release to the environment P304+340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing P312: Call a POISON CENTER or doctor/physician if you feel unwell P391: Collect spillage P501: Dispose of contents/container in accordance with local regulation
Supplemental hazard wording	EUH066: Repeated exposure may cause skin dryness or cracking EUH401: To avoid risks to human health and the environment, comply with the instructions for use

Justification for the classification assigned to the active substance (Regulation 1272/2008):

STOT SE 3: acute & 3-month inhalation toxicity studies in rats confirmed by human epidemiology data

No specific respiratory irritation (acute) study has been performed.

Epidemiological data of individuals exposed to products containing pyrethrins have revealed that respiratory symptoms such as bronchospasm, cough/choke, and dyspnea were more likely if the exposure included piperonyl butoxide (US-EPA, Memorandum, Review of Piperonyl butoxide Incident Reports, 2004). These symptoms are likely the reason for increased risk of moderate effects which typically would require medical attention. Other literature suggests that pyrethrin-based products may pose a hazard to asthmatics ([redacted] 1997, [redacted] 1999, [redacted] 2000).

Moreover, slight respiratory tract irritation evidenced as nasal discharge and laboured breathing accompanied by red foci in the lungs of 2/5 females was noted in the acute toxicity study by inhalation in rats ([redacted] 1991). In addition, in the 3-month inhalation study in rats red nasal

discharge and histopathological alterations in the larynx including slight squamous metaplasia with minimal hyperkeratosis and moderate inflammation were noted at 0.512 mg/L ([redacted] 1992). These findings are considered relevant as part of weight of evidence evaluation of the potential of Piperonyl butoxide to cause respiratory tract irritation.

Carc. 2: Mouse carcinogenicity study & lack of robust mechanistic data.

In the 18-month oral carcinogenicity study ([redacted] 1993) Piperonyl Butoxide neoplastic effects were observed in the liver including statistically significant, positive dose-related trend in the incidence of adenomas and the combined adenomas and carcinomas at 100 mg/kg b.w./day (males) and 300 mg/kg b.w./day (males and females). These findings were confirmed in an open literature study in mice ([redacted] 1994b), where Piperonyl Butoxide induced hepatocellular carcinomas in all treated groups in a dose-dependent manner when administered orally in the diet at daily doses of 6000, 12000 ppm.
The applicant submitted two (2) reports on a postulated MoA for the Piperonyl Butoxide-induced liver tumour formation in male mice and a testing strategy to substantiate this proposal. The proposed MoA is based on the assumption that Piperonyl Butoxide is a constitutive androstane receptor (CAR) activator in mouse liver and includes stimulation of microsomal CYP2b forms after treatment of mice with Piperonyl Butoxide, increased liver weight with morphological evidence of hepatocyte hypertrophy and a transient stimulation of replicative DNA synthesis. There are mechanistic data available supportive for this hypothesis (See Doc IIIA Section A6.10). This MoA is similar to that established for rodent tumour formation by phenobarbital and related compounds. The applicant submitted also a series of mechanistic studies (Phase I and Phase II) to support this hypothesis, whereas the final studies to demonstrate the soundness of the postulated MoA are still ongoing (Phase III). The results of Phase I and Phase II studies substantiate the hypothesis of a CAR-mediated formation of liver tumours not relevant to humans. However, the submission of the phase III studies (studies in cultured mouse and human hepatocytes) are still pending. The WG-II-2016 considered that based on the information available, not having the phase III studies, PBO should be considered as a potential carcinogen with a threshold mode of action. The overall NOAEL for the mouse carcinogenic effect is 30 mg/kg bw/day (LOAEL 100 mg/kg bw/day).

EUH066: 21-day dermal study in New Zealand White rabbits

Irreversible skin effects (erythema, edema, desquamation, fissuring, red raised areas) were observed in the repeated dose dermal preliminary toxicity study in rabbits ([redacted] 1992) from the lowest dose tested (100 mg/kg b.w./day).

Note: there is currently no harmonised classification for human health effects of Piperonyl Butoxide.

Aquatic Acute 1

Piperonyl Butoxide is classified as Aquatic Acute 1 based on its acute toxicity to aquatic invertebrates (i.e. 48-hour EC_{50} for *Daphnia magna*=0.51 mg/L; 96-hour LC_{50} for *Americamysis bahia* (formerly *Mysidopsis bahia*)=0.32 mg/L; 96-hour EC_{50} for *Crassostrea virginica*=0.23 mg/L).

Aquatic Chronic 1

Piperonyl Butoxide is classified as Aquatic Chronic 1 based on its chronic toxicity to aquatic invertebrates (21-day NOEC for *Daphnia magna*=0.030 mg/L*;* 28-day NOEC for *Chironomus riparius*=0.0148 mg/L) and the fact that it is not readily biodegradable.

Classification and labelling of the product AquaPy

Classification of AquaPy according to the (EC) Regulation 1272/2008

Classification	Carc. 2 Aquatic Acute 1 Aquatic Chronic 1
GHS Pictograms	
Signal Word	Warning
Hazard Statements	H351: Suspected of causing cancer H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects
Precautionary Statements	P201: Obtain special instructions before use P202: Do not handle until all safety precautions have been read and understood P280: Wear protective gloves/protective clothing/eye protection/face protection P273: Avoid release to the environment P308+313: IF exposed or concerned: Get medical advice/attention P391: Collect spillage P501: Dispose of contents/container in accordance with local regulation
	EUH208: "Contains Mixture of: - 3(2H)-isothiazolone, 5-chloro-2-methyl- with 2-methyl-3(2H)-isothiazolone (ratio 3:1) and poly[oxy(dimethylsilene)]. May produce an allergic reaction".
Other phrases	In case of indoor application the area should be well-ventilated. In case of outdoor application, all persons and animals should be excluded during treatment. Unprotected persons and animals should be kept away from treated areas until the product has dissipated. EUH401: To avoid risks to human health and the environment, comply with the instructions for use

Justification for the classification assigned to AquaPy (Regulation 1272/2008):

Classification of AquaPy as Carc. Cat.2 has been proposed since, the content of Piperonyl Butoxide in the product exceeds the concentration limit of 1% that trigger classification according to Regulation (EC) No. 1272/2008.

The EUH208 phrase is proposed based on the sensitizing properties of the respective non-active substances and since, its' concentration levels in the formulation are equal or above the concentration limits for elicitation.

Classification of AquaPy as Aquatic Acute 1 has been based on its acute toxicity to aquatic invertebrates (i.e. 48-hour EC50 for Daphnia magna=0.216 mg/L). It is noted that no reliable effect endpoint for the acute toxicity of AquaPy to fish is currently available. Classification as Aquatic Chronic 1 has been based on the sum of its components classified as Chronic 1. Due to the lack of adequate testing long-term toxicity data on the product as a whole, the classification for the long-term hazard was based on the summation of its classified components.

2.2. Summary of the Risk Assessment

2.2.1. Human Health Risk Assessment

2.2.1.1. Hazard identification

General Comment

The WG-II-2016 *ad hoc* follow up agreed that the Piperonyl Butoxide batches used in the toxicological studies are representative of the commercial product.

Toxicokinetics and dermal absorption

^{14}C-Piperonyl Butoxide was readily absorbed and within 72 hours nearly completely excreted in the urine and faeces, mainly as metabolised products. An oral absorption value of 100% has been set. Accumulation in tissues did not occur. Major pathways of metabolisation are identified, by oxidation and hydrolysis of the glycol ether side chain, the propyl side chain or the heterocyclic methylenedioxy-ring.

Piperonyl Butoxide is considered to be 100% bioavailable by the inhalation route by default.

A GLP study was conducted in human volunteers to determine the degree of dermal absorption of Piperonyl Butoxide from human skin. Radiolabelled ^{14}C-Piperonyl Butoxide was administered as a 3% (w/w) solution in isopropanol or as a 4% (w/w) solution in an aqueous formulation, to the forearm of four healthy volunteers.

Dermal absorption was found to be 2.4% for subjects administered Piperonyl Butoxide in isopropanol (3% w/w) and 0.58% for subjects administered Piperonyl Butoxide in aqueous formulation (4% w/w) taking into account the radioactivity detected in urine, faeces and tape-strips. As a worst case, a value of 2.4% based on the isopropanol data is proposed for risk assessment purposes. With regard to the intended in-use dilution (1.5%) and following the approach described in the EFSA Guidance on Dermal Absorption (2012), dermal absorption is set at 4.8%.

Acute toxicity, irritation/corrosivity and sensitisation

Based on the available data, Piperonyl Butoxide was found to be of low acute oral, dermal and inhalation toxicity. It was not irritating to rabbit skin and was slightly irritating to rabbit eyes.
With regard to respiratory irritation, epidemiological data of individuals exposed to products containing pyrethrins have revealed that respiratory symptoms such as bronchospasm, cough/choke, and dyspnea were more likely if the exposure included piperonyl butoxide. These symptoms are likely the reason for increased risk of moderate effects which typically would require medical attention. Indications of slight respiratory tract irritation were noted in the acute inhalation study in rats (nasal discharge, laboured breathing, red foci) and in the 3-month inhalation study in rats (red nasal discharge, histopathological alterations in the larynx including slight squamous metaplasia with minimal hyperkeratosis and moderate inflammation). These findings are considered relevant as part of weight of evidence evaluation of the potential of Piperonyl butoxide to cause respiratory tract irritation. The eCA proposes that Piperonyl Butoxide is classified as **STOT SE 3; H335: May cause respiratory irritation**.
Piperonyl Butoxide did not show potential for skin sensitisation.

Repeated dose effects

Piperonyl Butoxide is a potent **inhibitor** of cytochrome P450 enzymes (and of esterases). This is the proposed mechanism of acting as a synergist together with pyrethrins and synthetic pyrethroids, inhibiting the enzymatic degradation of these. Upon repeated exposure, Piperonyl Butoxide **induces** hepatic cytochrome P450 enzymes. This results, at high dose levels, in hepatocellular hypertrophy, cell proliferation and hepatotoxicity. This has been demonstrated in mechanistic studies in rodents.

Subchronic oral toxicity studies were conducted in mice, rats and dogs. The dog was the most sensitive species with an overall NOAEL of 16 mg/kg b.w./day (1-year study). Target organs were the liver (mouse, rat, dog) and kidneys (rat). The NOAEL derived from the 1-year dietary study in dogs was utilised in setting the medium-term AEL, long-term AEL and ADI values (agreed at WG-II-2016).

Dermal application of Piperonyl Butoxide at doses up to 1000 mg/kg bw/day for 21 days caused no systemic toxicity in rabbits. However, dermal effects (erythema, oedema, desquamation, fissuring and red raised areas) were noted from the lowest dose of 100 mg/Kg bw/day. There is no indication of reversibility of dermal observations of treated animals during exposure with Piperonyl Butoxide and the study design did not include a recovery period. It is proposed that based on the skin effects (erythema, edema, desquamation, fissuring, red raised areas), piperonyl butoxide should be assigned the additional hazard statement **EUH066; Repeated exposure may cause skin dryness or cracking.** The WG-II-2016 supported the proposal for the additional hazard statement of EUH066. In line with the Guidance on BPR: Volume III, Part B Risk Assessment Version 2.0 October 2015, Point 4.3.2, *"Local effects (irritation/corrosion, sensitisation) – Qualitative and semi-quantitative risk characterisation RC for local effects is triggered only when the biocidal product is classified for local effects. RC for local effects is not required when the active substance and/or co-formulants in a product are classified for local effects but are present at concentrations that do not trigger classification of the product according to the CLP criteria (Guidance on the Application of CLP Criteria)."* Based on the relevant concentration limits, EUH066 is not triggered for AquaPy and therefore local risk characterisation is not warranted for this product.

In a subchronic 90-day inhalation study in the rat, hepatotoxicity evidenced as decreased serum liver enzyme activity and increased relative liver weight, as well as kidney toxicity indicated by increased relative kidneys weight, were observed at the top dose of 0.512 mg/L. The target organs identified were the liver and kidneys. The NOAEL for systemic toxicity was set at 0.155 mg/L. Local effects included red nasal discharge of slight/moderate severity evidenced from the dose of 0.155 mg/L in females (14/15 animals) and at 0.512 mg/L in males (15/15 animals). Based on the nature and severity of the effect the RMS considers that no classification for specific target organ toxicity (STOT RE) is warranted. Other local effects considered to be adverse at 0.512 mg/L were histopathological alterations in the larynx including slight squamous metaplasia with hyperkeratosis (minimal) and inflammation (moderate). No classification for laryngeal effects is also warranted since the dose of 0.512 mg/L exceeds the concentration limit of 0.2 mg/L for STOT RE Category 2 classification of a mist according to Reg. 1272/2008. The NOAEL of 0.155 mg/L for both local and systemic effects may be converted to 178.3 mg/kg b.w./day, to allow comparison with the oral studies.

Genotoxicity

No indication for mutagenic potential was identified based on a complete *in vitro* testing genotoxicity battery (2 Ames tests, a chromosomal aberration and a gene mutation test in mammalian cells) and an *in vivo* micronucleus assay (of limited validity). In the lack of any genotoxic effect in the fully acceptable *in vitro* studies, Piperonyl Butoxide is considered not genotoxic and no further genotoxicity testing is required.

Carcinogenicity

Chronic toxicity and oncogenicity of Piperonyl Butoxide has been assessed in rats and mice.
In rats, Piperonyl Butoxide was not found to be carcinogenic in a two-year dietary study [redacted] 1987) at doses up to 500 mg/kg bw/day. The NOAEL was set 30 mg/kg bw/day on the basis of effects on the liver and kidneys. There was no evidence of a carcinogenic potential. However, in an open literature study in rats [redacted] 1994a) piperonyl butoxide induced hepatocellular carcinomas in males and females in a dose–dependent manner when administered orally in the diet at daily doses exceeding the MTD (greater than 6000 ppm) for 2 years. Findings from this latter study were not considered reliable, due to the excessive toxicity observed in animals of all dose groups evidenced primarily as gastric and caecal haemorrhage. Thus, overall it may be concluded that Piperonyl Butoxide is not carcinogenic in rats.
In mice, hepatotoxicity was evidenced after oral administration of Piperonyl Butoxide at doses

greater than 100 mg/kg b.w./day ([redacted] 1993). The NOAEL in this study was set at 30 mg/kg bw/day on the basis of liver effects. A positive dose related trend in the incidence of adenomas and the incidence of combined adenomas and carcinomas with statistical increases in the middle and high doses was observed in male mice. No statistical evaluation of the results for female mice was performed by the applicant. These findings were confirmed in an open literature study in mice ([redacted] 1994b), where piperonyl butoxide induced hepatocellular carcinomas in all treated groups in a dose-dependent manner when administered orally in the diet at daily doses of 6000, 12000 ppm. Overall, it might be concluded that Piperonyl Butoxide is carcinogenic in mice.

The applicant submitted two (2) reports on a postulated MoA for the Piperonyl Butoxide-induced liver tumour formation in male mice and a testing strategy to substantiate this proposal. The proposed MoA is based on the assumption that Piperonyl Butoxide is a constitutive androstane receptor (CAR) activator in mouse liver and includes stimulation of microsomal CYP2b forms after treatment of mice with Piperonyl Butoxide, increased liver weight with morphological evidence of hepatocyte hypertrophy and a transient stimulation of replicative DNA synthesis. This MoA is similar to that established for rodent tumour formation by phenobarbital and related compounds. The applicant submitted also a series of mechanistic studies (Phase I and Phase II) to support its hypothesis, whereas the final studies to demonstrate the soundness of the postulated MoA are still ongoing (Phase III). The results of Phase I and Phase II studies substantiate the hypothesis of a CAR mediated formation of liver tumours not relevant to humans.

The eCA awaits for the submission of the phase III studies (studies in cultured mouse and human hepatocytes) to reach a final conclusion on the robustness of the proposed MoA for mouse liver tumour formation by Piperonyl Butoxide.

The WG-II-2016 considered that based on the information available, not having the phase III studies, Piperonyl Butoxide should be considered as a potential carcinogen with a threshold mode of action. The NOAEL for mouse carcinogenic effect is 30 mg/kg bw/day (LOAEL 100 mg/kg bw/day). A statistical analysis of the incidence of hepatocellular adenomas in females is not required.

As a consequence of the WG-II-2016 agreement, classification of Piperonyl Butoxide as a carcinogen category 2 (labelling element: **Carc. 2; H351 Suspected of causing cancer)** is proposed.

Toxicity to reproduction and development

Piperonyl Butoxide did not show toxic effects on fertility in a two-generation reproductive toxicity study in the rat at dietary doses up to 500 mg Piperonyl Butoxide/kg bw/day. The NOAEL values for parental and offspring toxicity were both set at 100 mg Piperonyl Butoxide/kg bw/day, based on decreased body weight values at 500 mg/kg bw/day. In developmental toxicity studies in the rat and the rabbit, there was no evidence of embryotoxicity, foetotoxicity or teratogenicity at doses up to 1000 and 200 mg/kg bw/day, respectively. Maternal toxicity in rats was evidenced as clinical signs including perinasal encrustation and red urogenital discharge and significantly decreased food consumption, body weight and body weight gain from the dose of 500 mg/Kg b.w./day. The NOAEL for maternal toxicity in rats was set at 200 mg/kg bw/day. In rabbits, maternal body weight loss was observed during the treatment period among dams treated with 200 mg/kg b.w./day.

The NOAEL for maternal toxicity in rabbits was set at 100 mg/kg bw/day and it was agreed at the WG-II-2016 to be considered as the basis of the setting of the short-term AEL.

Neurotoxicity

In the submitted subchronic, chronic and reproductive toxicity studies there are no indications for a neurotoxic activity of Piperonyl Butoxide. During trilateral discussions of Piperonyl Butoxide (February, 2016), the applicant provided an acute neurotoxicity study in rats indicating a low neurotoxic potential for Piperonyl Butoxide at single oral doses up to 1000 mg/kg bw. No repeated dose neurotoxicity study is available and was not requested considering the outcome of the acute neurotoxicity study and the overall weight of evidence. The eCA considered that the waiving of a subchronic neurotoxicity study is acceptable. The WG-II-2016 agreed that there is no concern on neurotoxicity and no further information is required.

Immunotoxicity
Repeated dose and chronic toxicity studies did not reveal any immunotoxic effects of Piperonyl Butoxide. During the trilateral discussions of Piperonyl Butoxide (February, 2016), the applicant submitted "A waiver argument on immunotoxicity of Piperonyl Butoxide." The eCA considers that the summarized data presented by the applicant, indeed imply some uncertainty, especially at high doses, but no convincing evidence. The WG-II-2016 agreed that there is no concern on immunotoxicity and no further information is required.

Human data
According to the US-EPA, Memorandum, Review of Piperonyl butoxide Incident Reports, 2004 and based on data from Poison Control Centers, there appears to be a greater risk of moderate or major symptoms among those exposed to products containing pyrethrins and piperonyl butoxide than those exposed to pyrethrins alone. Respiratory symptoms and selected dermal symptoms were more likely if the exposure included piperonyl butoxide. In addition, based on open literature studies pyrethrin-based products may pose a hazard to asthmatics. The findings from analysis of symptoms from Poison Control Centers suggest that piperonyl butoxide adds to that risk. Overall, the US-EPA Memorandum recommends that the labelling of products containing Piperonyl Butoxide should advise handlers that respiratory irritation, rash, and itching can occur in sensitive individuals and that protective clothing should be used. Persons with asthma or other respiratory impairments should be advised to use extra caution to avoid inhalation or other exposure to Piperonyl Butoxide products.
According to the data reported by the applicant no increased health risk resulted from the use of piperonyl butoxide has been identified.

2.2.1.2. Effects assessment

a) Local effects

Piperonyl butoxide does not cause substantial site of contact toxicity and therefore classification as STOT RE is not warranted . This eCA proposal was agreed at the WG-II-2016, where it was concluded that it is not necessary to derive an AEC because no classification as STOT RE is proposed. However, as agreed during the trilateral discussions, a local inhalation **AEC** could be derived to be considered for (semi-)quantitative RC by Member States at product authorisation level when/if required, since there is sufficient data for AEC derivation. In this case, the NOAEL = 0.155 mg/L from the 3-month inhalation study in rats ([redacted] 1992), should be the basis for the estimation of the $AEC_{inhalation}$. It was also agreed to consider the assessment factor for respiratory exposure as indicated under point 4.3.2.4 of the Guidance on the BPR: Volume III Human Health, Part B Assessment, i.e. 2.5 (interspecies AF) and 10 (intraspecies AF). The resulting $AEC_{inhalation}$ is 0.0062 mg/L or 6.2 mg/m^3.

This AEC should then be compared with the external inhalation exposures, also expressed in mg/m^3 in a (semi-) quantitative RC, when/if considered necessary.

b) Systemic effects

Piperonyl butoxide is not considered to be neurotoxic, immunotoxic, mutagenic, or toxic to reproduction. It is not carcinogenic in rats. Concerning the carcinogenic potential of Piperonyl Butoxide in mice, a positive dose-related trend in the incidence of adenomas and the incidence of combined adenomas and carcinomas with statistical increases from the dose of 100 mg/kg bw/day was observed in males and females (only at 300 mg/kg bw/day). The WG-II-2016 considered that based on the information available on carcinogenicity, not having part of the mechanistic data (phase III studies), Piperonyl Butoxide should be considered as a potential carcinogen with a threshold mode of action. The NOAEL for mouse carcinogenic effect is 30 mg/kg bw/day (LOAEL 100 mg/kg bw/day). As a consequence of the WG-II-2016 agreement, classification of Piperonyl Butoxide as a carcinogen category 2 (labelling element: **Carc. 2; H351 Suspected of causing cancer**) is proposed.

After oral administration, target organs are the liver (mouse, rat, dog) and kidneys (rat). The dog appears to be the most sensitive species with an overall NOAEL of 16 mg/kg b.w./day based

on decreased body weight gain and food consumption and liver toxicity at 2000 ppm (approx. 53 mg/kg bw/d in males and 71 mg/kg bw/d in females) (1-year dietary study). The NOAEL derived from the 1-year dietary study in dogs is considered suitable for the medium-term AEL, long-term AEL and ADI setting (agreed at WG-II-2016). Moreover, in the rabbit teratology study, maternal body weight was decreased (4%) within the first day of dosing at the 200 mg/kg b.w./day. Thus, the NOAELmaternal of 100 mg/kg b.w./day from the rabbit teratology study was considered to be more relevant for the short-term AEL setting (agreed at the WG-II-2016).

Based on the results of the ADME studies, an oral absorption value of 100% should be incorporated in the calculation of the AEL values.

The default assessment factor of 100 [10 (interspecies variation) x 10 (intraspecies variation)] is considered appropriate.

Thus, the reference values are estimated as follows:

- ADI, medium-term long-term AEL = 16 / 100 x 100% = 0.16 mg/kg b.w./day
  For harmonisation with the WHO ADI (JMPR, 1995), the ADI, medium and long-term AELs were rounded to **0.2 mg/kg bw/day**
- short-term AEL = **1 mg/kg bw/day**

It should be noted that the AEL of 0.2 mg/kg b.w./day provides a margin of safety of 500 from the dose of 100 mg/kg bw/day where induction of eosinophilic adenomas in the liver of male mice had been observed in the 18-month carcinogenicity study.

2.2.1.3. Exposure assessment

Professional users

Production / formulation of active substance

Data on exposure during manufacture, formulation or packaging are not considered as core data requirements for the purposes of Annex I inclusion. Therefore, the eCA has not performed an assessment for occupational exposure during production of Piperonyl Butoxide. However, for transparency reasons, the assessment submitted by the applicant is presented below. It is noted that the dermal absorption and the AOEL value are the proposed values by the applicant.

Due to the low vapour pressure of the active ingredient and the production process occupational inhalative exposure is negligible.

Direct dermal contact with Piperonyl Butoxide or Aquapy is not foreseen. However, incidental contact is possible during transfer of the substance to the mixing vessel and during cleaning and disposal of the containers even while protective gloves are worn.

The TNsG, (2002) on Human Exposure to Biocidal Products give indicative values of 4.2 mg/min (75th percentile) for exposure of hands inside gloves. Assuming that the duration of the dermal exposure is 30 min/day, the dermal exposure is estimated to be 126 mg/day.

The highest exposure is during the first dilution step, towards Piperonyl Butoxide resulting in 126 mg Piperonyl Butoxide per person.

Assuming a worker of 60 kg body weight daily dermal exposure is calculated at 2.1 mg/kg b.w.

Considering a dermal penetration of 2 %, as shown in a study on dermal absorption of Piperonyl Butoxide ([redacted] 1995) the internal dose would amount to 0.042 mg Piperonyl Butoxide/kg bw/d.

This estimate is well below the AOEL of 0.2 mg/kg b.w./d.

In the next production steps the product is diluted further, therefore the exposure to the active substance Piperonyl Butoxide will be lower than in the case scenario discussed above. The dermal exposure is incidental and not a consequence of normal work practice; it occurs only occasionally, when a new batch is produced, and it may involve different persons for each batch.

Safety measures: protective gloves for solvents, solvent-resistant suit.

Application of AquaPy - Outdoor space treatment as fog by professional operators

Outdoor application of AquaPy is conducted by professional operators *via* ground ULV space application using either hand- held or vehicle mounted fogger and is intended for use 1-6 times per year with 4-weeks interval between applications. The applicant has used the German BBA-model and the UK POEM in order to estimate operator exposure (please refer to Doc. IIIB6 for applicant's detailed calculations).

Taking into account the intended uses of AquaPy, the eCA has considered as more relevant for the exposure assessment the following model: *"Fogging and misting model 2"* [Technical Notes for Guidance (2002)[2]].

With regard to exposure during mixing and loading and in case of outdoor application *via* hand-held fogger, the generic exposure values for pouring formulation into a portable vessel (EUROPOEM II database) have been used [Biocides Human Health Exposure Methodology, October (2015), p. 191].

In case of outdoor application *via* vehicle mounted fogger, the generic exposure values for pouring formulation into a fixed vessel (EUROPOEM II database) have been considered [Biocides Human Health Exposure Methodology, October (2015), p. 192].

Operator exposure during cleaning of the application equipment has been assessed using the surrogate values from BEAT model database for both hand-held & vehicle mounted applicaton scenarios.

Calculations have been performed considering a treated area of 150 ha/day for vehicle mounted applications and 5 ha/day for hand-held applications applications [Operational manual of the application of insecticides for Control of the mosquito vectors of Malaria and other diseases, WHO/CTD/VBC/96.1000, January, (1996), p.98]. The task duration used was 120 min for fogging according to the Biocides Human Health Exposure Methodology (ECHA, October 2015).

Inhalation absorption has been considered to be 100% and dermal absorption 2.4% for the concentrate and 4.8% for the in-use dilution. The exposure estimates [in mg/person/day] have been converted to systemic exposures [in mg/kg bw/day] considering a body weight of 60 kg. It is noted that AquaPy is intended for use outdoors 1-6 times per year with 4-weeks interval between applications and indoors 1-2 times per year with minimum interval between applications of 1 month. Therefore, use of the medium-term AEL = 0.2 mg/kg b.w./day for risk characterisation, is considered appropriate.

The following table summarises the exposure estimates for mixing/loading, application and cleaning of application equipment for AquaPy *via* fogging either with hand held or vehicle mounted fogging equipment.

Table 2.2.1.3-1:Estimated primary systemic operator exposure to piperonyl butoxide [mg/kg bw/day] during outdoor mixing/loading, application and cleaning for AquaPy *via* fogging with hand held fogging equipment/no PPE and with PPE

Systemic exposure in mg/kg bw/day resulting from	**Preparation and application of the in-use dilution via hand-held fogging** no PPE	 with PPE*
- Mixing and loading	0.0277	0.00281
- Application	0.081	0.050
- Cleaning application equipment	0.013	0.0055
Total systemic exposure [mg/kg bw/day]	0.1217	0.0583
% of the AEL$_{medium-term}$	**61**	**29**

*90% protection is assumed for gloves & 80% for coated coverall [Biocides Human Health Exposure Methodology, October (2015)]

[2] TNsG; Technical notes for guidance; Human exposure risk assessment to biocidal products, Guidance on exposure estimation, June 2002

Table 2.2.1.3-2:Estimated primary systemic operator exposure to piperonyl butoxide [mg/kg bw/day] during outdoor mixing/loading, application and cleaning for AquaPy *via* fogging with vehicle mounted fogging equipment/no PPE and with PPE

Systemic exposure in mg/kg bw/day resulting from	**Preparation and application of the in-use dilution *via* vehicle mounted fogging** no PPE	 with PPE*
- Mixing and loading	0.016	0.00181
- Application	0.081	0.050
- Cleaning application equipment	0.013	0.0055
Total systemic exposure [mg/kg bw/day]	0.11	0.05731
% of the $AEL_{medium-term}$	**55**	**29**

*90% protection is assumed for gloves & 80% for coated coverall [Biocides Human Health Exposure Methodology, October (2015)]

Conclusively, operator exposure levels during outdoor cold (ULV) fogging of AquaPy either with hand held or vehicle mounted equipment, are below the systemic medium-term AEL value of 0.2 mg/kg b.w./day even without the use of PPE. However, taking into account the toxicological properties of the formulation i.e. classified as Carc. 2 – H351, it is proposed to include in product label the precautionary statement P280 (Wear protective gloves/protective clothing/eye protection/face protection) in line with Reg. (EC) 1272/2008.

Application of AquaPy - Indoor space treatment as fog by professional operators

Indoor application of AquaPy is conducted by professional operators either *via* cold (ULV) or *via* thermal fogging using hand-held equipment and is intended for use 1-2 times per year with minimum interval between applications of 1 month.The assessment performed by the applicant was based on experimental data for professional indoor fogging application in a warehouse. However, it was agreed during the trilateral discussions between the WG members that it would be more preferable, as a Tier I approach, to use the following models:
"Misting at waist level using CDA (ULV) mist blower, TNsG: misting model 2" [Technical Notes for Guidance (2002), p 185] and "Thermal fogging, TNsG: Fogging & misting model 3" [Technical Notes for Guidance (2002), p 186].

With regard to exposure during mixing and loading and in case of indoor application *via* hand-held fogger, the generic exposure values for pouring formulation into a portable vessel (EUROPOEM II database) have been used [Biocides Human Health Exposure Methodology, October (2015), p. 191].

Operator exposure during cleaning of the application equipment has been assessed using the surrogate values from BEAT model database.

Calculations have been performed considering a space of 13372m^3. This area was one of the Units treated in the experimental studies submitted by the applicant and was considered as a worst case (Doc IIIB, appendix 1, p.54). The task duration used was 120 min for fogging according to the Biocides Human Health Exposure Methodology (ECHA, October 2015).

Inhalation absorption has been considered to be 100% and dermal absorption 2.4% for the concentrate and 4.8% for the in-use dilution. The exposure estimates [in mg/person/day] have been converted to systemic exposures [in mg/kg bw/day] considering a body weight of 60 kg. The medium-term AEL is considered to be 0.2 mg/kg b.w./day.

The following tables summarises the exposure estimates for mixing/loading, application and cleaning of AquaPy *via* fogging either *via* cold (ULV) or *via* thermal fogging equipment.

Table 2.2.1.3-3:Estimated primary systemic operator exposure to piperonyl butoxide [mg/kg bw/day] during mixing/loading and application of AquaPy *via* cold (ULV) fogging with hand held equipment/no PPE and with PPE

Systemic exposure in mg/kg bw/day resulting from	**Preparation and application of the in-use dilution *via* cold (ULV)fogging** no PPE	 with PPE*
- Mixing and loading	0.01232	0.00125
- Application	0.081	0.050
- Cleaning application equipment	0.013	0.0055
Total systemic exposure [mg/kg bw/day]	0.10632	0.0567
% of the $AEL_{medium-term}$	**53**	**28**

*90% protection is assumed for gloves & 80% for coated coverall [Biocides Human Health Exposure Methodology, October (2015)]

Table 2.2.1.3-4:Estimated primary systemic operator exposure to piperonyl butoxide [mg/kg bw/day] during mixing/loading and application of AquaPy *via* thermal fogging with hand-held fogging equipment/no PPE and with PPE

Systemic exposure in mg/kg bw/day resulting from	**Preparation and application of the in-use dilution *via* thermal fogging** no PPE	 with PPE*
- Mixing and loading	0.01232	0.00125
- Application	0.156	0.0008
- Cleaning application equipment	0.013	0.0055
Total systemic exposure [mg/kg bw/day]	0.1813	0.00755
% of the $AEL_{medium-term}$	**91**	**3.8**

*90% protection is assumed for gloves & 80% for coated coverall [Biocides Human Health Exposure Methodology, October (2015)]

Conclusively, operator exposure levels during indoor application of AquaPy either *via* cold (ULV) or *via* thermal fogging equipment, are below the systemic medium-term AEL value of 0.2 mg/kg b.w./day even without the use of PPE.
However, taking into account the toxicological properties of the formulation i.e. classified as Carc. 2 – H351, it is proposed to include in product label the precautionary statement P280 (Wear protective gloves/protective clothing/eye protection/face protection) in line with Reg. (EC) 1272/2008.

Non-professional users

AquaPy is intended for professional operators only.

Indirect exposure as a result of use

Outdoor space treatment as fog by professional operators
Considering the outdoor use of the product a person might accidentally re-enters an area before the fog has disappeared. For this exposure scenario inhalation exposure can be regarded as the most relevant exposure route. As a worst case tier one approach one might assume that shortly after application the fog covers a height of 1 m. Based on this approach an airborne residue concentration of 2.7 mg a.s./m^3 is calculated when considering the maximum application rate of 27 g a.s./ha. The exposure duration will be assumed to be 15 minutes as proposed in the EFSA Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (2014). Regarding the breathing rate a value of 1.25 m^3/hour will be assumed for the adult (60 kg), i.e. the same breathing rate as considered for the operator. For the child (toddler) a breathing rate of 1.26 m^3/hour and a bodyweight of 10 kg are assumed as proposed in the [Biocides Human Health Exposure Methodology, October (2015)].
The short-term AEL is considered to be 1 mg/kg b.w./day.

The corresponding estimations are presented in the following table.

Table 2.2.1.3-5: Estimated systemic secondary inhalation exposure when re-entering an outdoor area where AquaPy has been applied.

	Adult	Child (toddler)
Inhalation exposure (I):		
$S_{inhalation}$ (mg a.s./kg bw/day):	0.01406	0.08505
Proportion of short-term AEL (%)	**1.4**	**8.5**

Conclusively, incidental bystander (adult & child) inhalation exposure levels to piperonyl butoxide are below the systemic short-term AEL value of 1 mg/kg b.w./day.

Indoor space treatment as fog by professional operators

No bystanders are allowed entering premises during treatment and for at least 120 min after treatment. Ventilation of the premise should also be performed.

It was demonstrated that 90% of the applied dose was no longer airborne 40 minutes after application, and none was airborne after 120 minutes [redacted] (1996b), B.6.6/03 Appendix 1]. Therefore secondary exposure by the inhalative route is considered negligible.

Considering the indoor application rate of 4.5 mg/m^3 and a room height of 3 m the total application rate amounts to 13.5 mg a.s./m^2.

In a theoretical worst case tier one approach a 100% deposition of the fog is assumed, resulting in a floor surface loading of 13.5 mg a.s./m^2 corresponding to 0.00135 mg a.s./cm^2. It is reasonable to conclude that a re-entering infant represents the worst case. The short-term AEL is considered to be 1 mg/kg b.w./day.

Table 2.2.1.3-6: Estimated systemic secondary exposure when re-entering a room where AquaPy has been applied.

Infant	
Dermal exposure (D):	
S_{dermal} (mg a.s./kg bw/day:)	0.01458
S_{oral} (mg a.s./kg bw/day):	0.030375
S_{total} (mg a.s./kg bw/day):	0.0450
Proportion of short-term AEL (%)	4.5

Conclusively, exposure levels to piperonyl butoxide are below the systemic short-term AEL value of 1 mg/kg b.w./day.

Combined exposure

Since AquaPy is intended for professional operators and secondary exposure will be for non-professional there will be no relevant levels of combined exposure.

2.2.1.4. Risk characterisation

Professional users

Indoor and outdoor space treatment as fog by professional users of AquaPy leads to an acceptable risk even without the use of PPE. The calculated exposure levels for indoor application either *via* cold (ULV) or *via* thermal fogging equipment correspond to 53% and 91% of the medium-term AEL, respectively while, foroutdoor cold (ULV) application either with hand held or vehicle mounted fogging equipment exposure levels correspond to 61% and 55% of the medium-term AEL, respectively. However, taking into account the toxicological properties of the formulation i.e. classified as Carc. 2 – H351, it is proposed to include in product label the precautionary statement P280 (Wear protective gloves/protective clothing/eye protection/face protection) in line with Reg. (EC) 1272/2008. .

AquaPy is intended for professional operators only.

Indirect (secondary) exposure

The risk is considered to be acceptable for bystanders exposed to AquaPy during outdoor and indoor application as agreed at the BPC meeting.
No risk is anticipated for an infant that has re-entered to a treated room. The calculated exposure levels correspond to 4.5% of the short-term AEL.

A dietary risk assessment has not been performed for the representative product as exposure of food, feed and animal feeding stuffs has been excluded.

2.2.2. Environmental Risk Assessment

2.2.2.1. Fate and distribution in the environment

Hydrolysis: Piperonyl Butoxide is hydrolytically stable in solution in the dark at 25 °C at pH 5, 7 and 9 and its half-life under these conditions is greater than 500 days.

Photolysis: Photolysis of Piperonyl Butoxide in water was investigated in a study (Selim, 1995, report no. P0594010, Doc IIIA 7.1.1.1.2) and Piperonyl Butoxide was found to rapidly photolize in aqueous solution with a half-life of 8.4 hours.

Phototransformation in air: The chemical lifetime of Piperonyl Butoxide in the troposphere was calculated using the computer program Atmospheric Oxidation program V 1. 92. Based on the molecular structure of Piperonyl Butoxide, a half-life of 3.597 hrs has been estimated considering a 24 hr-day (based on an overall OH rate constant of 107.0380 x 10^{-12} cm^3/molecule sec and 0.5x10^6 OH radicals/cm^3).

Ready biodegradability: Piperonyl Butoxide was investigated for its ready biodegradability in a CO_2 evolution test based on OECD 301 B. Under the test conditions Piperonyl Butoxide is considered as not biodegradable within 28 days. Accordingly, PBO is classified as not readily biodegradable.

Aerobic soil degradation in soil: Two studies (GLP) have been considered as valid regarding the degradation of Piperonyl Butoxide in soil.

In Mayo, B.C., 1995, (report no. PBT 7/951484), the aerobic degradation of Piperonyl Butoxide has been tested in a sandy loam soil under dark conditions at 25^{0}C. Two major metabolites have been identified during the duration of the test. M8 was found at 9% of AR after 30 days and metabolite M12 was found at maximum of 16.6% at the same day. Moreover, three minor metabolites (M4, M11 and M16) have been observed at levels below 5% of AR.

Non-extractable ^{14}C-residues increased steadily and accounted for 37 % of the applied radioactivity after 128 days. After that, their rate decreased to 20 % at day 285.

Origin	Saunders County, Nebraska, USA
Soil type	sandy loam
Incubation temperature	25 ± 1°C
DT_{50} (days) [a] at 25^{0}C	14
DT_{50} (days) [a] at 12^{0}C	39.6

DT_{90} (days) [a]	50
Correlation coefficient (r)	0.9996

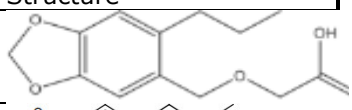
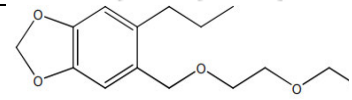
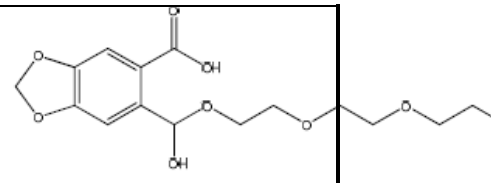
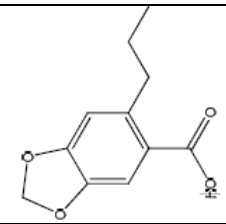
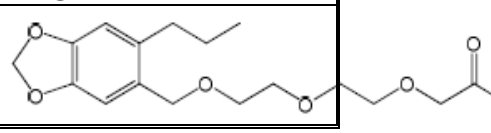
The second study (Derz, K., 2006 (report no. GAB-011/7-90)) was performed according to OECD-Guideline 307 under GLP. The aerobic degradation behaviour of Piperonyl Butoxide has been tested in three soil types (loamy sand, silt loam and sandy loam). Sampling was performed after the following incubation times: 0 d (immediately after application), 1 d, 3 d, 7 d, 14 d, 28 d, 50 d, 70 d, 97 d and 120 d after application. The calculated DT_{50} values in the three soils are presented in the following table.

Origin	IME 01-A, Hagen	IME 02-A, Soest	LUFA 3A, Not stated
Soil type	Loamy sand	Silt loam	Sandy loam
Incubation temperature	20 ± 2 °C		
DT_{50} (days) [a]	64	29	23
DT_{50} (days) at 12^0C	121.4	55	43.6
DT_{90} (days) [a]	212	97	76

Four major metabolites have been observed in the three soils. Metabolite **M12** (EN 1-93/3) amounted up to 16.1 % (soil IME 01-A) and 19.4 % of the applied radioactivity (soil IME 02-A). In LUFA soil detected in max amount of 7.5%. Metabolite **M2** was found up to 14.4% after 70 days in LUFA 3A, metabolite **EN 1-101/4** was detected in maximum amount of 6.6% of AR in LUFA 3A, metabolite **M1** amounted up to 5.9% in LUFA 3A. **M8** was found at 9% of AR after 30 days.

In general, a normalised geometric mean value of 58.3 days should be considered for risk assessment purposes. Furthermore, all the metabolites are summarised in the following table.

Table 2.2.2.1-1: Major soil metabolites of Piperonyl Butoxide

Code/Name	IUPAC name	Max.% AR/Reference	Structure
M1	[(6-propyl-1,3-benzodioxol-5-yl)methoxy]acetic acid	5.9/Derz (2006)	
M2	{2-[(6-propyl-1,3-benzodioxol-5-yl)methoxy]ethoxy}acetic acid	14.4/Derz (2006)	
M8	6-{[2-(2-butoxyethoxy)ethoxy](hydroxy)methyl}-1,3-benzodioxole-5-carboxylic acid	9/Mayo (1995)	
M12 (EN 1-93/3)	6-propylbenzo[d][1,3]dioxole-5- carboxylic acid	19.4/Derz (2006) 16.6/Mayo (1995)	
EN 1-101/4 (Metabolite F)	(2-{2-[(6-propyl-1,3-benzodioxol-5-yl)methoxy]ethoxy}ethoxy)acetic acid	6.6/Derz (2006)	

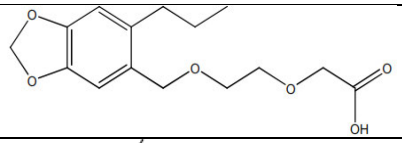
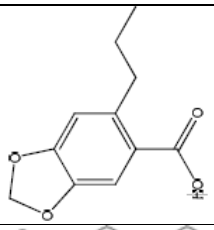
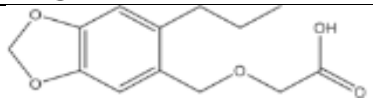
Anaerobic soil degradation: The degradation of Piperonyl Butoxide has been investigated under anaerobic conditions and a DT_{50} of 144 days at test temperature has been calculated. Metabolite F (EN 1-101 was identified as the major metabolite reaching a maximum concentration of about 36% on day 90.

Adsorption desorption Study: The Koc values in four soils varied between 788 and 9397. The arithmetic mean of 3745.3 L/kg (n=4) is proposed to be used for risk assessment purposes.

Aerobic aquatic degradation: Piperonyl Butoxide's degradation investigated in two water/sediment systems. Piperonyl Butoxide was degraded from the entire system with DT_{50} values calculated to be 102.4 and 104.3 days (should be used for risk assessment purposes) in creek and pond respectively at 12^0C.

One major metabolite (M2) was detected in a maximum value of 40.7% of AR after 100 days in creek water/sediment system (21.4% in pond system). M12 was found at max 6.6% of AR in in the total pond system and metabolite M1 reached up to 7.6% of total AR in pond system.

Table 2.2.2.1-1: Major soil metabolites of Piperonyl Butoxide

Code/Name	IUPAC name	Max.% AR/w/s system	Structure
M2	{2-[(6-propyl-1,3-benzodioxol-5-yl)methoxy]ethoxy}acetic acid	40.7/Creek	
M12 (EN 1-93/3)	6-propylbenzo[d][1,3]dioxole-5- carboxylic acid	6.6/Pond	
M1	[(6-propyl-1,3-benzodioxol-5-yl)methoxy]acetic acid	7.6/Pond	

Bioaccumulation: The log octanol:water partition coefficients of Piperonyl Butoxide (4.8; measured) and its metabolite M-12 (3.12; predicted *via* QSAR analysis) are above the trigger of 3 suggesting that the two substances may have significant potential for bioconcentration in both aquatic and terrestrial biota, with the possibility of bioaccumulation leading to secondary poisoning. The aquatic bioaccumulation potential of Piperonyl Butoxide was experimentally investigated using the bluegill sunfish *Lepomis macrochirus* ([redacted] 1992; A7.4.3.3). The kinetic (mean) BCF values in edible, non-edible and whole fish were calculated to be 99, 450 and 290 L/kg. The bioaccumulation potential of Piperonyl Butoxide in terrestrial organisms was predicted by using the relationship of Jager (1998) since no experimentally derived earthworm bioconcentration data were available. The earthworm bioconcentration factor ($BCF_{earthworm}$) was estimated to be 757 mg/kg. As regards metabolite M-12, the bioaccumulation potential in both aquatic (fish) and terrestrial (earthworms) organisms was predicted by using the equations developed by Veith et al. (1979) and Jager (1998), respectively. The fish and earthworm BCF values were estimated to be 89.5 L/kg and 15.8 mg/kg, respectively.

2.2.2.2. Effects assessment

The ecotoxicological properties of the active substance Piperonyl Butoxide were investigated in toxicity studies performed with representative species of non-target organisms inhabiting the terrestrial and aquatic compartments. Where necessary, the environmental effects assessment for the parent compound was based on predicted toxicity data resulting from standardized EU agreed equations (i.e. Equilibrium Partitioning Method).

The ecotoxicological properties of Piperonyl Butoxide major aquatic/sediment (M-1, M-2, M-12 (or EN 1-93/3)) and soil (M-1, M-2, M-8, M-12 (or EN 1-93/3), EN 1-101/4 (or Metabolite F)) metabolites were assessed on the basis of appropriate QSAR analyses. Where necessary, worst-case assumptions based on the available ecotoxicity data for the parent compound were employed.

General Comment: The ENV WG-II-2016 *ad hoc* follow up agreed that the Piperonyl Butoxide batches used in the ecotoxicological studies are representative of the proposed technical specification.

Effects assessment for the aquatic compartment

Effects on aquatic organisms

The toxicity of Piperonyl Butoxide to aquatic organisms was investigated through a number of acute and chronic toxicity tests with fish and aquatic invertebrates as well as toxicity tests on inhibitory effects on algae growth and aquatic microbial activity. The toxicity of major metabolites to aquatic organisms was investigated mainly via QSAR analysis.

The available acute toxicity data demonstrated that aquatic invertebrates were the most sensitive of the aquatic organisms tested towards Piperonyl Butoxide. In fact the acute effect endpoints calculated for the cladoceran freshwater flea *Daphnia magna* (48-hour EC_{50} 0.51 mg a.s./L), the shrimp-like marine crustacean *Americamycis bahia* (96-hour LC_{50} 0.32 mg a.s./L) and the eastern oyster *Crassostrea virginica* (96-hour EC_{50} 0.23 mg a.s./L) were approximately one order of magnitude lower than the respective endpoints calculated for fish (e.g. 96-hour LC_{50} of 3.94 mg a.s./L for *Cyprinodon variegatus*, 5.37 mg a.s./L for *Lepomis macrochirus* and 6.12 mg a.s./L for *Oncorhynchus mykiss*) and algae (e.g. E_rC_{50} of 3.89 and E_bC_{50} of 2.09 mg a.s./L for *Selenastrum capricornutum*). Based on the available acute toxicity data, Piperonyl Butoxide is characterized as toxic to fish and algae while as very toxic to aquatic invertebrates.

The acute toxicity to aquatic organisms was also investigated with Piperonyl Butoxide formulated as AquaPy. The acute effect endpoint (48-hour EC_{50}) of AquaPy to aquatic invertebrates represented by *Daphnia magna* was calculated to be 0.216 mg/L. The toxicity (72-hour EC_{50}) to the green algae *Pseudokirchneriella subcapitata* was determined to be 6.58 mg product/L based on inhibitory effects on growth rate and 3.1 mg product/L based on effects on biomass. No reliable fish acute effect endpoint for the biocidal product AquaPy could be determined.

The available chronic aquatic toxicity data on Piperonyl Butoxide demonstrated that aquatic and sediment-dwelling invertebrates were the most sensitive aquatic organisms tested under long-term water-borne exposure conditions. The chronic effect endpoints (NOEC) calculated were 0.18 mg a.s./L for fish (*Pimephales promelas*), 0.030 mg a.s./L for *Daphnia magna*, 0.824 mg a.s./L for the green algae *Selenastrum capricornutum* and 0.0148 mg a.s./L for *Chironomus riparius*.

Regarding major aquatic metabolites of Piperonyl Butoxide, limited testing toxicity data were available to assess their toxicity to aquatic organisms. In fact, there was only one study investigating the acute toxicity of metabolite M-12 (or EN 1-93/3) to the freshwater amphipod *Hyalella azteca*. The respective effect endpoint (96-hour LC_{50}) was calculated to be 31 mg/L. Due to the limited testing toxicity data set, the toxicity of Piperonyl Butoxide metabolites to

aquatic organisms was predicted via QSAR analysis (EpiSuite ECOSAR 1.11 calculation program). The respective QSAR results indicated that the most sensitive taxonomic group to Piperonyl Butoxide metabolites are aquatic invertebrates (Daphnia) and that metabolites are expected to be less toxic than the parent compound (table 2.2.2.2-1).

Table 2.2.2.2-1: QSAR modelling results for Piperonyl Butoxide metabolites

Test substance	Acute toxicity to fish – 96-h LC_{50} (mg/L)	Acute toxicity to Daphnia – 48-h EC_{50} (mg/L)	Toxicity to green algae – 96-h EC_{50} (mg/L)
PBO – testing toxicity data	3.94	0.51	2.09 (72-h E_bC_{50}) 3.89 (72-h E_rC_{50})
PBO – predicted by EpiSuite ECOSAR 1.11	2.4	0.38	3.1
M-1 – predicted by EpiSuite ECOSAR 1.11	57	2.8	32
M-2 – predicted by EpiSuite ECOSAR 1.11	118	3.3	59
M-8 – predicted by EpiSuite ECOSAR 1.11	3789	3.9	891
M-12 (or EN 1-93/3) – predicted by EpiSuite ECOSAR 1.11	16	2.3	12
EN 1-101/4 (or Metabolite F) – predicted by EpiSuite ECOSAR 1.11	241	3.8	105

The $PNEC_{aquatic}$ for Piperonyl Butoxide, i.e. 0.00148 mg a.s./L, was derived by applying an assessment factor of 10 to the lowest available NOEC of 0.0148 mg a.s./L for *Chironomous riparius*. It is noted that the PNECaquatic for Piperonyl Butoxide was agreed at the ENV wg-ii-2016. The $PNEC_{aquat\ c}$ for the aquatic metabolites M-1, M-2 and M-12 (or EN 1-93/3), i.e. 0.0028, 0.0033 and 0.0023 mg/L respectively, was derived by applying an assessment factor of 1000 to the lowest acute toxicity endpoint, i.e. Daphnia, estimated via QSAR analysis.

Effects on sediment-dwelling organisms

The toxicity of Piperonyl Butoxide to sediment-dwelling organisms was investigated in three chronic toxicity studies conducted with the freshwater endobenthic insects *Chironomus riparius* and *Chironomus dilutus* and the freshwater epibenthic amphipod *Hyalella azteca* and one acute toxicity study conducted with the estuarine endobenthic amphipod *Leptocheirus plumulosus*.

In the *Chironomus dilutus*, *Hyalella azteca* and *Leptocheirus plumulosus* tests Piperonyl Butoxide was spiked to the sediment. Although in the *Chironomus riparius* test Piperonyl Butoxide was spiked to the overlying water, a sediment-based NOEC was calculated as the sediment concentration of the test substance was monitored throughout the test. It is noted that the sediment-based NOEC endpoint for *Chironomus riparius* was agreed at the ENV WG-II-2016. The acute toxicity (10-day LC_{50}) of Piperonyl Butoxide to *Leptocheirus plumulosus* was determined to be > 86 mg/kg dwt. The chronic toxicity of Piperonyl Butoxide to *Chironomus riparius* (28-day NOEC), *Chironomus dilutus* (63-day NOEC) and *Hyalella azteca* (42-day NOEC) was determined to be 0.093, 0.44 and 39 mg/kg dwt, respectively.

During the ENV WG-II-2016 concerns were raised whether the lowest toxicity observed for *Chironomus riparius* is attributed to the test system design, i.e. a water spiked test system was used compared to the sediment spiked system used in the two other chronic toxicity tests. Taking into account:

(i) the insecticidal mode of action of Piperonyl Butoxide and the expected greater sensitivity of aquatic insects (i.e. chironomids) compared to other aquatic or sediment-dwelling organisms and

(ii) the fact that the NOEC endpoints for the two Chironomus species were considered not to be significantly different as their sensitivity difference is within a factor of less than 10

it was concluded at the ENV WG-II-2016 *ad hoc* follow up discussion that the derivation of the $PNEC_{sed}$ for Piperonyl Butoxide should be based on the lowest NOEC of 0.093 mg/kg dwt for *Chironomus riparius* by applying an assessment factor of 50 (as two long-term tests with species representing different living and feeding conditions, i.e. *Chironomus ripariusn* and *Hyalella Azteca*, were available). The $PNEC_{sed}$ for Piperonyl Butoxide was thus set at 0.0004 mg a.s./kg wwt.

No sediment toxicity data on Piperonyl Butoxide metabolites were available. At the ENV WG-II-2016 it was agreed that the aquatic risk assessment for metabolites should be considered sufficient to address any concerns related to the risk potentially posed to the sediment compartment.

Effects on STP microorganisms

Piperonyl Butoxide had no significant inhibitory effects on the respiration rate of activated sludge (representing combined carbonaceous and nitrogenous oxidation processes) up to and including the highest test concentration of 1000 mg a.s./L. Taking into that this concentration exceeded the water solubility of Piperonyl Butoxide (28.9 mg/L at 20.4^0C and pH 7.01), the NOEC for STP microorganisms was set equal to the water solubility value of 28.9 mg/L. Accordingly, the PNEC for STP microorganisms, i.e. 2.89 mg a.s./L, was derived by applying an assessment factor of 10 to the NOEC of 28.9 mg/L.

Effects on terrestrial organisms

Effects on soil organisms

The effects of Piperonyl Butoxide on soil organisms were investigated with a number of toxicity tests conducted with the three basic trophic levels of the soil compartment, e.g. plants (primary producers), earthworms (soil invertebrates) and soil microorganisms.

Piperonyl Butoxide had no adverse effects on soil microbial activity concerning nitrogen transformation and carbon mineralization up to and including the concentration of 28.8 mg a.s./kg standard soil dwt. Regarding soil invertebrates represented by the earthworm *Eisenia fetida*, the acute toxicity (14-day LC_{50}) of Piperonyl Butoxide was determined to be 143.8 mg a.s./kg standard soil dwt while its chronic toxicity (56-day NOEC) was determined to be 10.2 mg a.s./kg standard soil dwt. Regarding higher terrestrial plants, no adverse effects greater than 50% on biomass production were detected following application of Piperonyl Butoxide at rates up to 3250 g a.s./ha.

The effects on soil organisms were also investigated with Piperonyl Butoxide formulated as AquaPy. AquaPy had no adverse effects on soil microbial activity concerning nitrogen transformation and carbon mineralization up to and including the concentration of 26.64 mg/kg soil dwt. The acute toxicity (14-day LC_{50}) of AquaPy to soil invertebrates represented by the earthworm *Eisenia fetida* was determined to be greater than 1000 mg/kg soil dwt, while the short-term toxicity (21-day ER_{50}) to higher terrestrial plants was determined to be 6985 g/ha.

The $PNEC_{soil}$ for Piperonyl Butoxide was derived on the basis of both the available testing toxicity

data and the Equilibrium Partitioning Method. The EPM approach was followed as the available testing toxicity data were considered insufficient to address concerns related to the potentially higher or specific toxicity of Piperonyl Butoxide to soil-dwelling non-target arthropods. The experimental $PNEC_{soil}$, i.e. 0.181 mg a.s./kg wwt, was derived by applying an assessment factor of 50 to the lowest of the available long-term toxicity values, i.e. 56-day NOEC of 10.2 mg a.s./kg dwt for *Eisenia fetida*. As the calculated $PNEC_{soil}$ based on the Equilibrium Partitioning Method, i.e. 0.0980 mg/kg wwt, was lower than the calculated $PNEC_{soil}$ based on the testing toxicity, the former $PNEC_{soil}$ value was in the soil risk assessment.

No soil toxicity data on Piperonyl Butoxide metabolites are available. At the ENV WG-II-2016 it was agreed that the soil risk assessment for metabolites should be based on the assumption that they are as toxic to soil-dwelling organisms as the parent compound. This approach was considered sufficient to address any concerns related to the risk potentially posed to the soil compartment from Piperonyl Butoxide metabolites taking into account that (i) the $PNEC_{soil}$ for Piperonyl Butoxide was calculated by following a conservative approach (i.e. Equilibrium Partitioning Method) and (ii) the available QSAR data (table 2.2.2.2-1) indicated that Piperonyl Butoxide metabolites will be no more toxic to aquatic organisms than the parent compound; a similar toxicity pattern can reasonably be assumed for soil organisms.

Effects on other terrestrial non-target organisms

In addition to soil organisms, testing toxicity data were available for other groups of terrestrial organisms, e.g. birds, honeybees and other non-target arthropods.

The calculated acute oral and contact LD_{50}s of Piperonyl Butoxide to the honeybee *Apis mellifera* were 611.6 µg a.s./bee and 294 µg a.s./bee, respectively. Regarding the representative product AquaPy, the calculated acute oral and contact LD_{50}s to the honeybee *Apis mellifera* were 7.892 µg/bee and 2.767 µg/bee, respectively.

The effects of technical Piperonyl Butoxide and Piperonyl Butoxide formulated as AquaPy to non-target arthropods other than bees were investigated with the predatory mite *Typhlodromus pyri* and the parasitoid *Aphidius rhopalosiphi*. The 7-day LR_{50} of Piperonyl Butoxide to *T. pyri* was determined to be 0.319 kg a.s./ha while the 48-hour LR_{50} of Piperonyl Butoxide to *A. rhopalosiphi* was determined to be greater than 4.8 kg a.s./ha. Regarding the representative product AquaPy, the 7-day LR_{50} for *T. pyri* and the 48-hour LR_{50} for *A. rhopalosiphi* were determined to be 31.8 and 7.82 g/ha, respectively.

The avian toxicity of Piperonyl Butoxide was investigated in two acute oral, one short-term dietary and two long-term/reproductive toxicity tests with the representative species *Anas platyrhynchos* (mallard duck) and *Colinus virginianus* (northern bobwhite quail). The findings and effect endpoints derived from these studies demonstrated that Piperonyl Butoxide is not toxic to birds under acute, short-term or long-term exposure conditions. In fact, the acute oral LD_{50} was determined to be greater than 2250 mg a.s./kg bw, the short-term dietary LD_{50} was determined to be greater than 5620 mg a.s./kg bw and the chronic NOEC was determined to be 300 mg a.s./kg diet (equivalent to 27 mg a.s./kg bw/d for northern bobwhite quail and 47 mg a.s./kg bw/d for mallard duck).

A summary of the estimated PNEC values for the parent compound Piperonyl Butoxide and its major metabolites is provided in table 2.2.2.2-2.

Table 2.2.2.2-2: Summmary of PNEC values for Piperonyl Butoxide and its major metabolites

Substance	PNEC values for environmental compartments under concern					
	Surface water [$PNEC_{aquatic}$	Sediment [$PNEC_{sediment}$ (mg/kg	STP microorganisms [$PNEC_{STP(micro-}$	Soil [$PNEC_{soil}$ (mg/kg	Birds [$PNEC_{oral,}$ birds	Mammals [$PNEC_{oral,}$ mammals

	(mg/L)]	wwt)]	organisms) (mg/L)]	soil wwt)]	(mg/kg diet)]	(mg/kg diet)]
Piperonyl Butoxide (parent)	0.00148	0.0004	2.89	0.0980	10	20
Metabolite M-1	0.0028	- [1]	Not relevant	0.0980 [2]	Not relevant	Not relevant
Metabolite M-2	0.0033	- [1]	Not relevant	0.0980 [2]	Not relevant	Not relevant
Metabolite M-8	Not relevant	Not relevant	Not relevant	0.0980 [2]	Not relevant	Not relevant
Metabolite M-12 (or EN 1-93/3 or PBO acid)	0.0023	- [1]	Not relevant	0.0980 [2]	10 [2]	20 [2]
Metabolite EN 1-101/4 (or Metabolite F)	Not relevant	Not relevant	Not relevant	0.0980 [2]	Not relevant	Not relevant

[1] No $PNEC_{sed}$ calculation for major metabolites has been conducted; the risk to sediment-dwelling organisms from Piperonyl Butoxide metabolites is considered to be covered by the risk assessment for aquatic organisms

[2] No relevant toxicity data are available; as a worst-case approach, Piperonyl Butoxide metabolites have been considered as toxic to the respective non-target organisms as the parent compound

2.2.2.3. PBT and POP assessment

PBT assessment

Persistence criteria (P)

Piperonyl Butoxide is considered as not readily biodegradable The PBT assessment presented below covers the active substance Piperonyl Butoxide, the major soil and aquatic/sediment metabolites M-1, M-2, M-8, M-12 (or EN 1-93/3), EN 1-101/4 (or Metabolite F) and the two relevant impurities Dipiperonyl methane (DPM) and Dipiperonyl ether (DPE). No other impurities of the technical specification have been considered as the assessment of their PBT properties raised no particular environmental concern (see Doc II A1-A2 Confidential Data and Information).

. The ready biodegradability of Piperonyl Butoxide was tested following OECD Guideline 301B. The results of the two replicates (replicate I: 24%, replicate II: 48%) showed that Piperonyl Butoxide cannot be considered as readily biodegradable under aerobic conditions. No inherent biodegradability test has been conducted.

Data presented in Derz, K., 2006 (Doc IIIA 7.1.2.2.2) in two water-sediment systems, show that Piperonyl Butoxide degrades relatively slowly in the aquatic environment with a worst-case DT_{50} value of 104.3 days for "pond" whole system (102.4 days for "creek" system) at 12°C. Furthermore, water phase dissipation DT_{50} values of 51.2 days (pond) and 313 days (river) at 12°C. No reliable DT50 values could be derived for sediment. Worst case dissipation DT50 value for both systems exceed criteria for very persistent compounds (>60 d in freshwater and/or >180 d in freshwater sediment), in addition to that values for freshwater (creek system) do trigger additional concern.

Table 2.2.2.3-1: Half-lives of Piperonyl Butoxide in the water and sediment systems "Pond" and "Creek" at 12°C (Derz, K., 2006, Doc IIIA 7.1.2.2.2)

	DT_{50} (days) Pond	DT_{50} (days) Creek
Water phase (dissipation)	51.2	313
Entire system (degradation)	104.3	102.4

In addition to that soil aerobic degradation of Piperonyl Butoxide has been tested on four soils with a geomean DT_{50} value of 58.3 days to be calculated at 12^0C.

Table 2.2.2.3-2: Soil DT_{50} values for Piperonyl Butoxide

	DT_{50} at study temperature (days)	DT_{50} at 12^0C (days)
Mayo (1995) DocIIIA 7.2.1/01	14 (25^0C)	39.6
Derz (2006) DocIIIA 7.2.1/02	64 (20^0C)	121.4
Derz (2006) DocIIIA 7.2.1/02	29 (20^0C)	55
Derz (2006) DocIIIA 7.2.1/02	23 (20^0C)	43.6
Geometric mean		58.3

Based upon the data from the water/sediment study, Piperonyl Butoxide should be classified as vP compound.

Regarding the metabolites that identified in soil and/or water/sediment systems:

Metabolite M-2

In the WG-II 2016, it was agreed that metabolite M2 should be addressed based on QSAR data. The half-lives have been estimated with the PBT Profiler and the results are presented in the following table.

Table 2.2.2.3-3: DT_{50} values for metabolite M-2 as predicted by PBT profiler

Half-life (days)			
Water	Soil	Sediment	Air
38	75	340	0.19

Taking into consideration the estimated rate of degradation of M2 in sediment, metabolite fulfils the vP criterion.

Metabolite M-12 (EN 1-93/3)

EN 1-93/3 appears in both the submitted aerobic soil degradation studies with max. occurrence of 19.4% and 16.6% in Derz, 2006 and Mayo, 1995 (with code M12). No DT_{50} values were feasible to be derived for EN 1-93/3 from Derz, 2006. However, a DT_{50} value of 41.4 days at test temperature (78.5 days at 12^0C) has been calculated from data derived from Mayo, 1995. A SFO kinetic fit and a direct formation from the parent has been assumed.

Furthermore, EN 1-93/3 has been identified in the submitted water/sediment study in the two systems with max. occurrence of 6.6% AR and 6.1% AR in "Pond " and "Creek" respectively.

Table 2.2.2.3-4: DT_{50} values for metabolite M-12 (EN 1-93/3)

	DT_{50} (days) 20^0C	DT_{50} (days) 12^0C
Pond (SFO)	21.2	40.2
Creek (SFO)	93.4	177

Based on the aforementioned dissipation half-life metabolite M-12 (EN 1-93/3) fulfils vP criterion.

Metabolite M-8

M-8 is present in the soil aerobic study (Mayo, 1995) at 9% of AR. A DT50 value of 41.3 days at 25^0C (116.8 days at 12^0C) assuming a SFO kinetic fit and a direct formation from the parent. Based on this value, M8 does not fulfil the P criterion.

Metabolite M-1 & EN 1-101/4

For metabolites M1 and metabolite F (EN 1-101/4) half-lives have been estimated using the PBT Profiler and the results are presented in the following table.

Table 2.2.2.3-5: DT_{50} values for metabolite M-1 as predicted by PBT profiler

Half-life (days)			
Water	Soil	Sediment	Air
15	30	140	0.22

Table 2.2.2.3-6: DT_{50} values for metabolite EN 1-101/4 (Metabolite F) as predicted by PBT profiler

Half-life (days)			
Water	Soil	Sediment	Air
38	75	340	0.16

Based on the estimated half-lives it can be assumed that M1 is a P compound and EN 1-101/4 is a vP compound.

Regarding the impurities Dipiperonyl methane (DPM) and Dipiperonyl ether (DPE), half-lives in water, soil, sediment and air have been estimated using the PBT Profiler and the results are presented in the following table.

Table 2.2.2.3-7: DT_{50} values for impurities DPM and DPE as predicted by PBT profiler

	Half-life (days)			
	Water	Soil	Sediment	Air
Dipiperonyl methane (DPM)	60	120	540	0.13
Dipiperonyl ether (DPE)	60	120	540	0.12

Dipiperonyl methane (DPM) and Dipiperonyl ether (DPE) should be considered as vP molecules based on the estimated half-lives.

Bioaccumulation criteria (B)

The fish bioconcentration factor (BCF_K) for Piperonyl Butoxide was experimentally determined to be 290 L/kg (whole fish; [redacted] 1992; A7.4.3.3), e.g. lower than the both trigger values of 2000 and 5000 L/kg. Thus, nor B neither vB criterion was found to be fulfilled for Piperonyl Butoxide.

Regarding Piperonyl Butoxide metabolites, no testing data on their bioaccumulation potential have been provided. However, considering that the log K_{OW} values estimated via QSAR analysis (Doc IIA, Section 4.1.3) for all metabolites (M-1, M-2, M-8, M-12 (or EN 1-93/3), EN 1-101/4 (or Metabolite F)) are below the trigger of 4.5 and no specific uptake mechanism apart from lipophilic partitioning is known or suspected, Piperonyl Butoxide metabolites were considered as not fulfilling the B or vB criterion.

Regarding the impurities Dipiperonyl methane (DPM) and Dipiperonyl ether (DPE), QSAR analysis and more specifically the US EPA PBT profiler has been used to estimate their bioaccumulation potential. Based on the estimated BCF values, i.e. 11000 L/kg for Dipiperonyl methane (DPM) and 12000 L/kg for Dipiperonyl ether (DPE), it was assumed that both impurities meet the vB criterion.

Toxicity criteria (T)

Based on the available mammalian toxicity data, Piperonyl Butoxide is classified as carcinogenic (category 2) and STOT SE 3 (H335: May cause respiratory irritation). Regarding long-term aquatic toxicity, the lowest available chronic NOEC for Piperonyl Butoxide, e.g. 0.0148 mg/L for *Chironomus riparius*, is greater than the trigger value of 0.01 mg/L. Since no classification of concern has been assigned based on the available mammalian toxicity data and the long-term aquatic toxicity is below the established threshold value, T-criterion was considered as not fulfilled for Piperonyl Butoxide.

Regarding Piperonyl Butoxide metabolites, no relevant testing long-term mammalian or aquatic toxicity data were available. Thus, screening information and more specifically their acute aquatic toxicity as predicted via QSAR analysis (EpiSuite ECOSAR 1.11) was considered. In addition, for metabolite M-12 (or EN 1-93/3), the experimentally derived acute toxicity endpoint (96-hour LC_{50} 31 mg/L; Shaw, 2014) for the freshwater amphipod *Hyalella azteca* was additionally taken into account. As none of the available acute toxicity endpoints was below the trigger value of 0.01 mg/L, it was concluded that Piperonyl Butoxide metabolites do not meet the T-criterion.

Regarding the impurities Dipiperonyl methane (DPM) and Dipiperonyl ether (DPE), QSAR analysis and more specifically the US EPA PBT profiler was used to estimate their long-term aquatic toxicity. Based on the estimated fish chronic endpoints, i.e. 0.00074 mg/L for Dipiperonyl methane (DPM) and 0.0018 mg/L for Dipiperonyl ether (DPE), it was assumed that both impurities meet the-T criterion.

Table 2.2.2.3-8: Overall conclusion on the PBT assessment

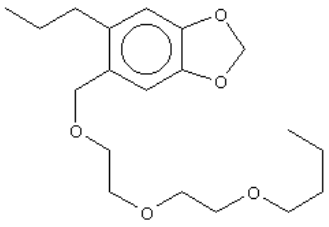
Substance	PBT properties		
	Persistence (P)	Bioaccumulation (B)	Toxicity (T)
Piperonyl Butoxide (parent)	vP	not B, not vB	not T
Metabolite M-1	P	not B, not vB	not T
Metabolite M-2	vP	not B, not vB	not T
Metabolite M-8	not P, not vP	not B, not vB	not T

Metabolite M-12 (or EN 1-93/3)	vP	not B, not vB	not T
Metabolite EN 1-101/4 (or Metabolite F)	vP	not B, not vB	not T
Impurity Dipiperonyl methane (DPM)	vP	vB	T
Impurity Dipiperonyl ether (DPE)	vP	vB	T

In line with the ECHA Guidance Document on IR&CSA, Part C: PBT/vPvB assessment (Version 2.0, November 2014), if a substance contains one or more constituents, impurities and/or additives with PBT/vPvB properties in individual amounts ≥ 0.1 % (w/w), the substance must be considered as PBT/vPvB and hence subjected to emission characterisation and risk characterisation in accordance with Article 14 (4) of REACH Regulation (EC) No 1907/2006. As the individual concentrations of DPM and DPE in the proposed technical specification are above the limit of 0.1 % w/w, i.e. 1.95 and 0.90 % w/w respectively, it was agreed at the ENV-II-2016 *Ad hoc* follow-up discussion that further data are required in order to enable a definite conclusion on the specific exclusion or substitution criteria of the active substance. It was further agreed that this conclusion should be reflected in the BPC Opinion. In these circumstances, the Commission will consider the possibility to approve the active substance setting a requirement in the approval regulation that the missing data information shall be submitted by a certain date.

POP assessment

Chemical Identity

Name	Piperonyl Butoxide
CAS No	51-03-6
Chemical name (IUPAC)	5-{[2-(2-butoxyethoxy)ethoxy]methyl}-6-propyl-1,3-benzodioxole
Structural formula	
Chemical class	

With regard to the proportion of non-active isomers or impurities, Piperonyl Butoxide is put on the market with 94% w/w minimum purity. Given this, Piperonyl butoxide does not fulfil criterion (f) of Art 10.

Persistency

Piperonyl Butoxide is concluded to be persistent and fulfils persistency criteria for POP assessment since the half-life of Piperonyl Butoxide in water exceeds the 60 days trigger (313 days at 12^0C in creek system) in one of the two water/sediment systems. Furthermore, in soil Piperonyl Butoxide is degraded with a geometric mean DT_{50} value of 58.3 days at 12^0C. Based

on the above mentioned DT50 values, Piperonyl Butoxide should be considered as persistent in water.

Bioaccumulation

Piperonyl Butoxide was concluded to be not bio-accumulative. The bioconcentration factor (BCF_K) for Piperonyl Butoxide in fish (*Lepomis macrochirus*) was experimentally determined to be 290 L/kg, e.g. lower than the trigger value of 5000 L/kg.

Potential for long-range environmental transport

Long-range environmental transport is not expected. Based on overall OH rate constant of 0.5E6 OH radicals/cm^3 a half-life of 3.597 hrs using a 24-hour days. Based on the estimated half-life in air there is no potential for long range transport.

Adverse effects

No classification of concern has been assigned to PBO based on the available mammalian toxicity data, i.e. carcinogen (cat. 1A or 1B), germ cell mutagenic (cat. 1 or 1B), toxic to reproduction (cat. 1A, 1B or 2), STOT RE 1 or STOT RE 2. Therefore, the interim criteria for the determination of endocrine-disrupting properties are not fulfilled.

Although PBO has been classified as *Very toxic to aquatic life with long-lasting effects* (H410), the lowest chronic NOEC for aquatic organisms was determined to be 0.0148 mg a.s./L (*Chironomus riparius*), i.e. greater than the trigger value of 0.01 mg/L (T-criterion).

Conclusion: There is no evidence indicating that Piperonyl Butoxide has the POPs-like characteristics (outlined in Annex D 'Information Requirements & Screening Requirements' of the Convention Stockholm Convention on Persistent Organic Pollutants 2001) such that global control is necessary.

2.2.2.4. Exposure assessment

The environmental exposure has been assessed using all the valid submitted studies and the Organisation for Economic Co-operation and Development (OECD) Task Force documents; Emission Scenario Document (ESD) for 'Insecticides, acaricides and products to control arthropods (PT 18) for household and professional use' (July 17, 2008), Part II of the Technical Guidance Document on Risk Assessment (TGD; EC, 2003), guidance from MOTA (Manual of Technical Agreements, Vs. 4, 2010) and TAB (September 2015) were also included to derive the PEC values.

Aquapy is intended for professional outdoor and indoor use an insecticide to control flying insects (Mosquitoes and houseflies) for indoor use, in domestic premises, public buildings and flying insects (Mosquitoes), for outdoor use (amenity areas and woodlands).

Professional pest controls operators apply Aquapy by fogging (cold and thermal).

Releases into the environment can take place from processes at any stage of the life-cycle of a substance. However, the local scale environmental emissions associated with the indoor use for Aquapy, as a professional fogging product, are considered for PECs calculations. Regarding outdoor use of the product, PECs have been calculated for the use of Aquapy on woodlands and amenity areas. The direct routes of potential environmental exposure following the use of the biocidal product AquaPy according to the intended uses are summarised in the following table:

Table 2.2.2.4-1: Relevant Environmental compartments for each application method

	Environmental Compartments

	Air	STP	Soil	Surface water/sediment	Groundwater
Indoor applications	**(+)**	**++**	**+**	**+**	**+**
Outdoor applications	**(+)**	**(+)**	**++**	**++**	**+**

++ Primarly exposed, + Secondarily exposed, (+) Potentially exposed

For a detailed presentation of the results and the used scenarios please refer to the corresponding IIB documents.

2.2.2.5. Risk characterisation

The environmental risk characterisation for the active substance Piperonyl Butoxide has been based on the proposed use pattern of the biocidal product AquaPy, an EW (emulsion, oil in water) formulation containing 30 g/L pyrethrins and 135 g/L Piperonyl Butoxide.

Using the Predicted No Effect Concentrations (PNEC) estimated in Document IIA (Section 4.3) and the Predicted Environmental Concentrations estimated in Document IIB (Section 3.3), PEC/PNEC ratios were calculated in order to assess the environmental risk associated with the intended use of the active substance Piperonyl Butoxide. Separate PEC/PNEC ratios were calculated for each intended use (i.e. indoor use, single outdoor use and multiple outdoor use) of formulated Piperonyl Butoxide as AquaPy. PEC/PNEC ratios less than 1 indicate no unacceptable risk, while PEC/PNEC ratios greater than 1 indicate an unacceptable risk to the environmental compartment under concern.

The risk characterization for the aquatic compartment (including surface water organisms, sediment-dwelling organisms and STP microorganisms) and the terrestrial compartment (including soil organisms and top predators exposed via the food chain) has been conducted on the basis of the respective PEC/PNEC calculations. The risk to the groundwater has been assessed via comparison of the calculated $PEC_{goundwater}$ values with the threshold concentration of 0.1 µg/L stipulated under Drinking Water Directive. The risk for other beneficial arthropods was qualitatively assessed by comparing the respective effect concentrations with the intended outdoor application rate of the representative product AquaPy. No risk assessment for bees following outdoor use of AquaPy has been conducted as, at the moment, no specific guidance is available for biocidal products on how to perform the bee risk assessment. The risk to bees following indoor use of formulated Piperonyl Butoxide was concluded to be negligible as the active substance is not expected to be systemic in plants.

As the current dossier is intended for the inclusion of Piperonyl Butoxide into Annex I of the Biocidal Product Regulation (BPR, Regulation (EU) 528/2012), the environmental effect, exposure and risk assessments are focused on Piperonyl Butoxide alone. At Piperonyl Butoxide approval stage, no environmental risk assessment based on the ecotoxicity endpoints for the representative product AquaPy is needed and therefore it has not been conducted. However, at product authorisation level synergism should be addressed when the product under consideration contains other insecticides besides Piperonyl Butoxide.

The risk characterization (PE/PNEC) ratios calculated for the environmental compartments under concern considering the intended indoor and outdoor uses of formulated Piperonyl Butoxide as AquaPy are presented in the following tables (tables 2.2.2.5-1 – 2.2.2.5-6).

Table 2.2.2.5-1: Summary of PEC/PNEC ratios for Piperonyl Butoxide (parent)

Scenario/Compartment	PEC/PNEC		
	Indoor use	Outdoor use / Single application	Outdoor use / Multiple applications
STP microorganisms	0.017	not relevant	not relevant
Surface water – aquatic organisms	3.3	0.96	2.8 (no mitigation measure) 0.06 (30 m unsprayed buffer zone)**
Sediment-dwelling organisms	998	16 (no mitigation measure) 0.56 (30 m unsprayed buffer zone)*	45 (no mitigation measure) 0.94 (30 m unsprayed buffer zone)**
Soil organisms	0.79	0.37	1.1
Fish-eating birds	0.14	0.04	0.12
Fish-eating mammals	0.07	0.02	0.06
Earthworm-eating birds	0.05	0.0004	0.001
Earthworm-eating mammals	0.02	0.0002	0.0006

* Considering 30m distance, for fruit crops (late application) which corresponds to 0.54% drift value, single application (Rautmann et al., 2001)

** Considering 30m distance, for fruit crops (late application) which corresponds to 0.36% drift value, threefold application (Rautmann et al., 2001)

Table 2.2.2.5-2: Summary of PEC/PNEC ratios for metabolite M-1

Scenario/Compartment	PEC/PNEC		
	Indoor use	Outdoor use / Single application	Outdoor use / Multiple applications
Surface water – aquatic organisms	0.09	0.03	0.08
Sediment-dwelling organisms	Covered by the risk assessment for aquatic organisms		
Soil organisms	0.04	0.02	0.05

Table 2.2.2.5-3: Summary of PEC/PNEC ratios for metabolite M-2

Scenario/Compartment	PEC/PNEC		
	Indoor use	Outdoor use / Single application	Outdoor use / Multiple applications
Surface water – aquatic organisms	0.52	0.16	0.45
Sediment-dwelling organisms	Covered by the risk assessment for aquatic organisms		
Soil organisms	0.12	0.05	0.15

Table 2.2.2.5-4: Summary of PEC/PNEC ratios for metabolite M-8

Scenario/Compartment	PEC/PNEC		
	Indoor use	Outdoor use / Single application	Outdoor use / Multiple applications
Soil organisms	0.09	0.04	0.11

Table 2.2.2.5-5: Summary of PEC/PNEC ratios for metabolite M-12 (or EN 1-93/3)

Scenario/Compartment	PEC/PNEC		
	Indoor use	Outdoor use / Single application	Outdoor use / Multiple applications
Surface water – aquatic organisms	0.09	0.03	0.07
Sediment-dwelling organisms	Covered by the risk assessment for aquatic organisms		
Soil organisms	0.11	0.05	0.14
Fish-eating birds	0.0018	0.0006	0.0015
Fish-eating mammals	0.0009	0.0003	0.0008
Earthworm-eating birds	0.0011	0.00005	0.0001
Earthworm-eating mammals	0.00055	0.00003	0.00005

Table 2.2.2.5-6: Summary of PEC/PNEC ratios for metabolite EN 1-101/4 (or Metabolite F)

Scenario/Compartment	PEC/PNEC		
	Indoor use	Outdoor use / Single application	Outdoor use / Multiple applications
Soil organisms	0.06	0.03	0.08

The conclusions reached regarding the potential environmental risks from the parent compound Piperonyl Butoxide and its major aquatic/sediment and soil metabolites following the intended uses of the representative product AquaPy are summarized below.

Piperonyl Butoxide:

Aquatic compartment (including STP, surface water and sediment)

The risk to STP microorganisms has been calculated to be acceptable following the intended indoor use of the representative product AquaPy (no exposure of STP microorganisms is anticipated following the outdoor use of the representative biocidal product). The risk to aquatic organisms (surface water) has been calculated to be acceptable following the proposed outdoor uses of AquaPy, but unacceptable for the intended indoor use. Regarding outdoor use/single application scenario, the aquatic risk has been found to be acceptable without considering any mitigation measures while the aquatic risk for the outdoor use/multiple (threefold) applications scenario has been found to be acceptable only when a 30 m unsprayed buffer zone between the treated area and surface water was considered. The risk to sediment-dwelling organisms has been calculated to be unacceptable for all intended uses when no mitigation measures were considered. However, an acceptable risk for the sediment compartment following the intended outdoor uses (single and multiple (threefold) applications) has been indentified when a 30 m unsprayed buffer zone between the treated area and surface water was considered.

Terrestrial compartment (including soil, groundwater, fish- and earthworm eating predators, bees and other non-target arthropods)

The risk to soil organisms has been calculated to be acceptable for the indoor and outdoor use/single application scenarios. Regarding the outdoor use/multiple applications scenario, the calculated PEC/PNEC ratio was above the trigger value of 1, i.e. 1.1. As the latter value is only marginally above the trigger of 1 and taken into account the conservative approach used for the $PNEC_{soil}$ derivation (e.g. Equilibrium Partitioning Method), the respective risk is considered to be acceptable. Regarding groundwater, the calculated PEC values has been found to be below the threshold value of 0.1 µg/L indicating no unacceptable risk to drinking water following application

of AquaPy according to the proposed use pattern.

The risk to fish- and earthworm- eating predators has been calculated to be acceptable following all intended uses (indoor, outdoor/single application, outdoor/multiple applications) of the representative product AquaPy.

The risk to bees has been assessed to be acceptable following the intended indoor use of the representative product AquaPy. Regarding outdoor uses, no risk assessment for bees has been conducted as, at the moment, no specific guidance is available for biocidal products on how to perform the bee risk assessment. The risk to other non-target (beneficial) arthropods has been assessed to be acceptable following the intended indoor and outdoor uses.

Major metabolites of Piperonyl Butoxide:

The risk to aquatic and sediment-dwelling organisms from metabolites M-1, M-2 and M-12 (or EN 1-93/3), the risk to soil organisms from metabolites M-1, M-2, M-8, M-12 (or EN 1-93/3) and EN 1-101/4 (or Metabolite F) and risk to fish- and earthworm- eating predators from metabolite M-12 (or EN 1-93/3) has been calculated to be acceptable following the intended indoor and outdoor (single application/multiple applications) uses of AquaPy.

Overall, an acceptable risk for all environmental compartments under concern has been identified only for the outdoor use/single application scenario provided that:

- A 30 m unsprayed buffer zone between the treated area and the water body is established in order to protect aquatic and sediment-dwelling organisms

2.2.3. Assessment of endocrine disruptor properties

No endocrine specific studies, e.g. *in vitro* or *in vivo* screening assays or *in vivo* confirmatory tests, have been submitted to investigate the potential endocrine mode of action of the active substance. Therefore, the assessment of the potential endocrine disrupting activity of Piperonyl Butoxide is based on the available mammalian toxicity data and available information and evidence from the scientific literature.

Standard mammalian toxicology studies with Piperonyl Butoxide such as repeated dose toxicity, long-term toxicity and carcinogenicity, reproductive and developmental toxicity, have not provided any indication of endocrine activity that could be attributed to Piperonyl Butoxide administration, including effects on the sexual hormone system and on thyroid activity. Non-standard studies on specific endocrine mechanisms in mammals have not been conducted and were not considered necessary.

In addition, based on a scientific literature search conducted by the eCA there are no findings or evidence to raise endocrine related concern associated with Piperonyl Butoxide. However, further information to assess the potential for endocrine disruption of Piperonyl Butoxide may be required when EU harmonised guidelines are established for test methods and risk assessment. The only currently available criteria for identifying an endocrine disruptor are the interim provisions of Art. 5, paragraphs 1(d) & 3 to the BPR. There is no harmonized classification for Piperonyl Butoxide with regard to human health effects. The WG-II-2016 (human health) proposed that Piperonyl Butoxide should be classified as a carcinogen category 2 based on liver neoplastic effects observed in mice, but not as a reproductive toxicant. Therefore, the conditions of the interim provisions of Art. 5 concerning endocrine disrupting properties in relation to human health, are not met.
Piperonyl butoxide is placed in the CoRAP list to be evaluated by SE for potential endocrine disruption. However, this evaluation has not started yet and the source documents that will be considered are not known. When this evaluation becomes available it will be considered in line

with the legislative requirements in force at the time.
In agreement with the eCA' s evaluation, the US EPA stated the following regarding the potential for Piperonyl Butoxide to cause endocrine disruption (US EPA, June 2006): "In the available human health toxicity studies on Piperonyl Butoxide, there were no toxicologically significant evidence of endocrine disruptor effects. When the appropriate screening and/or testing protocols have been developed, Piperonyl Butoxide may be subject to additional screening and/or testing".

2.3. Overall conclusions

The outcome of the assessment for Piperonyl Butoxide in product-type 18 is specified in the BPC opinion following discussions at the [number of BPC meeting] meeting of the Biocidal Products Committee (BPC). The BPC opinion is available from the ECHA website.

2.4. List of endpoints

The most important endpoints, as identified during the evaluation process, are listed in Appendix I.

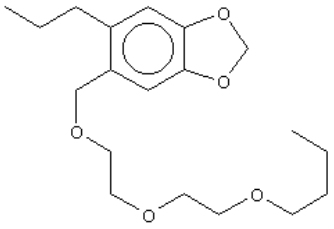
Appendix I: List of endpoints

Chapter 1:Identity, Physical and Chemical Properties, Classification and Labelling

Active substance (ISO Name)	Piperonyl Butoxide (synergist)
Product-type	PT 18 (insecticides, acaricides and products to control other arthropods)

Identity

Chemical name (IUPAC)	5-{[2-(2-butoxyethoxy)ethoxy]methyl}-6-propyl-1,3-benzodioxole
Chemical name (CA)	5-[[2-(2-butoxyethoxy)ethoxy]methyl]-6-propyl-1,3-benzodioxole
CAS No	51-03-6
EC No	200-076-7
Other substance No.	CIPAC no. 33
Minimum purity of the active substance as manufactured (g/kg or g/l)	940 g/kg
Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg)	Safrole: max. content <0.004% w/w Dihydrosafrole: max. content <0.0085% w/w Dipiperonyl methane: max. content 1.95% w/w Dipiperonyl ether: max. content 0.9%w/w Isosafrole: max. content <0.004% w/w Methyl dihydrosafrole: max. content 0.5%w/w Piperonyl Butoxide-x (Piperonyl Butoxide homologue): max. content 0.47 % w/w ortho-Piperonyl Butoxide (Piperonyl Butoxide homologue): max. content 0.51 % w/w N.N-dimethylformamide: max. content <0.04% w/w Dichloromethane: max. content <0.05% w/w
Molecular formula	$C_{19}H_{30}O_5$
Molecular mass	338.43 g/mol

Structural formula	

Physical and chemical properties

Melting point (state purity)	Practical experience has shown that the purified active substance Piperonyl butoxide is a liquid both at ambient temperature and even at – 10 °C
Boiling point (state purity)	203 °C at 2.78 mbar (purity 94.47%)
Thermal stability / Temperature of decomposition	300°C
Appearance (state purity)	Transparent oily liquid at 20 °C (typically around 93%)
Relative density (state purity)	1.058 g/mL at 20 °C (purity 96.76%)
Surface tension (state temperature and concentration of the test solution)	Result: 35.79 mN/m (as supplied) Temperature: 25 °C result: 50.39 mN/m (1% solution) Temperature: 20 °C
Vapour pressure (in Pa, state temperature)	2.11×10^{-5} Pa at 60 °C The calculated vapour pressure at 25 °C will be less than 1.33×10^{-5} Pa
Henry's law constant (Pa m^3 mol^{-1})	1.648×10^{-4} Pa m^3/mole
Solubility in water (g/l or mg/l, state temperature)	Solubility: 36.1 mg/L at 8.4 ºC and pH = 7.04 28.9 mg/L at 20.4 ºC and pH = 7.01 23.1 mg/L at 33.4 ºC and pH = 7.02 Solubility: 30.7 mg/L at 20.4 ºC and pH = 4.06 32.8 mg/L at 20.4 ºC and pH = 6.12 28.9 mg/L at 20.4 ºC and pH = 7.01 30.5 mg/L at 20.4 ºC and pH = 8.86

Solubility in organic solvents (in g/l or mg/l, state temperature)	temperature: 15 °C and 25 °C Result: n-Hexane > 30 % w/v Toluene > 30 % w/v 1,2-Dichloroethane > 30 % w/v 2-Propanol > 30 % w/v Acetone > 30 % w/v Ethyl acetate > 30 % w/v
Stability in organic solvents used in biocidal products including relevant breakdown products	-
Partition coefficient (log P_{OW}) (state temperature)	Result: log P_{OW}= 4.8 temperature: 20 °C pH: 6.5
Dissociation constant	Not required, because Piperonyl Butoxide contains no dissociative groups
UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength)	UV/Vis spectrum acceptable. It showed two relevant maximum absorbances at 237 nm and 290 nm with molecular extinction ε = 7532 and 6081 respectively. No absorbance above 290 nm. IR, MS and NMR spectra acceptable and consistent with the structure of piperonyl butoxide. A comparison of the spectra submitted with the ones published in the NIST database showed no differences.
Flammability or flash point	Flash point: 179.25 °C => Piperonyl Butoxide not flammable Self-ignition temperature: 265 °C
Explosive properties	The test item (purity 95.38 %) has no danger of explosion.
Oxidising properties	The test item has no oxidizing properties.
Auto-ignition or relative self ignition temperature	The self-ignition temperature of the test item is 265 °C.

Classification and proposed labelling

with regard to physical hazards	None.
with regard to human health hazards	STOT SE 3; H335 Carc. 2; H351 EUH066

with regard to environmental hazards	

Chapter 2:Methods of Analysis

Analytical methods for the active substance

Technical active substance (principle of method) Impurities in technical active substance (principle of method)	A fully validated GC/FID analytical method has been submitted for the determination of pure Piperonyl Butoxide and its impurities in Piperonyl Butoxide technical material. Piperonyl Butoxide and its ten impurities were identified by GC/MS. Representative chromatograms have been submitted and are acceptable.

Analytical methods for residues

Soil (principle of method and LOQ)	20 g of the soil sample were weighed into a 250 mL glass bottle. 10 mL water and 100 mL acetonitrile were added, the flask closed with a screw cap and shaken on a flatbed shaker for at least 6 hours. Thereafter, at least 10 g of sodium chloride were added and the flasks were shaken again for approx. 1 min to separate the phases. An aliquot of about 1 mL was transferred into a 2 mL single-use syringe fitted with a 0.45 µm Nylon filter and the extract was filtered into a HPLC vial (1.8 mL). The final extracts were diluted 1:10 with acetonitrile (100 µL final extract + 900 mL acetonitrile) and used for HPLC/MS-MS analysis. Two ion transitions (SRM 356→177 for quantification and SRM 356→119 for confirmation) have been validated. LOQ: 0.05 mg/kg
Air (principle of method and LOQ)	Sampling of Piperonyl Butoxide on the front filter of the adsorbent tube, consisting of two units (front and back-up bed) filled with Tenax as adsorbent material. Sampling of air under constant flow. The humidity was > 80% in average and the temperature was 35 ± 2 °C. The sampling time was 8 h. Extraction of Piperonyl Butoxide from the adsorbent was made with 5 mL acetone on a flatbed shaker for 60 min at 100 rpm at a temperature around 20 °C. Analysis of Piperonyl Butoxide concentrations was performed by using GC/MS. LOQ is 5.83 µg/m^3

Water (principle of method and LOQ)	Surface water samples were diluted with acetonitrile to contain 25% of acetonitrile (v/v) to ensure analyte solubility in the analytical sample. In the current study, 1 mL water sample was used and diluted with 250 µL acetonitrile. After shaking this analytical sample, an aliquot is transferred into an HPLC vial and used directly for analysis by HPLC-MS/MS. Two ion transitions (SRM 356→177 for quantification and SRM 356→119 for confirmation) have been validated. LOQ: 0.1 µg/L Provided that the proposed method has been successfully validated for surface water at the LOQ required for drinking water (0.1µg/L), no further validation in drinking water is required.
Body fluids and tissues (principle of method and LOQ)	Not required as Piperonyl Butoxide is not indicated to be toxic or highly toxic.
Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	Piperonyl Butoxide is an active substance in PT 18 (insecticides) used in public and private areas, as well as in areas where foodstuffs and other goods are stored, prepared and packaged. Therefore, residues are possible and potential risks have to be assessed at product authorization level.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	Piperonyl Butoxide is an active substance in PT 18 (insecticides) used in public and private areas, as well as in areas where foodstuffs and other goods are stored, prepared and packaged. Therefore, residues are possible and potential risks have to be assessed at product authorization level.

Chapter 3:Impact on Human Health

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption:	Rapid and almost complete [>92% based on urinary (35.65%) and faecal excretion (59.61%) at 72 hours after single oral administration] 100% value used in risk assessment

Rate and extent of dermal absorption*:	Low dermal absorption on human volunteers 2.4% for the concentrate (considering radioactivity detected in urine, faeces and tape-strips) 4.8% for the in-use dilution (following the pro-rata approach)
Rate and extent of inhalation absorption:	100% by default
Distribution:	Highest concentration at GI contents, carcass and liver
Potential for accumulation:	Low potential for body accumulation
Rate and extent of excretion:	Rapid and higher than 90% at 72 hours mainly *via* faeces
Toxicologically significant metabolite(s)	Parent compound

* the dermal absorption value is applicable for the active substance and might not be usable in product authorization

Acute toxicity

Rat LD_{50} oral	> 2000 mg/kg bw (♂), >5000 mg/kg bw (♀)
Rat LD_{50} dermal	> 2000 mg/kg bw (♂ & ♀)
Rat LC_{50} inhalation	> 5.9 mg/L/4h (♂ & ♀ ; whole body exposure)

Skin corrosion/irritation	Non-irritant/corrosive
Eye irritation	Non-irritant
Respiratory tract irritation	Irritant **[STOT SE 3; H335]**
Skin sensitisation (test method used and result)	Non-sensitizer (modified 9-induction Buehler method)
Respiratory sensitisation (test method used and result)	Not assessed

Repeated dose toxicity

Short term/Subchronic

Species / target / critical effect	• **Systemic effects (oral, inhalation):** Liver: **dog** clinical chemistry changes, increased liver wt, hepatocellular hypertrophy; **mouse** increased liver wt, hepatocellular hypertrophy; **rat** increased liver wt Kidneys: **rat** increased kidneys wt • **Local effects:** Dermal: **rabbit** erythema, oedema, desquamation, fissuring, red raised areas Respiratory: **rat** red nasal discharge, larynx, histopathology
Relevant oral NOAEL / LOAEL	16 mg/kg bw/day (1-year, dog)
Relevant dermal NOAEL / LOAEL	Syst.: > 1000 mg/kg bw/day (21-days, rabbit) Local: <100 mg/kg bw/day (21-days, rabbit) **[EUH066]**
Relevant inhalation NOAEL / LOAEL	Systemic: 0.155 mg/L (3-month, rat) Local: 0.155 mg/L (3-month, rat)

Long term

Species/ target / critical effect	Liver: **rat** increased liver wt, hypertrophy of hepatocytes) **mouse** hepatocellular hyperplasia and necrosis, adenomas (males, females), carcinomas (males) Kidney: **rat** increased kidney wt, increased incidence of chronic interstitial glomerulonephritis
Relevant oral NOAEL / LOAEL	30 mg/kg bw/day (2 year, rat; 18 months, mouse)
Relevant dermal NOAEL / LOAEL	No relevant study submitted. Not required.
Relevant inhalation NOAEL / LOAEL	No relevant study submitted. Not required.

Genotoxicity	No genotoxic potential

Carcinogenicity

Species/type of tumour	Mouse: hepatocellular adenomas (males, females) and carcinomas (males) **[Carc. 2; H351]**
Relevant NOAEL/LOAEL	30 mg/kg bw/day (18 months, mouse)

Reproductive toxicity

Developmental toxicity

Species/ Developmental target / critical effect	No developmental effects at maternally toxic doses (rat, rabbit)
Relevant maternal NOAEL	100 mg/kg bw/day (rabbit) 200 mg/kg bw/day (rat)
Relevant developmental NOAEL	200 mg/kg bw/day (rabbit) 1000 mg/kg bw/day (rat)

Fertility

Species/critical effect	No effects of fertility (rat)
Relevant parental NOAEL	100 mg/kg bw/day (rat)
Relevant offspring NOAEL	100 mg/kg bw/day (rat)
Relevant fertility NOAEL	500 mg/kg bw/day (rat)

Neurotoxicity

Species/ target/critical effect	Not tested. Not required.

Developmental Neurotoxicity

Species/ target/critical effect	No concern; no further data required.

Immunotoxicity

Species/ target/critical effect	Not tested. Further discussion is required on the need of further investigation of the immunotoxic potential of Piperonyl Butoxide considering the effects from open literature studies.

Developmental Immunotoxicity

Species/ target/critical effect	Not tested.

Other toxicological studies

None available

Medical data

No evidence of anomalies or medical situations to be kept under control was notified and no cases existed in which alterations are strictly related to exposure to substances used in the Ravenna plant.

US-EPA, Memorandum, Review of Piperonyl butoxide Incident Reports, 2004: a greater risk of moderate or major symptoms among those exposed to products containing pyrethrins and piperonyl butoxide than those exposed to pyrethrins alone.
Recommendation/warning to handlers: respiratory irritation, rash, and itching can occur in sensitive individuals; use protective clothing.

Summary

	Value (mg/kg bw/day)	Study	Safety factor
$AEL_{long\text{-}term}$	0.2	1-year dietary dog	100*
$AEL_{medium\text{-}term}$	0.2	1-year dietary dog	100*
$AEL_{short\text{-}term}$	1	developmental rabbit	100*
ADI[3]	0.2	1-year dietary dog	100
ARfD	Not set. Not required.		

** assuming 100 oral absorption*

*** in agreement with JMPR, 1995*

MRLs

Relevant commodities	Not set. ~~Not required.~~

Dermal absorption

Study (*in vitro/vivo*), species tested	*In vivo* study on human volunteers
Formulation (formulation type and including concentration(s) tested, vehicle)	• 3% a.s. (w/w) in isopropanol: ≤3 % • 4% a.s. (w/w) in water based solution: ≤1 %
Dermal absorption values used in risk assessment	2.4% for the concentrate (considering radioactivity detected in urine, faeces and tape-strips) 4.8% for the in-use dilution (following the pro-rata approach)

Acceptable exposure scenarios (including method of calculation)

Formulation of biocidal product	AquaPy (150 g/L Piperonyl Butoxide, EW)
Intended uses	Intended for indoor and outdoor use as fog against mosquitoes and houseflies.
Industrial users	Not assessed. Not relevant.

[3] If residues in food or feed.

Professional users	Outdoor cold (ULV) application: - Hand-held: 61% of the $AEL_{medium-term}$ (No PPE) - Vehicle mounted: 55% of the $AEL_{medium-term}$ (No PPE) Indoor: - Cold (ULV) fogging: 53% of the $AEL_{medium-term}$ (No PPE) - Thermal fogging: 91% of the $AEL_{medium-term}$ (No PPE)
Non professional users	Not intended.
General public	**Bystanders:** Outdoor (adult): 1.4% of the $AEL_{short-term}$ Outdoor (toddler): 8.5% of the $AEL_{short-term}$ Indoor (adults): covered by infants Indoor (infant): 4.5% of the $AEL_{short-term}$
Exposure via residue in food	Piperonyl Butoxide is an active substance in PT 18 (insecticides) used in public and private areas, as well as in areas where foodstuffs and other goods are stored, prepared and packaged. Therefore, residues are possible and potential risks have to be assessed at product authorization level.

Chapter 4: Fate and Behaviour in the Environment

Route and rate of degradation in water

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)	Stable at pH 5, pH 7, pH 9 with DT_{50}>500 days (25^0C)
pH 5	Stable
pH 9	Stable
Other pH: *7*	Stable
Photolytic / photo-oxidative degradation of active substance and resulting relevant metabolites	$t_{1/2E}$= 8.4 hours (pH 7, 25°C) Hydroxymethyl dihydrosafrole: 54.5% of AR Corresponding aldehyde of the alcohol: 12.2% of AR
Readily biodegradable (yes/no)	No
Inherent biodegradable (yes/no)	No study submitted.
Biodegradation in freshwater	
Biodegradation in seawater	No study submitted.
Non-extractable residues	-

Distribution in water / sediment systems (active substance)	Pond system: water: 84.3% (day 1) sediment: 40.2% (day 62)	Creek system: Water: 81.8% (day 0) Sediment: 29.3% (day 62)
DT50 water (12^0C)	51.2 days	313 days
DT50 sediment (12^0C)	Not allocated	Not allocated
DT50 whole system (12^0C)	104.3 days	102.4 days
Distribution in water / sediment systems (metabolites)	M2 metabolite	
	Pond system: water: 9.8 % (day 62) sediment: 12.9% (day 120)	Creek system: Water: 30.4% (day 100) Sediment: 10.3% (day 100)

Route and rate of degradation in soil

Mineralization (aerobic)	Not measured
Laboratory studies (range or median, with number of measurements, with regression coefficient)	
DT_{50lab} (20°C, aerobic):	DT_{50lab} (20°C, aerobic): 23, 29, 64 days DT_{50lab} (25°C, aerobic): 14 days
DT_{90lab} (20°C, aerobic):	DT_{90lab} (20°C, aerobic): 212, 97, 76 days DT_{90lab} (25^0C, aerobic): 50 days
DT_{50lab} (10°C, aerobic):	DT_{50lab} (10°C, aerobic): -
DT_{50lab} (20°C, anaerobic):	DT_{50lab} (20°C, anaerobic): 144 days
degradation in the saturated zone:	-
Field studies (state location, range or median with number of measurements)	-
DT_{50f}:	-
DT_{90f}:	-
Anaerobic degradation	-
Soil photolysis	-
Non-extractable residues	25.1% after 120 days (geomean value, n=4), 37% after 128 days.
Relevant metabolites - name and/or code, % of applied a.i. (range and maximum)	M1 (max: 5.9%) M2 (max: 14.4%) M8 (max: 9%) M12 (EN1-93/3) (max: 19.4%) EN 1-101/4 (Metabolite F) (max: 6.6%)
Soil accumulation and plateau concentration	-

Adsorption/desorption

Ka , Kd Ka_{oc} , Kd_{oc}	Average values: Ka: 12.09 mL/g, Ka_{OC}: 3745.3 mL/g, Kd: 15.7 mL/g, Kd_{OC}: 4813 mL/g
pH dependence (yes / no) (if yes type of dependence)	No

Fate and behaviour in air

Direct photolysis in air	No test
Quantum yield of direct photolysis	No test
Photo-oxidative degradation in air	Atkinson model (via AOPWIN vs 1.92) DT_{50}: 3.597 hrs (24-hr day, 0.5E6 OH/cm^3)
Volatilization	-

Reference value for groundwater

According to BPR Annex VI, point 68	0.0001mg/L (Directive 98/83/EC)

Monitoring data, if available

Soil (indicate location and type of study)	No data available
Surface water (indicate location and type of study)	No data available
Ground water (indicate location and type of study)	No data available
Air (indicate location and type of study)	No data available

Chapter 5: Effects on Non-target Species

Toxicity data for aquatic species (most sensitive species of each group)

Species	Time-scale	Endpoint	Toxicity
Fish			
Cyprinodon variegatus	96-hour – flow-through	LC_{50}	3.94 mg a.s./L (m.m.)
Lepomis macrochirus	96-hour – Flow through	LC_{50}	5.37 mg a.s./L (m.m.)
Oncorhynchus mykiss	96-hour – Flow through	LC_{50}	6.12 mg a.s./L m.m.)
Pimephales promelas	35-day – flow-through	NOEC	0.18 mg a.s./L (m.m.)
Invertebrates			
Daphnia magna	48-hour – flow-through	EC_{50}	0.51 mg a.s./L (m.m.)
Daphnia magna	48-hour – static	EC_{50}	0.216 mg AquaPy/L (m.m.)
Americamycis bahia	96-hour – flow-through	EC_{50}	0.32 mg a.s./L (m.m.)

Crassostrea virginica	96-hour – flow-through	EC_{50}	0.23 mg a.s./L (m.m.)
Daphnia magna	21-day – flow-through	NOEC	0.030 mg a.s./L (m.m.)
Algae			
Selenastrum capricornutum	72-hour – static	E_rC_{50}	3.89 mg a.s./L (m.m.)
		E_bC_{50}	2.09 mg a.s./L (m.m.)
		NOE_rC	0.824 mg a.s./L (m.m.)
Pseudokirchneriella subcapitata	72-hour – static	E_rC_{50}	6.58 mg AquaPy/L (m.m.)
		E_bC_{50}	3.1 mg AquaPy/L (m.m.)
		NOE_rC	0.976 mg AquaPy/L (m.m.)
Sediment – dwelling organisms			
Leptocheirus plumulosus	10-day (spiked sediment system)	LC_{50}	> 86 mg a.s./kg dwt (m.m.)
Chironomus riparius	28-day (spiked water system)	NOEC	0.0148 mg a.s./L (m.m.*) 0.0933 mg a.s./kg dwt (m.m.*)
Chironomus dilutus	63-day (spiked sediment system)	NOEC	0.44 mg a.s./kg dwt (m.m.)
Hyalella azteca	42-days (spiked sediment system)	NOEC	39 mg a.s./kg dwt (m.m.)
Microorganisms			
Aerobic microorganisms	3-hour – static	NOEC	28.9 mg a.s./L

m.m.: based on mean measured concentrations

* based on time-weighted geometric mean concentrations

Effects on earthworms or other soil non-target organisms

Acute toxicity to earthworms (*Eisenia fetida*)	14-day LC_{50} = 143.8 mg a.s./kg standard soil dw 14-day LC_{50} >1000 mg AquaPy/kg soil dw
Reproductive toxicity to earthworms (*Eisenia fetida*)	56-day NOEC = 10.2 mg a.s./kg standard soil dw

Effects on soil micro-organisms

Nitrogen mineralization, carbon mineralization	28-day NOEC = 28.8 mg a.s./kg standard soil dw; 28-day EC_{50} > 28.8 mg a.s./kg standard soil dw 28-day NOEC = 26.6 mg AquaPy/kg soil dw; 28-day EC_{50} > 26.6 mg AquaPy/kg soil dw

Effects on terrestrial vertebrates

Acute toxicity to mammals	Refer to Chapter 3

Acute toxicity to birds	14-day LD_{50} > 2250 mg a.s./kg bw
Dietary toxicity to birds	5-day LD_{50} > 5620 mg a.s./kg bw diet
Reproductive toxicity to birds	NOEC = 300 mg a.s./kg diet (equivalent to 27 mg a.s./kg bw/d for Northern bobwhite quail and 47 mg a.s./kg bw/d for mallard duck)

Effects on honeybees

Acute oral toxicity	48-hour LD_{50} = 611.6 µg a.s./bee 48-hour LD_{50} = 7.892 µg AquaPy/bee
Acute contact toxicity	48-hour LD_{50} = 294 µg a.s./bee 72-hour LD_{50} = 2.767 µg AquaPy/bee

Effects on other beneficial arthropods

Effects on *Typhlodromus pyri*	7-day LR_{50} = 0.319 kg a.s./ha 7-day LR_{50} = 0.0318 kg AquaPy/ha
Effects on *Aphidius rhopalosiphi*	48-hour LR_{50} > 4.80 kg a.s./ha 48-hour LR_{50} = 0.000782 kg AquaPy/ha

Bioconcentration

Bioconcentration factor (BCF) fish	BCF_K 99 (edible tissue) BCF_K 450 (non edible tissue) BCF_K 290 (whole body)
Depration time (DT_{50})	1.3 days (whole fish)
Depration time (DT_{90})	4.2 days (whole fish)
Level of metabolites (%) in organisms accounting for > 10 % of residues	Not applicable

Chapter 6: Other End Points

Effects on higher terrestrial plants

Vegetative vigour	ER_{50} > 3250 g a.s./ha ER_{50} = 6984 ml AquaPy/ha (equivalent to 6985 g AquaPy/ha)

Appendix II: List of Intended Uses

Piperonyl Butoxide (PBO) has been evaluated for its intended uses as an insecticide (PT 18); Data on efficacy of Piperonyl Butoxide were provided and accepted in support of the intended uses.

Object and/or situation	Product name	Organisms controlled	Formulation		Application			Applied amount per treatment			Re marks:
			Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	number min max	interval between applications (min)	g a.s./L min max	water L/m² min max	g a.s./m² min max	
Outdoor											
Amenity areas, woodlands	AquaPy (3 % w/v Pyrethrins + 13.5 % w/v Piperonyl Butoxide)	Diluted with water or paraffin oil against adult mosquitoes	EW	30 g Pyrethrins + 135 g PBO/lt product	Ground ULV (Ultra Low Volume) space spray application (cold fogging) using hand held or vehicle mounted cold fogger. AquaPy can be used diluted with water or paraffin oil (9 parts water or oil + 1 part product) For ULV aerosol applications the generator should be capable of	1-6 During mosquito season (April to October). Treatments should be repeated if necessary after applying appropriate surveillance methods.	4 weeks	Diluted with water or oil (9 parts water or oil + 1 part product): (final solution contains 10% AquaPy) 13.5 g PBO/lt final spray solution	Diluted with water: 0.2 ml final spray solution /m² (containing 10% product) 0.18 ml water + 0.02 ml product/ m² Diluted with paraffin oil:	Diluted with water (10% in the final solution or 1 part product + 9 parts water): 0.0027 gr PBO/ m² or 27.0 gr PBO /ha or 0.02 ml product /m² Diluted with	When outdoor spraying, the best conditions occur during early morning or late afternoon and evening when still air conditions are most likely to prevail and thermal

Object and/or situation	Product name	Organisms controlled	Formulation		Application			Applied amount per treatment			Re marks:
			Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	number min max	interval between applications (min)	g a.s./L min max	water L/m² min max	g a.s./m² min max	
					producing a distribution of droplets with a maximum volume median diameter (VMD) value of 50 microns and preferably below 25 microns.				0.125 ml final spray solution /m² (containing 10% product): 0.1125 ml oil + 0.0125 ml product/ m² (0.125 - 0.2 mL spray /m²)	paraffin oil (final solution with10% product or 1 part product + 9 parts oil): 0.0017 gr PBO/ m² or 17.0 gr PBO /ha or 0.0125 ml product /m² (0.0017-0.0027 g as / m²) (17.0 – 27.0 g as/ha) (0.0125 – 0.02 mL product /m²)	currents are minimal.

Object and/or situation	Product name	Organisms controlled	Formulation		Application			Applied amount per treatment			Re marks:
			Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	number min max	interval between applications (min)	g a.s./L min max	water L/m^2 min max	g a.s./m^2 min max	
Indoor											
Public Buildings,	AquaPy (3 % w/v Pyrethrins + 13.5 % w/v Piperonyl Butoxide)	Flying insects. Space spray application against adult mosquitoes and houseflies.	EW	30 g Pyrethrins + 135 g PBO/lt product	Space spray by misting (cold fogging) or thermal fogging using mist generator or thermal fogger respectively. The product is applied after dilution with water (9 parts water + 1 part product – 10% aqueous solution) For mist applications the machine should be capable of producing droplets with a VMD of 50 microns or below	Space spray into an infected room will be applied if necessary. Treatments should be applied as frequently as the situation demands. More frequent applications may be necessary for heavy infestations 1 - 2	Min. 1 month	Diluted with water (9 parts water + 1 part product): 13.5 gr PBO/ lt final spray solution (final solution contains 10% AquaPy)	Diluted with water: 0.33 ml final spray solution / m^3 (containing 10% product) 0.3 ml water + 0.033 ml product/ m^3 0.33 mL diluted product /m^3	Diluted with water (final solution with10% product or 1 part product + 9 parts water): 0.033 ml product/m^3 or 0.0045 gr PBO/ m^3 0.0045 g/m^3 (0.033 mL product / m^3)	The doors and windows should be closed and the ventilation systems should be shut off before spraying and for 30 minutes after treatment. The spray cloud should be distributed evenly throughout the area. Treatments should be timed to coincide with the

Object and/or situation	Product name	Organisms controlled	Formulation		Application			Applied amount per treatment			Re marks:
			Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	number min max	interval between applications (min)	g a.s./L min max	water L/m^2 min max	g a.s./m^2 min max	
											time of maximum flight activity for the target insects.

Appendix III: List of studies

Data protection is claimed by the applicant in accordance with Article 60 of Regulation (EU) No 528/2012.

Reference list by Author

Efficacy (active substance: Piperonyl Butoxide)

Section point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protection claimed yes/no	Owner
Colli, M.	A5.10.2/14	2010	Laboratory tests to determine the synergistic effect of Piperonyl Butoxide on Delthametrin against mosquitoes Culex pipiens pipens L. (Diptera, Culicidae). Biotecnologie BT, Final Report ET038/10.	yes	Endura S.p.A
Dove W.	A5.10.2/15	1947	Piperonyl Butoxide, a new and safe insecticide for the household and field. The American Journal of Tropical Medicine 27(3), pp 339-345.		Open literature
Glynne-Jones G. D.		1998	Piperonyl Butoxide. The Insecticide Synergist. Editor: Jones D. G., Published by Academic Press		Open literature
Luepkes, K.-H	A5.10.2/07	2007	Biological Test Report: Efficacy of formulations against House Dust Mites. Biogenius GmbH, Germany, Report No. BIO073/07.	yes	Endura S.p.A
Luepkes, K.-H.	A5.10.2/08	2010	Biological Test Report: Efficacy of various aerosols against House fly Musca domestica in 20 m^3 chambers. Biogenius GmbH, Germany, Report No. BIO058/10.	yes	Endura S.p.A
Luepkes, K.-H	A5.10.2/13	2011	Biological Test Report: Efficacy of various aerosols various insect species by different test methods. Biogenius GmbH, Germany, Report No. BIO103/10.	yes	Endura S.p.A

Section point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protection claimed yes/no	Owner
Luepkes, KH.	A5.10.2/19	2014	Insecticidal efficacy after direct spray treatment of Piperonyl Butoxide (PBO) dissolved in acetone against House fly, Musca domestica, and German cockroach, Blattella germanica. Biogenius GmbH, Germany, Report No. BIO122/14.	yes	Endura S.p.A
Luepkes, KH.	A5.10.2/20	2014	Insecticidal/Residual efficacy after treatment of tiles with Piperonyl Butoxide (PBO) dissolved in acetone against House fly, Musca domestica, and German cockroach, Blattella germanica. Biogenius GmbH, Germany, Report No. BIO123/14.	yes	Endura S.p.A
Luepkes, KH.	A5.10.2/21	2014	Aerosol efficacy of Piperonyl Butoxide (PBO) dissolved in acetone in 20 m^3 chambers against House fly, Musca domestica, and House mosquito, Culex quinquefasciatus. Biogenius GmbH, Germany, Report No. BIO124/14.	yes	Endura S.p.A
Moores G.		2009	Piperonyl Butoxide - The Mode of Action of Piperonyl Butoxide and the Science of Insecticide Resistance, Rothamsted Research, UK, Sponsored by Endura S.p.A.		Endura S.p.A
Radecki, C.	A5.10.2/18	2014	Determination of the insecticidal effect of Piperonyl Butoxide (PBO) by topical application against House flies, Musca domestica, and House mosquitoes, Culex quinquefasciatus. Biogenius GmbH, Germany, Report No. BIO121/14.	Yes	Endura S.p.A
Thomson, D.H.	A5.10.2/06	1998	Screening test on effect of PBO on the House Dust Mite. Medical Entomology Centre Ltd., Cambridge, Report No. JM/MIT/1	yes	Endura S.p.A
Van der Linde, D.	A5.10.2/09	2000	Aerosol tests. South African Bureau of Standards, Pretoria, South Africa, Report No. 1371903/S126	yes	Endura S.p.A
Van der Linde, D.	A5.10.2/10	2001	Aerosol tests. South African Bureau of Standards, Pretoria, South Africa, Report No. 1576159/T124.	yes	Endura S.p.A

Section point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protection claimed yes/no	Owner
Van der Linde, D.	A5.10.2/11	2000	Aerosol tests. South African Bureau of Standards, Pretoria, South Africa, Report No. 1510184/T084.	yes	Endura S.p.A
Van der Linde, D.	A5.10.2/12	1993	Aerosol test KD 5-10-15 min. South African Bureau of Standards, Pretoria, South Africa, Report No. 561/89520/J17	yes	Endura S.p.A

Efficacy (product: AquaPy)

Bowron, M.J.	B5.10.2/05	1993	An evaluation of Aqua Pybuthrin in dry deposit and wet walkover tests against Blattella germanica Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. REPE 93-C8 GLP: no Published: no	no	BES
Lucas, J.R., Bowron, M.J.	B5.10.2/03	1994 b	An evaluation of the biological performance of AquaPy when applied in a 42 m3 chamber as a mist against houseflies and clothes moths Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. GB94-0019 GLP: no Published: no	no	BES
Lucas, J.R., Bowron, M.J.	B5.10.2/04	1994c	Thermal fogging of AquaPy (TF2578) against Culex quinquefasciatus Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. GB94-0033 GLP: no Published: no	no	BES

Lucas, J.R., Bowron, M.J	B5.10.2/01.	1994a	An evaluation of the biological activity of AquaPy (TF2578) when applied out of doors as a ULV space spray against caged Musca domestica and Culex quinquefasciatus Roussel Uclaf Environmental Health, Berkhamstedt Bayer ES Report-no. GB94-0121 GLP: no Published: no	no	BES
Tolosa, M.	B5.10.2/02	2006	Field conditions evaluation of adulticide efficacy by terrestrial application of the AquaPy preparation (aqueous emulsion based on 30 g pyrethrins and 135 g piperonyl butoxide/L)on mosquito pests Ochlerotatus caspius, Oc. dedritus, Aedes vexans (Diptera-Culicidae) EID Méditerranée Bayer ES Report-no. EID 05049 GLP: no Published: no	yes	BES

Section point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protection claimed yes/no	Owner
[redacted]	A6.9/01	2014	An Acute Neurotoxicity Study of Piperonyl Butoxide in Rats by Oral (Gavage) Administration. [redacted] GLP: Yes Published: No	Yes	PBTF II
[redacted]	A 6.4.1/03	1992	SUB-ACUTE TOXICITY OF PIPERONYL BUTOXIDE IN F344 RATS - [redacted] GLP: no Published: yes	no	Open literature
[redacted]	A 6.1.1/01	1991a	ACUTE ORAL TOXICITY, LD50 - RATS [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.1.2/01	1991b	ACUTE DERMAL TOXICITY, SINGLE LEVEL - RABBITS [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.1.1/01	1991a	ACUTE ORAL TOXICITY, LD50 - RATS [redacted] GLP: yes Published: no	no	PBTF II
[redacted]	A 6.3.1/01	1993a	EVALUATION OF PIPERONYL BUTOXIDE IN AN EIGHT WEEK TOXICITY STUDY IN DOGS [redacted] GLP: yes Published: no	yes	PBTF II

[redacted]	A 6.3.2/01	1992	21-DAY REPEATED DOSE DERMAL TOXICITY STUDY WITH PIPERONYL BUTOXIDE IN RABBITS [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.4.1/02	1993b	EVALUATION OF PIPERONYL BUTOXIDE IN A ONE YEAR CHRONIC DIETARY TOXICITY STUDY IN DOGS [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.4.1/02	1993b	EVALUATION OF PIPERONYL BUTOXIDE IN A ONE YEAR CHRONIC DIETARY TOXICITY STUDY IN DOGS [redacted] GLP: yes Published: no	no	PBTF II
[redacted]	A 6.7/01	1987	A 24-MONTH DIETARY TOXICITY AND CARCINOGENICITY STUDY OF PIPERONYL BUTOXIDE IN THE ALBINO RAT [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.7/02	1993	CHRONIC DIETARY ONCOGENICITY STUDY WITH PIPERONYL BUTOXIDE IN CD-1 MICE [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.1.3/01	1991	AN ACUTE INHALATION TOXICITY STUDY OF PIPERONYL BUTOXIDE IN THE RAT [redacted] GLP: yes Published: no	yes	PBTF II

[redacted]	A 6.18/01	1995	PIPERONYL BUTOXIDE (PBO) (EU) A REVIEW OF TOXICOLOGY [redacted] GLP: no Published: no	yes	PBTF II
[redacted]	A 6.10/05	1996	AN INVESTIGATION OF THE EFFECT OF PIPERONYL BUTOXIDE ON UNSCHEDULED DNA SYNTHESIS IN CULTURED HUMAN LIVER SLICES [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.6.1/01	1991	PIPERONY BUTOXIDE IN THE SALMONELLA/MAMMALIAN-MICROSOME REVERSE MUTATION ASSAY (AMES TEST) WITH A CONFIRMATORY ASSAY [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.2/01	1991	ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME) STUDIES OF PIPERONYL BUTOXIDE IN THE RAT [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.10/02	1998	A WEIGHT OF EVIDENCE EXPERT REPORT REGARDING MOUSE BENIGN HEOATIC PROLIFERATIVE LESION [redacted] GLP: no Published: no	yes	PBTF II
[redacted]	A 6.10/07	1991	GENOTOXICITY TEST ON PIPERONYL BUTOXIDE IN THE ASSAY FOR UNSCHEDULED DNA SYNTHESIS IN RAT LIVER PRIMARY CELL CULTURES WITH A CONFIRMATORY ASSAY [redacted] GLP: yes Published: no	yes	PBTF II

Moretto, A.	A 6.18/02	1995	PIPERONYL BUTOXIDE - A MONOGRAPH PREPARED BY THE JOINT FAO/WHO MEETING ON PESTICIDES RESIDUES (GENEVA 1995) Istituto di Medicina del Lavoro, Padua, Italy Joint FAO/WHO Meeting on Pesticides Residues (Geneva 1995) Report-no. not applicable GLP: no Published: yes	no	Open literature
[redacted]	A 6.6.2/01	1991	MEASURING CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS WITH MULTIPLE HARVESTS UNDER CONDITIONS OF METABOLIC ACTIVATION [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.4.3/01	1992	A SUBCHRONIC (3-MONTH) INHALATION TOXICITY STUDY OF PIPERONYL BUTOXIDE IN THE RAT VIA WHOLE-BODY EXPOSURES [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.10/01	1997	EFFECT OF PIPERONYL BUTOXIDE ON CELL REPLICATION AND XENOBIOTIC METABOLISM IN THE LIVERS OF CD-1 MICE AND F344 RATS [redacted] GLP: no Published: yes	no	Open literature
[redacted]	A 6.6.2/01	1991	MEASURING CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS WITH MULTIPLE HARVESTS UNDER CONDITIONS OF METABOLIC ACTIVATION [redacted] GLP: yes Published: no	yes	PBTF II

[redacted]	A 6.4.3/01	1992	A SUBCHRONIC (3-MONTH) INHALATION TOXICITY STUDY OF PIPERONYL BUTOXIDE IN THE RAT VIA WHOLE-BODY EXPOSURES [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.10/01	1997	EFFECT OF PIPERONYL BUTOXIDE ON CELL REPLICATION AND XENOBIOTIC METABOLISM IN THE LIVERS OF CD-1 MICE AND F344 RATS - [redacted] GLP: no Published: yes	no	Open literature
[redacted]	A 6.6.2/01	1991	MEASURING CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS WITH MULTIPLE HARVESTS UNDER CONDITIONS OF METABOLIC ACTIVATION [redacted] Report-no. 14413-0-437c GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.4.3/01	1992	A SUBCHRONIC (3-MONTH) INHALATION TOXICITY STUDY OF PIPERONYL BUTOXIDE IN THE RAT VIA WHOLE-BODY EXPOSURES [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.10/01	1997	EFFECT OF PIPERONYL BUTOXIDE ON CELL REPLICATION AND XENOBIOTIC METABOLISM IN THE LIVERS OF CD-1 MICE AND F344 RATS - [redacted] GLP: no Published: yes	no	-

[redacted]	A 6.6.2/01	1991	MEASURING CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY (CHO) CELLS WITH MULTIPLE HARVESTS UNDER CONDITIONS OF METABOLIC ACTIVATION [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.6.4/01	1989	MUTAGENICITY TESTS ON PIPERONYL BUTOXIDE [redacted] Report-no. not applicable GLP: no Published: no	yes	Endura S.p.A
Tagliani, A.	A 6.12.5/01	2004	CLINICAL SIGNS AND SYMPTOMS OF POISONING AND DETAILS OF CLINICAL TESTS not applicable Endura S.p.A Report-no. not applicable GLP: no Published: no	yes	Endura S.p.A
[redacted]	A 6.7/03	1993	CHRONIC TOXICITY STUDY OF PIPERONYL BUTOXIDE IN F344 RATS INDUCTION OF HEPATOCARCINOMA [redacted] GLP: no Published: no	no	Open literature
[redacted]	A 6.6.3/01	1986	EVALUATION OF PIPERONYL BUTOXIDE IN THE CHO/HGPRT MUTATION ASSAY WITH AND WITHOUT METABOLIC ACTIVATION [redacted] GLP: yes Published: no	yes	PBTF II
[redacted]	A 6.2/03	1994	HUMAN IN VIVO PERCUTANEOUS ABSORPTION OF PYRETHRIN AND PIPERONYL BUTOXIDE - [redacted] GLP: no Published: yes	no	Open literature

Human Health Risk Assessment (product: AquaPy)

Section point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protection claimed yes/no	Owner
Boucaud, B.	B6.6/01	2006	OCCUPATIONAL MEDICAL EXPERIENCES WITH PYRETHRE not applicable Bayer ES Report-no. not applicable GLP: no Published: no	yes	BES

Environmental Risk Assessment (active substance: PBO)

Authors	Section point/ reference number	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protection claimed yes/no	Owner
Balluff, M.	A7.5.1.3/01	2006	A GREENHOUSE LIMIT TEST TO DETERMINE THE EFFECTS OF PIPERONYL BUTOXIDE ON THE VEGETATIVE VIGOUR OF SIX SPECIES OF PLANTS GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Endura S.p.A Report-no. 20064004/S1-FGVV GLP: no Published: no	yes	Endura S.p.A
Bealing, D.J.	A7.4.1.4/01	2002	PIPERONYL BUTOXIDE: DETERMINATION OF INHIBITION OF RESPIRATION OF ACTIVATED SLUDGE Covance Laboratories Ltd., Harrogate, UK Endura S.p.A Report-no. 2145/2-D2149 GLP: no Published: no	yes	Endura S.p.A
Bocksch, S.	A7.5.4.1/01	2006	ASSESSMENT OF SIDE EFFECTS OF PIPERONYL BUTOXIDE TO THE HONEY BEE, APIS MELLIFERA L. IN THE LABORATORY GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Endura S.p.A	yes	Endura S.p.A

			Report-no. 20061066/S1-BLEU GLP: yes Published: no		
Bosse, D.	A7.3.1/01	1999	Substance Piperonyl Butoxide - Calculation of the Indirect Photolysis Reaction Using the Incremental Method af Atkinson and the Program AOPWIN, Version 1.80 Hoechst AG, Frankfurt am Main, Germany Endura S.p.A Report-no. 99.0001 GLP: no Published: no	yes	Endura S.p.A
[redacted]	A7.5.3.1.1/01	1991	AN ACUTE ORAL TOXICITY STUDY WITH PIPERONYL BUTOXIDE IN THE NORTHERN BOBWHITE QUAIL [redacted] Report-no. 306-103 GLP: yes Published: no	yes	PBTFII
Daly, D.	A7.1.3/02	1991	Soil/Sediment Adsorption-Desorption of Piperonyl Butoxide ABC Laboratories, Columbia, Missouri, USA Endura S.p.A Report-no. 38360 GLP: yes Published: no	yes	PBTFII
Derz, K.	A7.1.2.2.2/01		Aerobic transformation of Piperonylbutoxide (PBO) in water/sediment systems (OECD 308) Fraunhofer Institut, Schmallenberg-Grafschaft, Germany Endura S.p.A Report-no. GAB-011/7-92 GLP: yes Published: no	yes	PBTFII
Derz, K.	A7.2.2/01		Degradation rates of Piperonyl Butoxide and selected metabolites in soil under aerobic conditions Fraunhofer Institut, Schmallenberg-Grafschaft, Germany Endura S.p.A Report-no. GAB-011/7-90 GLP: yes Published: no	yes	Endura S.p.A
Elsom, L.F.	A7.1.3/01	1995b	14C-Piperonyl Butoxide Adsorption/Desorption on Soil Huntingdon Life Sciences Limited, Huntingdon, UK Endura S.p.A Report-no. PBT 10A/950775 GLP: yes Published: no	yes	PBTFII
Elsom, L.F.	A7.2.3.2/01	1995c	14C-Piperonyl Butoxide Soil Column	yes	PBTFII

			Leaching of non-Aged and Aged Residues of 14C-Piperonyl Butoxide Huntingdon Life Sciences Limited, Huntingdon, UK Endura S.p.A Report-no. PBT 10B/950899 GLP: yes Published: no		
[redacted]	A7.5.3.1.2/01	1991a	A DIETARY LC50 STUDY WITH PIPERONYL BUTOXIDE IN THE NORTHERN BOBWHITE QUAIL [redacted] GLP: yes Published: no	yes	PBTFII
[redacted]	A7.5.3.1.2/02	1991b	A DIETARY LC50 STUDY WITH PIPERONYL BUTOXIDE IN THE MALLARD [redacted] GLP: yes Published: no	yes	PBTFII
[redacted]	A7.4.1.1/03	1992a	A 96-HOUR FLOW-THROUGH ACUTE TOXICITY TEST WITH PIPERONYL BUTOXIDE IN THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*). [redacted] GLP: yes Published: no	To be confirmed by the applicant	PBTFII
[redacted]	A7.4.1.1/01	1992b	A 96-HOUR FLOW-THROUGH ACUTE TOXICITY TEST WITH PIPERONYL BUTOXIDE IN SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) [redacted] GLP: yes Published: no	yes	PBTFII
[redacted]	A7.4.1.1/02	1992c	A 96-HOUR FLOW-THROUGH ACUTE TOXICITY TEST WITH PIPERONYL BUTOXIDE IN THE BLUEGILL (LEPOMIS MACROCHIRUS) [redacted] GLP: yes Published: no	yes	PBTFII
Holmes, C.M., Smith,	A7.4.1.2/01	1992d	A 48-HOUR FLOW-THROUGH ACUTE TOXICITY TEST WITH PIPERONYL	yes	PBTFII

G.J.			BUTOXIDE IN DAPHNIDS (DAPHNIA MAGNA) Wildlife International, Ltd., Easton, Maryland, USA Endura S.p.A Report-no. 306A-105A GLP: yes Published: no		
Holmes, C.M., Smith, G.J.	A7.4.1.2/02	1992f	A 96-HOUR FLOW-THROUGH ACUTE TOXICITY TEST WITH PIPERONYL BUTOXIDE IN MYSID SHRIMP (MYSIDOPSIS BAHIA) Wildlife International, Ltd., Easton, Maryland, USA Endura S.p.A Report-no. 306A-109 GLP: yes Published: no	no	PBTFII
Holmes, C.M., Smith, G.J.	A7.4.1.2/04	1992	A 96-HOUR SHELL DEPOSITION TEST WITH PIPERONYL BUTOXIDE IN THE EASTERN OYSTER (*CRASSOSTREA VIRGINICA*). Wildlife International, Ltd., Easton, Maryland, USA Endura S.p.A Report-no. 306A-110A GLP: yes Published: no	To be confirmed by the applicant	PBTFII
Holmes, C.M., Smith, G.J.	A7.4.3.4/02	1992	A FLOW-THROUGH LIFE-CYCLE TOXICITY TEST WITH PIPERONYL BUTOXIDE IN DAPHNIDS (*DAPHNIA MAGNA*). Wildlife International, Ltd., Easton, Maryland, USA Endura S.p.A Report-no.: 306A-104 GLP: yes Published: no	To be confirmed by the applicant	PBTFII
Kirkpatrick, D.	A7.1.1.1.1/01	1995	Piperonyl Butoxide Hydrolysis as a Function of pH at 25°C Huntingdon Research Centre Ltd., Huntingdon, UK Endura S.p.A Report-no. PBT 4/943285 GLP: no Published: no	yes	PBTFII
Kölzer, U.	A7.5.1.1/01	2006a	ASSESSMENT OF THE SIDE EFFECTS OF PIPERONYL BUTOXIDE ON THE ACTIVTY OF THE SOIL MICROFLORA GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany Endura S.p.A Report-no. 20051329/01-ABMF GLP: yes Published: no	yes	Endura S.p.A
Kölzer, U.	A7.5.1.2/01	2006b	ACUTE TOXICITY OF PIPERONYL BUTOXIDE ON EARTHWORMS, EISENIA FETIDA	yes	Endura S.p.A

			USING AN ARTIFICIAL SOIL TEST GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany Endura S.p.A Report-no. 20051329/01-NLEf GLP: yes Published: no		
Kölzer, U.	A7.5.2.1/01	2006c	SUBLETHAL TOXICITY OF PIPERONYL BUTOXIDE TO THE EARTHWORM EISENIA FETIDA IN ARTIFICIAL SOIL GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany Endura S.p.A Report-no. 20051329/01-NREf GLP: yes Published: no	yes	Endura S.p.A
[redacted]	A7.4.3.2/01	1994	PIPERONYL BUTOXIDE TECHNICAL, TASK FORCE BLEND PB200 - THE TOXICITY TO FATHEAD MINNOW (PIMEPHALES PROMELAS) DURING AN EARLY LIFE STAGE EXPOSURE [redacted] GLP: yes Published: no	yes	PBTFII
Mattock S.D.	A7.4.1.3/01	2002	PIPERONYL BUTOXIDE: INHIBITION OF GROWTH THE ALGA SELENASTRUM CAPRICORNUTUM Covance Laboratories Ltd., Harrogate, UK Endura S.p.A Report-no. 2145/1-D2149 GLP: yes Published: no	yes	PBTFII
Mayo, B.C.	A7.2.1/01	1995a	Piperonyl Butoxide Aerobic Soil Metabolism Huntingdon Life Sciences Limited, Huntingdon, UK Endura S.p.A Report-no. PBT 7/951484 GLP: yes Published: no	yes	PBTFII
Picard, C.R.	A7.4.3.5.1/02	2013a	Piperonyl Butoxide – Life-Cycle Toxicity Test Exposing Midges (Chironomus dilutus) to a Test Substance Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods. Smithers Viscient, Massachusetts, USA Report No. 13513.6137 GLP: yes Published: no	yes	PBTFII
Picard, C.R.	A7.4.3.5.1/03	2013b	Piperonyl Butoxide - 42-Day Toxicity Test Exposing Freshwater Amphipods (Hyalella azteca) to a Test Substance	yes	PBTFII

			Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods, Smithers Viscient, 790 Main Street, Wareham, Massachusetts Report no. 13513.6138 Published: no GLP: yes		
Picard, C.R.	A7.4.3.5.1/04	2015	Piperonyl Butoxide – 10-day toxicity test exposing estuarine amphipods (Leptocheirus plumulosus) to a test substance applied to sediment under static conditions, Smithers Viscient, 790 Main Street, Wareham, Massachusetts Report no. 13513.6179 Published: no GLP: yes	yes	PBTFII
Picard, C.R.	A7.4.3.5.1/05	2015	Piperonyl Butoxide – 28-day Sediment-Pore Water Equilibration Trial, Smithers Viscient, 790 Main Street, Wareham, Massachusetts Report no. 13513.6155 Published: no GLP: no	yes	PBTFII
Putt, A.E.	A7.4.3.4/01	1994	PIPERONYL BUTOXIDE TECHNICAL TASK FORCE BLEND PB200 - THE CHRONIC TOXICITY TO DAPHNIA MAGNA UNDER FLOW-THROUGH CONDITIONS Spingborn Lab. Inc., Wareham, Massachusetts 02571, USA Endura S.p.A Report-no. 94-5-5270 GLP: yes Published: no	yes	PBTFII
Roberts, C.A., Swigert, J.P.	A7.4.1.2/03	1995	PIPERONYL BUTOXIDE : A 96-HOUR FLOW-THROUGH ACUTE TOXICITY TEST WITH THE SALTWATER MYSID (*MYSIDOPSIS BAHIA*). Wildlife International, Ltd., Easton, Maryland, USA Endura S.p.A Report-no. 306A-111A GLP: yes Published: no	yes	PBTFII
[redacted]	A7.5.3.1.3/01	1995b	PIPERONYL BUTOXIDE: EFFECTS ON REPRODUCTION ON THE MALLARD DUCK AFTER DIETARY ADMINISTRATION [redacted] GLP: yes Published: no	yes	PBTFII
Selim, S.	A7.1.1.1.2/01	1995	Isolation and Identification of Major Degradates of Piperonyl Butoxide	no	PBTFII

			(PBO) following aqueous photolysis Biological Test Center, Irvine, CA 92713-9791-USA Endura S.p.A Report-no. P0594010 GLP: no Published: no		
Stäbler, D.	A7.4.3.5.1/01	2006	ASSESSMENT OF SIDE EFFECTS OF PIPERONYL BUTOXIDE ON THE LARVAE OF THE MIDGE, CHIRONOMUS RIPARIUS WITH THE LABORATORY TEST METHOD GAB Biotechnologie GmbH, Niefern-Öschelbronn, Germany Endura S.p.A Report-no. 20051329/01-ASCr GLP: yes Published: no	yes	Endura S.p.A
[redacted]	A7.4.3.3.1/01	1992	A BIOCONCENTRATION STUDY WITH PIPERONYL BUTOXIDE IN TH BLUEGILL (LEPONIS MACROCHIRUS) [redacted] GLP: yes Published: no	yes	PBTFII
Warmers, C.	A7.5.4.1/02	2006b	PB80 EC-NF: A RATE RESPONSE TEST TO STUDY THE EFFECTS ON THE PREDATORY MITE, TYPHLODROMUS PYRI SCHEUTEN (ACARI, PHYTOSEIIDAE) UNDER EXTENDED LABORATORY CONDITIONS GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Endura S.p.A Report-no. 20061006/01-NETp GLP: yes Published: no	yes	Endura S.p.A
Warmers, C.	A7.5.4.1/03	2006d	PB80 EC-NF: A DOSE RATE RESPONSE TEST TO STUDY THE EFFECTS ON THE APHID PARASITOID, APHIDIUS RHOPALOSIPHI DE STEFANI PEREZ (HYMENOPTERA, BRACONIDAE) UNDER EXTENDED LABORATORY CONDITIONS GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Endura S.p.A Report-no. 20061006/01-NEAp GLP: yes Published: no	yes	Endura S.p.A
Williams, M.D.	A7.1.2.2.2/02	1991c	Aerobic Aquatic Metabolism of Piperonyl Butoxide ABC Laboratories, Columbia, Missouri, USA	yes	PBTFII

			Endura S.p.A Report-no. 38549 GLP: yes Published: no		
Williams, M.D.	A7.1.2.2.2/03	1991d	Anaerobic Aquatic Metabolism of Piperonyl Butoxide ABC Laboratories, Columbia, Missouri, USA Endura S.p.A Report-no. 38507 GLP: yes Published: no	yes	PBTFII
Williams, M.D.	A7.2.2.4/01	1991f	Anaerobic Soil Metabolism of Piperonyl Butoxide ABC Laboratories, Columbia, Missouri, USA Endura S.p.A Report-no. 38585 GLP: yes Published: no	yes	PBTFII

Environmental Risk Assessment (product: AquaPy)

Authors	Section point/ reference number	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data protection claimed yes/no	Owner
Balluff, M.	B7.8.6/01	2006	AQUAPY: A GREENHOUSE TOXICITY STUDY TO DETERMINE THE EFFECTS OF A 30 G AI/L PYRETHRINE AND 135 G AI/L PIPERONYL BUTOXIDE EW FORMULATION ON THE VEGETATIVE VIGOUR OF SIX SPECIES OF PLANTS GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20054072/S2-FGVV GLP: yes Published: no	yes	BES
Bealing, D.J.	A7.4.1.4/01	2002	PIPERONYL BUTOXIDE: DETERMINATION OF INHIBITION OF RESPIRATION OF ACTIVATED SLUDGE Covance Laboratories Ltd., Harrogate, UK Endura S.p.A Report-no. 2145/2-D2149 GLP: no Published: no	YES	Endura S.p.A
Bocksch, S.	B7.8.2/01	2005	ASSESSMENT OF SIDE EFFECTS OF AQUAPY ® (PBO+PYR EW 135+30A G) TO THE HONEY BEE, Apis mellifera L., in the Laboratory GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-BLEU GLP: yes Published: no	yes	BES
Bowman, B.	A3.2/01	1989	DETERMINATION OF THE VAPOR PRESSURE OF PIPERONYL BUTOXIDE ANALYTICAL BIO-CHEM. LAB., INC., COLUMBIA, MISSOURI, USA ENDURA S.P.A Report-no. 38007 GLP: yes Published: no	YES	Endura S.p.A
Dengler, D.	B7.7.1.1/03	2005	TESTING OF TOXIC EFFECTS OF AQUAPY ON THE SINGLE CELL GREEN ALGA PSEUDOKIRCHNERIELLA SUBCAPITATA (FORMERLY SELENASTRUM CAPRICORNUTUM) GAB Biotechn. GmbH & IFU Umweltanalytik GmbH, Germany Bayer ES Report-no. 20051085/01-AAPs GLP: yes Published: no	yes	BES

Kölzer, U.	B7.8.4/01	2005a	ACUTE TOXICITY OF AQUAPY ON EARTHWORMS, EISENIA FETIDA USING AN ARTIFICIAL SOIL TEST GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-NLEf GLP: yes Published: no	yes	BES
Kölzer, U.	B7.8.5/01	2005b	ASSESSMENT OF THE SIDE EFFECTS OF AQUAPY ON THE ACTIVITY OF THE SOIL MICROFLORA GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-ABMF GLP: yes Published: no	yes	BES
Large, R.	A7.2.1/02	1996	Piperonyl Butoxide Aerobic Soil Metabolism. Review of Proposed Structure of Metabolite M8 in HRC PBT 7/951484 - Endura S.p.A Report-no. 9610/8926 GLP: no Published: no	yes	PBTFII
[redacted]	B7.7.1.1/01	2005a	ACUTE TOXICITY TESTING OF AQUAPY IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) (TELEOSTEI, SALMONIDAE) [redacted] GLP: yes Published: no	yes	BES
Stäbler, D.	B7.7.1.1/02	2005b	ASSESSMENT OF TOXIC EFFECTS OF AQUAPY ON DAPHNIA MAGNA USING THE 48 H ACUTE IMMOBILISATION TEST GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-AADm GLP: yes Published: no	yes	BES
Warmers, C.	B7.8.3/01	2005a	AQUAPY: TOXICITY TO THE PREDATORY MITE, TYPHLODROMUS PYRI SCHEUTEN (ACARI, PHYTOSEIIDAE) IN THE LABORATORY (RATE RESPONSE TEST) GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-NLTp GLP: yes Published: no	yes	BES

Warmers, C.	B7.8.3/02	2005b	AQUAPY: ACUTE TOXICITY TO THE APHID PARASITOID APHIDIUS RHOPALOSIPHI DE STEFANI PEREZ (HYMENOPTERA, BRACONIDAE) IN THE LABORATORY (RATE RESPONSE TEST) GAB Biotechn. GmbH & GAB Analytik GmbH, Niefern-Öschelbronn Bayer ES Report-no. 20051085/01-NLAp GLP: yes Published: no	yes	BES