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Ecotoxicity of wood preservatives (SOLTOX)

Current knowledge and evaluation of potential toxicity for soil organisms

April 2015

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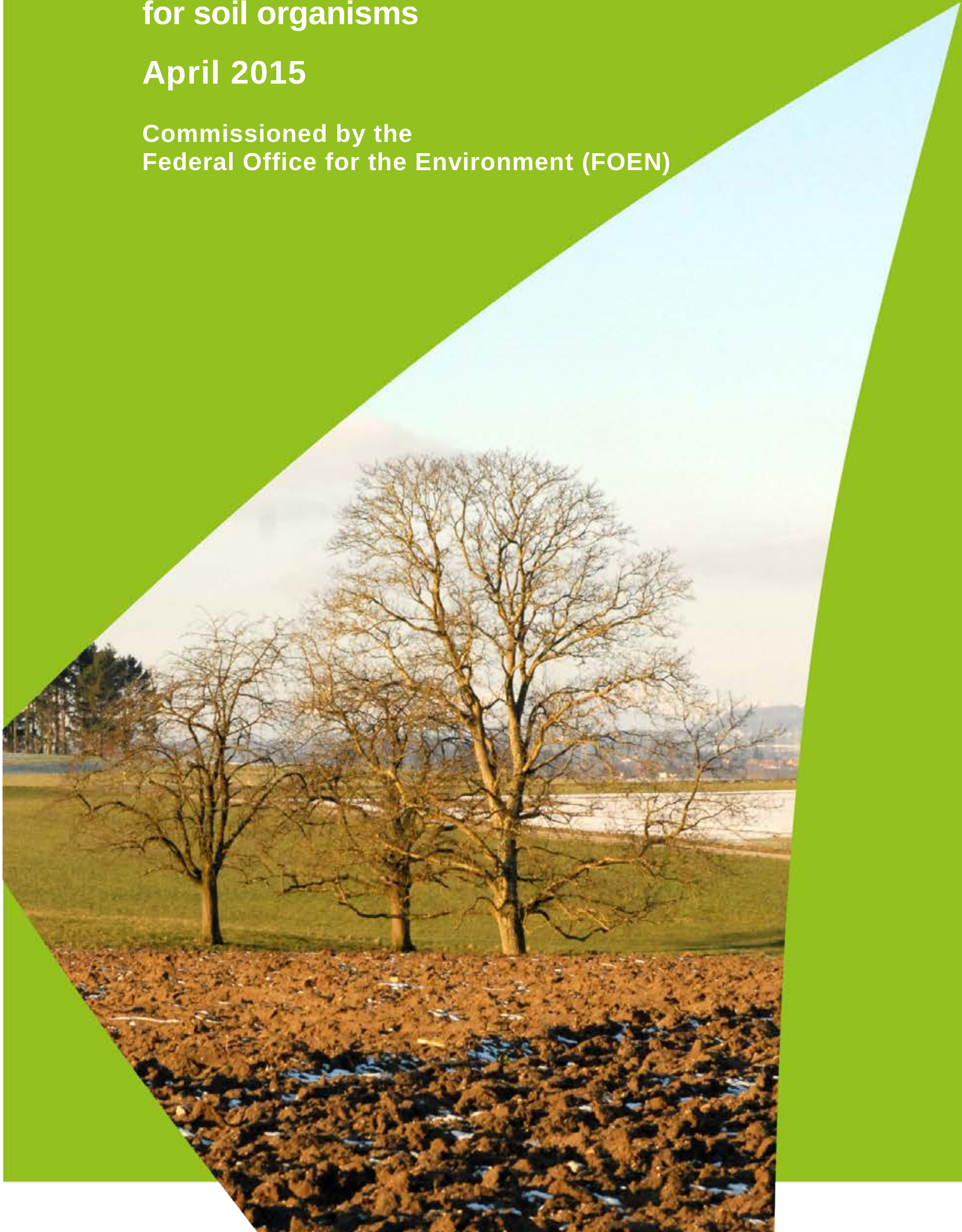

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Summary

Wood preservatives are biocidal products used to protect wood building materials from wood-destroying or wood-disfiguring organisms. Active ingredients such as metallic salts, quaternary ammonium salts, carbamates and azoles are frequently employed. Solid timber or wood based products are used for diverse service situations and are therefore classified according to different "Use Classes", as defined by the European standard EN 335: 2013. Situations for which wood product is above ground and exposed to the weather or in direct contact with ground are classified under use class 3 and 4, respectively. In this case, the chemical substances employed are likely to reach the soil compartment either by leaching from the treated wood surface or by direct contact between the wood and the soil. They may then pose a potential risk for the soil organisms, the essential functions they performed and thus for the soil ecosystem in general. Currently, the available toxicity data for terrestrial organisms regarding the active ingredients present in wood preservatives are still scarce.

The aim of this project was to synthesize the available knowledge regarding the toxicity for terrestrial organisms of representative active ingredients contained in wood preservatives and authorized for use in Switzerland, and to provide missing data where necessary. Therefore, a market analysis was performed to determine the active ingredients the most frequently used (calculated as the number of entries of active ingredients registered in wood preservatives authorized in Switzerland) and present on the Swiss market. More than 60% of the number of entries of active ingredients were represented by only 4 substances, i.e. IPBC, Propiconazole, Permethrin, and Tebuconazole, all belonging to use class 3. Boric acid, quaternary ammonium salts and copper based salts represented more than 15% of the number of entries for use class 4.

Four wood preservatives containing the selected active ingredients, either present as a single substance or in a mix, were chosen for the ecotoxicological evaluation. They contained: 1) IPBC, 2) Propiconazole, 3) Boric acid and Copper(II)hydroxide, with Chromium as fixing agent; referred to as "CuCrB" 4) Copper(II)carbonate-Copper(II)hydroxide, Didecylpolyoxethylammoniumborat (DPAB) and Boric acid; referred to as "Quats". A review of the toxicity data available for terrestrial organisms for the chosen active ingredients was made. Literature was also searched for mixture toxicity data regarding the active ingredient combinations present in the corresponding wood preservatives. The literature search showed a lack of information regarding the toxicity of IPBC, Propiconazole and DPAB for terrestrial organisms. Toxicity of Boric acid and Copper based salts was better documented but no information on mixture toxicity was found for the active ingredient combinations. Therefore, collembolan reproduction tests and earthworm avoidance tests were performed in order to determine effect concentrations, either for the single active substance or for the active ingredient mixture contained in the wood preservatives.

For IPBC, similar range of toxicity were obtained for collembolans and earthworms, with EC50s of 40 mg a.i./kg d.w. and of 33 mg a.i./kg d.w., respectively. Earthworms seemed to be more sensitive to Propiconazole than collembolans with EC50s of 52 mg a.i./kg d.w. and 192 mg a.i./kg d.w., respectively. The 2 wood preservatives containing a mix of active ingredients showed a quite high toxicity for collembolans but even greater for earthworms taking into account that 2 or more active ingredients were present in the biocidal products. For CuCrB, EC50s of 239 mg/kg d.w. and of 11 mg/kg were found for collembolans and earthworms, respectively. For Quats, the EC50 was of 873 mg/kg for collembolans and of 47 mg/kg d.w. for earthworms. The toxicity data found in the literature for the individual active ingredients themselves were much greater than the corresponding concentrations of these substances present in the wood preservative for which a toxicity was observed.

Leaching data in field situation for the selected active ingredients contained in wood preservatives are nearly unavailable and data on measured environmental soil concentrations were not found. This project will provide the basis for future studies aiming at characterizing the impact of wood preservatives on soil organisms in microcosms or in field conditions.

Content

6 Indices

1 Introduction

Wood ageing and degradation by insects and microorganisms belong to the natural life cycle of wood. Wood species have not the same sensitivity to degradation depending on their anatomical features, extractive contents and position in the trunk. Most of the species in Europe are sensitive to fungal attacks, especially if they are brought in contact with the soil. For wood outdoor applications like for examples façades, decks, poles, barrier or railway sleepers, the wood need to be protected from biological attack to reduce quality loss and incidents. For this purpose, wood preservative treatments are employed. Wood preservatives are defined by the European Committee for Standardisation (CEN, 35th Meeting of CEN/TC 38) as "active ingredient(s) or preparations containing active ingredient(s) which are applied to wood or wood-based products themselves, or which are applied to non-wood substrates (e.g. masonry and building foundations) solely for the purpose of protecting adjacent wood or wood-based products from attack by wood-destroying organisms (e.g. dry rot and termites)" (OECD 2013). Wood preservatives are often combinations of two or more active ingredients to increase the efficacy of the product formulation.

Most of the usual wood preservatives used are leached into the environment, especially into water and soil. They may then pose a potential risk for the soil organisms, the essential functions they performed and thus for the soil ecosystem services in general. Currently, ecotoxicity data regarding wood preservatives are mainly available for aquatic organisms. Data for soil organisms are still relatively scarce.

The objective of this project was to synthesize the available information on the ecotoxicity for soil organisms of the most relevant active ingredients authorized for use in Switzerland in wood preservatives and to provide missing data where needed. The following actions have been undertaken:

- The market situation of wood preservatives in Switzerland has been analysed in order to define the current tendency in the evolution of the type of active ingredients used and the quantity (number of entries) of these substances present on the market. Four wood preservatives, containing the most relevant active ingredients employed in wood preservatives for Switzerland have been chosen accordingly. Active ingredients were present as a single substance or in a mix in the different wood preservatives selected. (Section 2.3).
- A bibliographic review has been conducted to collect physical and chemical data for the active ingredients of the selected wood preservatives (mobility, persistence, bioaccumulation...) and their available toxicity data (effect and no effect concentrations) for terrestrial organisms (Section 2.5). Data on mixture toxicity was search as well for the active ingredients present in combination for the corresponding wood preservative.
- As toxicity data for terrestrial organisms were scarce or lacking for part of the selected active ingredients and as mixture toxicity data were not found for the concerned wood preservatives, standardized collembolan reproduction and earthworm avoidance tests were conducted for each of the 4 wood preservatives in order to obtain a part of the missing information (Section 4).

The project will provide the basis for future studies aiming at characterizing the impact of wood preservatives on soil organisms in microcosms or in field conditions.

This study was achieved in cooperation with the Institute for Materials and Wood Technology from the Bern University of Applied Sciences and Ecotox Centre of Eawag/EPFL.

2 Background Information

Biocidal products used for wood preservation belong to the Product Type 8 (PT8; "Wood preservatives")" according to the Annex 10 of the Swiss Ordinance on Biocidal Products (OPBio; RS 813.12) and Annex V of the Biocidal Products Regulation (BPR, (Regulation (EU) 528/2012) regulating their placing on the market and use. According to the Swiss product register for chemicals (https://www.rpc.admin.ch; March 2015), 231 products are listed as wood preservatives and authorized for use in Switzerland. It represents 32 active ingredients (state August 2014; Annex II of the OPBio; RS 813.12), about 13 of which being the most frequently used in PT8 (Burkhardt and Dietschweiler 2013, Empa-Lignum 2014). As biological agents likely to attack wood material are insects (e.g. wood boring beetles) and/or fungi (e.g. blue mould, decay, soft rot, disfiguring fungi), the majority of these active ingredients are either insecticides or fungicides, generally present in a mixture in wood preservatives (OECD 2003). In order to rate the biological hazards to which wood commodities can be exposed and to allow the choice of appropriate wood preservatives and treating methods, a system of "Hazard or Use Classes" has been defined.

2.1 Use classes and preservation systems

Depending on the environmental conditions (humidity, soil contact, type of exposure, duration of exposure) and on the related biological hazards, use classes are defined from 1 (wood inside a construction, not exposed to weather and wetting) to 5 (permanent contact with salt water) according to the European standard EN_335 (2013). This study will focus on active ingredients used in hazard class 3 (wood not covered and not in contact with the ground, continually exposed to weather or protected from the weather but subject to frequent wetting) and 4 (wood in contact with the ground) because they correspond to a possible risk of leaching into the soil. Naturally non-durable wood species are treated with the following typical active ingredients for class 3 and 4 in Switzerland (Empa-Lignum 2014) (Table 1):

Table 1: Typical biocidal active ingredients used in class 3 and 4 for Switzerland.

Active ingredients typically employed in use class 3:	**Active ingredients typically employed in use class 4:**
Iodopropynyl butylcarbamate (IPBC)	Boric acid
Propiconazole	Quaternary Ammonium salts (Quats)
Tebuconazole	Copper based salts
Permethrin and/or Cypermethrin	Creosote
Dichlofluanid and Tolyfluanid	

Most of the commercialized wood preservatives contain several of these active ingredients combined in a mix, water or solvent-based. Some of them are oil-based (for example creosote). Active ingredients of the class 4 can be mixed in a product of the class 3 and vice-versa.

2.2 Estimation of the emissions of biocidal active ingredients into the environment

Different wood treatment processes exist to ensure adequate application, penetration and retention of the chemical preservatives in the selected wood species to guarantee its preservation. After wood impregnation, the biocidal active ingredients often act via slowly release on the material surface. However, if wood materials are exposed to outdoor weathering, this can also cause leaching of substances into environmental compartments. Leaching rates for estimation of emissions is a required data set for authorisation of active ingredients and biocidal products according to the Biocidal Products Directive 98/8/EC (EU_98/8/EC 1998). Throughout the service life of the wood product and under constant exposure conditions, the release of biocidal substances into the environment does not occur as a single event but is a rather continuous process with decreasing emission rates over time. This leaching process can be evaluated either by performing laboratory tests or outdoor exposure experiments and should discriminate between early and later stages of this process (Schoknecht and Töpfer 2012).

For PT8, laboratory leaching tests are proposed by CEN (DIN_CEN 2008a, b) and OECD (OECD 2007). The exposure conditions of the tests are designed for different wood service life conditions, mainly for either occasional or permanent contact with water, more rarely for contact with soil. The AWPA E20-04 (AWPA_E20-04 2004) procedure is one of the only standard method for determining the leachability of wood preservative active ingredients in permanent contact to wet soil. Semi-field tests also exist (e.g. the NT Build 509 procedure (NT_BUILD-509 2005), and aimed at investigating leaching processes closely linked to service life conditions (natural weathering) over the long-term (minimum one year, most likely 2 years). However, public data available for this kind of studies are scarce (see Schoknecht and Töpfer (2012) for more details about biocidal active ingredient leaching characterization).

These leaching rates can then be used further as input data in exposure assessment (emission rates, distribution and PEC) (OECD 2003).

2.3 Market situation of wood preservatives in Switzerland

In order to support the evaluation of the ecotoxicological risks of wood preservatives for soil organisms, their market situation can be observed. This makes possible to define current trends of the type of active ingredients used and the quantity of these substances present on the market. On the other side, the evolution of the market of wood products for outdoor applications can also give indications of the potential increase or decrease of the utilization of a wood preservative.

2.3.1 Comparison of the market evolution of wood preservatives between 2011 and 2014

In order to observe the evolution tendency on the market of the main active ingredients used in Switzerland (especially typical active ingredients for the use class 3 and 4) the data collected in 2011 by Burkhardt and Dietschweiler (2013) have been compared to the newest dataset from 2014 (Empa-Lignum 2014). These data are based on the amount of entries of each active ingredient for each registered wood preservative. The following figure (Figure 1) highlights the changes in the amount of entries. The most relevant active ingredients are IPBC, Propiconazole, Permethrin and Tebuconazole for the class 3 and Boric acid and Quats for the class 4. The occurrence of Fluorine based substances (Dichlofluanid and Tylofluanid) is decreasing. The entries for IPBC, Propiconazole and Tebuconazole were sensibly increasing between 2011 and 2014. IPBC is also used for other applications such as for the treatment of non-wood-based claddings and masonry (Burkhardt and Dietschweiler 2013). In consequence, it can be considered as the most relevant active ingredient.

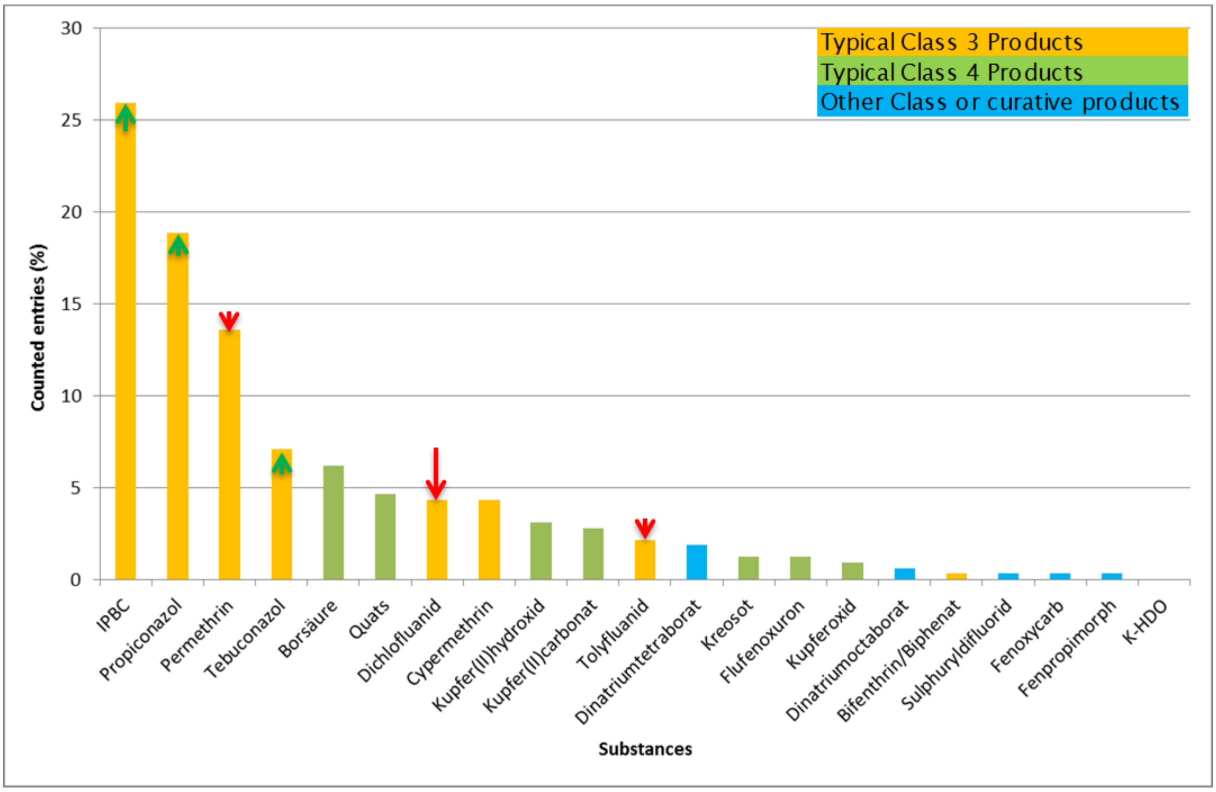

Figure 1: Counted entries of active ingredients in the registered wood preservatives for Switzerland (the length of the arrows represents the relative change between 2011 and 2014).

2.3.2 Market evolution of the different wood products which need a protection by wood preservation systems

Data concerning the evolution of the volumes of wood treated with the active ingredients cited above were not found. However, it is possible to observe trends of the wood building market. As most of the wood preservatives containing IPBC are employed in use class 3, the objects mostly represented in this category are wooden façades. The quantity of wood construction projects, especially multi-storey buildings, has increased in the last year and is predicted to increase even more in the next year as reported by Neubauer-Letsch et al. (2014). This means that the volume of treated wood for the cladding of the multi-storey buildings should also increase as well as the treated wood used for decks around these buildings.

Concerning the wooden products which are in contact with the soil (mainly railway sleepers and utility poles) it seems to be a relatively stable market. They might be decreasing sensibly in the future due to the replacement of wooden sleepers by concrete sleepers and due to the burying of electrical and communication lines.

2.4 Selection of wood preservatives

The market of wood preservatives is evolving slowly and shows that active ingredients used mostly for the utilization of wood in class 3 are the most relevant. From the tendencies mentioned above, we can consider IPBC, Propiconazole and Tebuconazole as key active ingredients for the class 3 and the Boric acid, Quats and Copper-based salts as key active ingredients for the class 4, for consideration of ecotoxic risks to the soil.

In the end, 4 different wood preservatives were selected for soil ecotoxicity testing. Two of them contained only an active ingredient. The two other ones corresponded to a mix of active ingredients, as is frequently the case. Indeed, wood preservatives are often combinations of 2 or more active ingredients and then mixture toxicity is an issue of concern. They contained the following active ingredients: 1) IPBC, 2) Propiconazole, 3) Copper(II)hydroxide and Boric acid with Chromium as fixing agent (i.e. Copper-Chromium-Boron salts) and 4) Copper(II)carbonate-Copper(II)hydroxide, Didecylpolyoxethylammoniumborat and Boric acid. They are described more in details in Section 3.1.

2.5 Proprieties, use and ecotoxicity of the selected biocidal active ingredients

The main properties of the selected biocidal active ingredients are summarized in the table below (Table 2).

Table 2: Main properties and use of the selected biocidal substances.

Substance class	**Active substance**	**Synonyms**	**CAS N°**	**Biocidal effect**
Carbamate	Iodocarb	IPBC, 3-Iodo-2-propynyl N-butylcarbamate; 3-Iodo-2-propynyl butylcarbamate; butyl-3-iodo-2-propynyl ester; Iodopropynyl butylcarbamate	55406-53-6	Fungicide (PT8) Bactericide Algaecide
Triazole	Propiconazole	-	60207-90-1	Fungicide (PT8)
Metallic salts	Boric acid (H_3BO_3)	Orthoboric acid; Boracic acid, Boron hydroxide	10043-35-3	Fungicide (PT8) Insecticide (PT8) Acaricides Algaecides Herbicides
	Copper hydroxide ($Cu(HO)_2$)	Copper hydroxide; Cuprous hydroxide; Copper monohydroxide; Copper(II)hydroxide	20427-59-2	Fungicide (PT8) Insecticide (PT8) Algaecide Bactericide Nematocide Molluscicide
	Copper(II) carbonate--Copper(II) hydroxide (1:1) ($CuCO_3.Cu(OH)_2$)	-	12069-69-1	
Quaternary ammonium salts	Didecylpolyoxethylammoniumborat	DPAB, polymeric betain; Copper amine preservative	214710-34-6	Fungicide (PT8) Insecticide (PT8)

2.5.1 IPBC

IPBC is a broad based biocide and is used in several product types (e.g. PT 6, 7, 8, 9,10, 11, 13 (Schoknecht and Töpfer 2012). It is employed as preservative in wood products, but also in surface and building materials, paint, adhesives, metal cutting fluids, emulsions, plastics, textiles, inks, paper coatings and personal care products and cosmetics. It is also applied in ventilation and air conditioning ducts and equipments as well as in heating to control fungi and mold (USEPA 1997). IPBC is expected to be rapidly degraded into soil with DT50lab (aerobic) of 2.1 hours at 22°C, 4.7 hours at 12°C, and 8.6 hours at 5° C. Due to fast degradation of IPBC observed in laboratory studies (DT50lab), field studies are not required. IPBC has a medium to high mobility potential in soil (Koc[1] = 126 ml/g) and based on its log Kow[2] value of 2.8, its bioaccumulation potential is considered as not significant (EU 2008, 2013b).

Because of the use and application of IPBC, exposure is not expected to be significant for wildlife and toxicity data for terrestrial vertebrates, honey bees and other non-target organisms are not required for this Product Type (PT8) (EU 2008, 2013b). Toxicity data for soil organisms are scarce and were only found for earthworms (acute studies only), terrestrial plants and microorganisms. They were all obtained from the Assessment Reports on active substance evaluation for IPBC in PT6 and PT8 (EU 2008, 2013b) and are given in the table below (Table 3). No data on IPBC toxicity for collembolans were found in the literature.

However, toxicity data for other carbamates compounds are available and show a relatively high toxicity for collembolans and earthworms. A few toxicity data for carbamates are provided in the Table 4.

Table 3: IPBC toxicity data for soil organisms.

Organisms (species)	**Endpoints**	**Toxicity value**	**Study type**	**Substrate**	**Reference**
Earthworms (*Eisenia fetida*)	Mortality (14 days)	LC50 > 1000 mg/kg d.w. soil	Lab	artificial soil	EU (2008) EU (2013b)
Microorganisms (-)	Carbon mineralization	EC50 = 312.5 mg/kg d.w. soil	Lab	n.r.	
Terrestrial plants (*Avena sativa*)	Fresh weight reduction	EC50 of 4.92 mg/kg d.w. soil	Lab	n.r.	

n.r. = not reported; d.w. = dry weight

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[1] Koc is a binding coefficient commonly used to describe a pesticide's tendency to sorbs to organic carbon. A high Koc value (greater than 1000) indicates the pesticide sorbs strongly to organic carbon and is not likely to be transported, except with eroding soil particles. Pesticides with low Koc values (less than 300 to 500) are less strongly adsorbed and tend to be transported more readily with runoff water (Gillespie et al. 2011).

[2] According to U.S. Environmental Protection Agency (2009): "the logarithm of the octanol-water partition coefficient (Kow) represent the ratio of the solubility of a compound in octanol (a non-polar solvent) to its solubility in water (a polar solvent). The higher to Kow, the more non-polar the compound. The Log Kow is generally used to quantifies the lipophilicity of a substance and is assumed to be an index of the ability to pass through biological membranes and to bio-accumulate in living organisms. It is utilized as a measure of the affinity of a pesticide for the biota. Bio-accumulating pesticides have a Log Kow >3 (Gillespie et al. 2011).

Table 4: Carbamate toxicity data for collembolans and earthworms

Organisms **(species)**	**Carbamates**	**Endpoints**	**Toxicity value**	**Reference**
Collembolans (*Folsomia candida*)	Fenoxycarb	Reproduction (28 days)	EC50 = 113 mg a.i/kg d.w. NOEC = 12.5 mg a.i./kg d.w.	Campiche et al. (2006)
	Phenmedipham		EC50 = 10.1 mg ai./kg NOEC 5.0 mg a.i/kg	Amorim et al. (2005)
	Carbofuran		EC50 = 0.09 mg a.i/kg d.w.	Heupel (2000)
Earthworms (*Eisenia fetida*)	Carbaryl	Mortality (7 days & 14 days)	LC50 = 152.2 mg a.i/kg d.w. LC50 = 133.5 mg a.i/kg d.w.	Wang et al. (2012)
	Carbosulfan		LC50 = 146.8 mg a.i/kg d.w. LC50 = 130.1 mg a.i/kg d.w.	
	Isoprocarb		LC50 = 69.4 mg a.i/kg d.w. LC50 = 60.8 mg a.i/kg d.w.	
	Metolcarb		LC50 = 108.1 mg a.i/kg d.w. LC50 = 93.8 mg a.i/kg d.w.	
	Promecarb		LC50 = 31.23 mg a.i/kg d.w. LC50 = 28.43 mg a.i/kg d.w.	

a.i. = active ingredients; d.w. = dry weight

2.5.2 Propiconazole

Propiconazole is registered for use as wood preservative but also to control fungi in agriculture and on turf and ornamentals (USEPA 2006, Health_Canada 2012). A DT50lab of 43 days at 20°C is measured for degradation of Propiconazole in soils. For field studies, a DT50field of 49 days is obtained (geometric mean dissipation, Central Europe conditions). Accumulation in soil under Northern European condition cannot be excluded. With a Koc of 944 ml/g (arithmetic mean from 9 soils), Propiconazole is of limited mobility and is expected to adsorb to soil. Regarding its bioaccumulation potential, a log Kow of 3.72 (25°, pH 6.6) is measured for Propiconazole.

Toxicity data to terrestrial species are available for 3 trophic levels, i.e. earthworms (acute and chronic studies), terrestrial plants and microorganisms (Table 5) and are reported in several evaluation dossiers and assessment reports (EU 2003, 2013a, ANSES 2014). Propiconazole also being used as an active ingredient in Plant Protection Products (PPP), a few toxicity data can be found in this context. For example, Frampton and Wratten (2000) have investigated the effects of Propiconazole on epigeic species of Collembola in arable crops (see Table 5 for details). No other data were found in the literature on the toxicity of Propiconazole to soil organisms.

Table 5: Propiconazole toxicity data for soil organisms.

Organisms **(species)**	**Endpoints**	**Effect/no effect concentration**	**Study type**	**Sub-strate**	**Reference**
Earthworms (*Eisenia fetida*)	Mortality	LC50 = 686 mg a.i./kg d.w. (= 205 mg ai/kg w.w.; at 3.4% organic matter and using conversion factor of 0.88 from d.w. to w.w.) (LC50corr[1] = 343 mg/kg)	Lab	n.r	EU (2013a) EU (2003) ANSES (2014)
	Reproduction	NOEC = 0.998 mg a.i./kg (NOECcorr[1] = 0.417 mg/kg)			
Microorganisms (-)	Nitrogen mineralization	EC50 > 1.67 mg a.i./kg d.w. (= 2.16 mg ai/kg w.w. at 3.4% organic matter and using a conversion factor of 0.88 from d.w. to w.w.)	Lab.	n.r.	EU (2013a)
		NOEC = 1.67 mg a.i./kg d.w. (= 2.16 mg ai/kg w.w.; 3.4% organic matter and using a conversion factor of 0.88 from d.w. to w.w.)			
Terrestrial plants (n.r.)	Seedling emergency and survival	EC50 = 4.32 of mg a.i./kg w.w. (at 3.4% organic matter)	Lab.	n.r.	EU (2013a)
	Reproduction	NOEC = 0.96 mg a.i./kg d.w. soil (= 1.69 mg a.i./kg w.w. soil (at 3.4% organic matter)			
Collembolans (higher taxa)	Abundance	Transient negative effect at field application rate of 0.5L/ha[2] (Tilt 250EC, 25% a.i. EC)	Field	Natural soil	Frampton and Wratten (2000)

[1] LC50corr/NOECcorr = endpoint corrected due to log Kow >2.0

[2] According to the FOCUS model (Sanco 2006), this would correspond to an initial PEC of 0.17 mg a.i./kg soil (worst case, immediately after application, considering a mixing depth of 5 cm and bulk density of 1.5 g/cm^3)

a.i. = active ingredient; n.r. = not reported; d.w. = dry weight; w.w = wet weight;

2.5.3 Boric acid

Apart from its use as wood preservative, Boric acid (H_3BO_3) is also employed in a wide varieties of sites such as sewage systems, transportation and storage facilities, food and non-food crops, veterinary institutions and animal housing, swimming pool, outdoor residential areas, and indoor sites such as homes, hospitals, and commercial buildings (USEPA , HHS 2010). Boron is a widely occurring element in minerals found in the earth's crust and cannot be degraded in the environment. Boric acid therefore does not comply with the persistence criteria assessment in soil (Annex VI of the Biocides Directive) and is considered as persistent (EU 2009, HHS 2010).

The toxicity of H_3BO_3 for terrestrial organisms is well documented. Indeed, Boric acid is the recommended reference substance for earthworm avoidance test (ISO 2008b) and collembolan reproduction test (OECD 2009) and has also been proposed as reference substance for other soil organisms (Becker et al. 2011). Some relevant EC50s for Boric acid for soil species are given in the table below (Table 6).

Table 6: H_3BO_3 toxicity data for soil organisms.

Organism (species)	**Endpoint**	**EC50 (mg/kg d.w. soil)**	**Substrate**	**Reference**
Microorganisms (-)	Nitrogen trans-formation	>2400	LUFA 2.3[1]	Becker at al. (2011)
Terrestrial plants *(Avena sativa)*	Biomass (fresh weight);	182		
	Shoot length	308		
Terrestrial plants *(Brassica napus)*	Biomass (fresh weight);	175		
	Shoot length	357		
Nematodes *(Caenorhabditis elegans)*	Reproduction	747	LUFA 2.2	
Enchytraieds *(Enchytraeus crypticus)*	Reproduction	220	OECD artificial soil	
Enchytraeids *(Enchytraeus luxuriosus)*	Reproduction	228		
Earthworms *(Eisenia fetida)*	Reproduction	484		
Earthworms *(Eisenia andrei)*	Avoidance	700	Lufa 2.2	Ecotox Centre data (unpublished)
Collembolans *(Folsomia candida)*	Avoidance	1441	OECD artificial soil	Becker at al.(2011)
Collembolans (*Folsomia candida and Folsomia fimetaria*)	Reproduction	100	n.r.	OECD (2009)
Isopods *(Poecilus cupreus)*	Food uptake	1342	LUFA 2.1	Becker at al. (2011)

[1] LUFA 2.1, 2.2, 2.3 are European natural field soil often used for soil ecotoxicity testing

d.w. = dry weight

2.5.4 Copper-based salts

Copper-based salts are employed as biocide for a large variety of use and more specifically as fungicide and insecticide for wood preservation (see Table 2). As for Boric acid, persistence criteria are not applicable to Copper(II)hydroxide and Copper carbonate (EU 2011a, b).

Regarding the toxicity of Copper to soil organisms, a large amount of studies can be found in the literature as well. As an example, the ECOTOX database from the US Environmental Protection Agency (US EPA; epa.gov/ecotox/) listed more than 60 scientific references, for the toxicity of CuCl2 only, for terrestrial species. In a study performed by Scott-Fordsmand et al. (2000), the toxicity of Copper for collembolans was studied for freshly spiked soil and for long-standing contaminated soil from a timber impregnating factory (contamination occurred between 1911 and 1924). The results obtained from this study (Table 7) underline the importance of taking soil ageing into account, as it can be expected that testing freshly spiked soils can overestimate the toxicity of chemical compounds to soil organisms in comparison to long-term equilibrated soils. Toxicity data found for earthworms are also summarized in Table 7.

Table 7: Cu toxicity data for soil organisms.

Organism (species)	**Endpoint**	**Effect/no-effect concentrations**	**Substrate**	**Reference**
Collembolans (*Folsomia fimetaria*)	Reproduction (21 days)	LOEC; NOEC; EC10; EC50 > 2912 mg Cu/kg d.w.	Natural contaminated soil with 3.9 to 5.5% humus; Long standing contamination (>70 years; > 2,911 mg Cu/kg d.w.)	Scott-Fordsmand et al.(2000)
	Mortality (21 days)	LOEC; NOEC; LC10; LC50 > 2912 mg Cu/kg d.w.		
	Reproduction (21 days)	EC10 = 337 mg Cu/kg d.w. soil EC50 = 994 mg Cu/kg d.w. soil NOEC = 400 Cu/kg d.w. soil LOEC = 600 mg Cu/kg d.w. soil	Natural soil with 3.9 to 5.5% humus, only containing background Cu concentrations (15 mg Cu/kg) Freshly spiked with CuCl2 (1 day before experiment)	
	Mortality (21 days)	LC10 = 813 mg Cu/kg d.w. soil LC50 = 2141 mg Cu/kg d.w. soil NOEC = 1000 Cu/kg d.w. soil LOEC = 2000 mg Cu/kg d.w. soil		
Earthworms (*Eisenia fetida*)	Mortality (14 days)	LC50 = 836 mg/kg d.w. soil NOEC = 293 mg/kg d.w. soil	OECD artificial soil	Spurgeon and Hopkin (1995)
		LC50 = 683 mg/kg d.w. soil	OECD artificial soil	Spurgeon et al. (1994)
	Mortality (56 days)	LC50 = 555 mg/kg d.w. soil NOEC = 210 mg/kg d.w. soil	OECD artificial soil	Spurgeon et al. (1994)
	Cocoon production (56 days)	EC50 = 53.3 mg/kg d.w. soil NOEC = 32 mg/kg d.w. soil		
	Cocoon production (21 days)	LC50 = 716 mg/kg d.w. soil NOEC = 29 mg/kg d.w. soil	OECD artificial soil	Spurgeon and Hopkin (1995)
Earthworms (*Lumbricus rubellus*)	Cocoon production (6 weeks)	NOEC = 54 mg Cu/kg LOEC = 131 Cu/kg	agricultural sandy soil (loamy sand) 5.7 % organic matter	Ma (1984)

d.w. = dry weight

2.5.5 Polymeric betain (DPAB)

Polymeric betain was developed as a co-biocide for Chromium-free Copper based wood preservatives and is shown to be efficient against fungi and insects. Only very limited information is available regarding this active ingredients and toxicity values for soil species were not found. For aquatic organisms, a LC50 (96h static) of 0.89 mg/L and a NOEC of 0.54 mg/L are reported for the Bluegill sunfish. An EC50 of 0.39 mg/L and a NOEC of 0.06 mg/L are given for *Daphnia magna* (Härtner et al. 2008, 2009).

2.5.6 Chromium

The presence of hexavalent Chromium (Cr(VI)), used as fixing agent, in the CuCrB formulation must be noted. Cr(VI) is not intended to have a biocidal role in wood preservation but could still have toxic effects on soil organisms, in case of leaching for example. Cr(VI) is reduced to trivalent chromium Cr(III) after wood impregnation. A few studies reporting the toxicological effects of Cr(III) and Cr(VI) on soil invertebrates can be found in the literature, the majority focusing on Cr(III). Toxicity values are reported in the table below (Table 8). In most of case and based on the comparison of toxicity data, Cr (VI) appeared to be generally more toxic than Cr (III) (Sivakumar and Subbhuraam 2005).

Table 8: Cr(III) and (VI) toxicity data for soil organisms.

Organism (species)	**Endpoint**	**Effect/no-effect concentrations**	**Substrate**	**Reference**
Earthworms (*Eisenia fetida*)	Mortality (14 days)	LC50 = 222 to 257 mg Cr(VI)/kg LC50 = 1656 to 1902 mg Cr(III)/kg	10 different soil types	Sivakumar and Subbhuraam (2005)
	Cocoon production (21 days)	EC50 = 892 mg Cr(III)/kg	OECD artificial soil	Lock and Janssen (2002)
	DNA damage (2 days) (comet assay on the earthworm coelomocytes)	Genotoxic effects of Cr (III) and Cr (VI) at 491 mg/kg soil (representative concentration for industrial contaminated site)	OECD artificial soil	Bigorgne et al. (2010)
Earthworms (*Eisenia andrei*)	Cocoon production (21 days)	NOEC = 32 mg Cr(III)/kg LOEC = 100 mg Cr(III)/kg	OECD artificial soil	van Gestel et al. (1993)
Enchytraieds (*Enchytraeus albidus*)	Reproduction (42 days)	EC50 = 637 mg Cr (III)/kg d.w. soil	OECD artificial soil	Lock and Janssen (2002)
Collembolans (*Folsomia candida*)	Reproduction (28 days)	EC50 = 604 mg Cr (III)/kg d.w. soil	OECD artificial soil	Lock and Janssen (2002)

d.w. = dry weight

3 Material and Methods

The ecotoxicity of the 4 selected wood preservatives to soil organisms was assessed using the two following tests:

- Collembolan Reproduction test with *Folsomia fimetaria* according to OECD guideline n° 232; (OECD 2009)
- Earthworm Avoidance test with *Eisenia andrei* according to ISO standard 17512-1 (ISO 2008b)

Based on the market analysis (section 2.3), the 4 following wood preservatives containing the bellow mentioned active ingredients were tested:

- IPBC
- Propiconazole
- Copper(II)hydroxide and Boric acid (i.e. Copper-Chromium-Boron salts). Hereinafter referred as "CuCrB".
- Copper(II)carbonate-Copper(II)hydroxide (1:1), DPAB and Boric acid (i.e. Copper-Amine salts). Hereinafter referred as "Quats"

(see Section 2.5 for details on the active ingredients).

3.1 Selection and preparation of the test concentrations

Based on the ecotoxicity review (Section 2.5) and in order to determine both NOEC/LOEC and ECx for each of the selected wood preservatives (i.e. for a single active ingredient or for a mix of active ingredients), 9 treatment concentrations within a geometric series (spacing factor of 3.2) were selected for both collembolans and earthworms toxicity tests. The chosen concentration ranges are given in table below (Table 9).

Table 9: Concentration ranges of the 4 selected wood preservatives for collembolan and earthworm toxicity tests (nominal concentrations)

Biocide formulation	**Tested concentrations[1]**	**Active ingredient(s)**
IPBC	0.10 to 1099.51 a.i. mg/kg d.w. soil	> 98% 3-Iod-2-propinyl-N-butylcarbamate (IPBC); as a powder
Propiconazole	0.10 to 1099.51 a.i. mg/kg d.w. soil	50% Propiconazole; 50% dipropylene glycol monomethyl ether; as a solution
CuCrB	0.004 to 49.15 g/kg d.w. soil	15% Copper(II)hydroxide; 4% Boric acid; Chromium (VI) as fixing agent; as a solution
Quats	0.004 to 42.60 g/kg d.w. soil	20% Copper(II)carbonate-Copper(II)hydroxide (1:1); 10% Didecylpolyoxethylammoniumborat; 8% Boric acid; as a solution

a.i. = active ingredient; d.w. = dry weight

[1] Concentrations were expressed in amount (mg) of the active ingredient when only one single substance was present in the wood preservative and in amount (mg) of the wood preservative preparation if a mix of active ingredients was present.

A negative control (nanopure water) without test substances was also prepared as well as an additional solvent control for IPBC and Propiconazole (see below) . Additionally, an EC50 positive control using Boric acid (H_3BO_3) as reference substance was used to ensure the performance of the test systems[3]. H_3BO_3 is the recommended reference substance for both collembolan reproduction and earthworm avoidance tests according to ISO 17512-1 and OECD guideline n° 232, respectively. An EC50 of 100 mg/kg d.w. soil was used for the collembolans (ISO 17512-1) while an EC50 of 700 mg/kg d.w. soil was used for the earthworms (Ecotox Centre data).

Each concentration of the corresponding wood preservative was tested in 4 replicates while the negative and solvent controls were tested in 8 replicates. The positive control with H_3BO_3 was tested in 5 replicates. The European natural field soil LUFA 2.2 (Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Speyer, Germany; loamy sand type, 43.5 % Water Holding Capacity (WHC), pH $CaCl_2$ 5.4 and 1.59% organic carbon (C), batch n° 0315) was used as substrate for all experiments.

For CuCrB and Quats, the wood preservatives were applied to the soil using water as a carrier while an organic solvent (i.e. acetone) was used for both non-water soluble IPBC and Propiconazole wood preservative. Briefly, the volume of CuCrB and Quats wood preservative required to obtain the desired concentration were dissolved in volume of ultrapure water corresponding to the 50% LUFA 2.2 Water Holding Capacity (WHC) for collembolan tests, and to the 60% WHC for the earthworms tests. The stock solutions were then added to the soil and mixed thoroughly the first day of the test (day 0). For IPBC and Propiconazole, the amount or volume of wood preservative required to obtained the desired concentration were dissolved in a small volume of acetone equivalent to 10% of the total mass of soil required per concentration one day prior the beginning of the test (day -1). Solvent stock solutions were then mixed to a fraction of LUFA 2.2 soil corresponding to 10% of the total soil amount per concentration. Acetone was allowed to evaporate overnight under a fume hood and each LUFA 2.2 contaminated soil fraction was mixed to the remaining soil amount of the corresponding concentration. Finally a volume of ultrapure water corresponding to the 50% LUFA 2.2 WHC for collembolan tests, and to the 60% WHC for the earthworms was added to the soil and mixed thoroughly the first day of the test (day 0).

3.2 Collembolan Reproduction Test with *Folsomia fimetaria*

Toxic effects of the selected wood preservatives were assessed on the reproductive output and adult mortality of the *Folsomia fimetaria* soil arthropod (Collembola) after 21 days. Collembolan experiments were conducted according to the OECD guideline n° 232 (OECD 2009). The springtails used for the test were provided by Dr Paul Henning Krogh from the Terrestrial Ecology Group, Aarhus University, Silkeborg, Denmark and have been successfully bred in the Ecotox Centre laboratory in Lausanne since 2009. For all assays, adult *F. fimetaria* between 23-26 day old were used.

Glass containers were filled with 30g of LUFA soil (wet weight, 50% WHC) and 20 adults *F.fimetaria*. i.e. 10 males and 10 females, were introduced per test vessel (day 0 of the test) for each test concentration, negative control, solvent control and H_3BO_3 positive control. The glass containers were then closed with a plastic lid and left for 21 days in a climatic chamber (20 ± 2 °C, light-dark cycle 16:8h and 400 to 800 lux). Collembolans were fed once during the test with dry beaker yeast on day 14 and the test vessels were opened twice a week for aeration. At the end of the test period (i.e. day 21), collembolans were extracted from the soil using a controlled

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[3] "Reference toxicant are used to provide insight into mortalities or changes in organism sensitivity that may occur as a result of acclimation, disease, loading density or handling stress. Concurrent tests using a reference toxicant should be implemented at regular intervals (e.g. monthly for cultures maintained in-house) and for each new batch of test organisms obtained" In "Handbook of Ecotoxicology, Second Edition, Lewis Publishers 2003"

temperature gradient extraction technique (MacFayden 1962) and counted to assess adult mortality and reproduction.

3.3 Earthworm avoidance test with *Eisenia andrei*

Toxic effect of the selected wood preservatives were assessed by measuring the avoidance behavior of the earthworms *Eisienia andrei* after 48h. Earthworm experiments were conducted according to the ISO standard 17512-1(ISO 2008a). According to this standard, avoidance endpoint is as sensitive as the reproduction endpoint in regards to the experiences gained in a laboratory comparison test with eight contaminated soils. The earthworms were obtained from Lombrico-Wurmhandel, Germany (www.wurmhandel.de). Adult *E. andrei* between 300 and 600 mg and with a well developed clitella were used for all assays.

Polystyrene plastic containers (capacity 1L; 160*110*60 mm) were divided vertically into 2 identical parts using a plastic divider. One half of the test container was filled with 450 g (wet weight, 60% WHC) of LUFA 2.2 untreated soil (nanopure water, control soil) and the second half was filled with 450g (wet weight, 60% WHC) of LUFA 2.2 soil spiked with the wood preservatives (test concentrations), H_3BO_3 (positive control) or acetone (solvent control). For the negative control (without test substances), both parts of each container were filled with LUFA 2.2 control soil. The plastic separator was then removed and 10 adults worms were placed on the separating line of each test container. The vessels were closed with a fine mesh and a perforated lid and left for 48h under controlled conditions (20 ± 2 °C, light-dark cycle 16:8h and 400 to 800 lux). The earthworms were not fed during the test. At the end of the test period (i.e. 48h), lids were opened and the dividers were replaced in the middle without moving the test containers. The number of worms was determined in each compartment of the vessels. The avoidance response of the worms was calculated according to the following equation (Garcia et al. 2008, ISO 2008b):

$$Avoidance\ (\%) = \frac{Ncontrol - Ntreatment}{10} \times 100$$

where $Ncontrol$ = the number of worms observed in the control soil, $Ntreatment$ = the number of worms observed in the treated soil and 10 = total number of worms per replicate.

3.4 Statistical analysis

Prior to analysis, data were tested for variance homogeneity (Cochran's test) and normal distribution (D'Agostino & Pearson omnibus normality test; $p \leq 0.05$). The median effective concentration (EC50s) values and their associated lower and upper 95% confidence limits were estimated using logistic functions (regression analysis). These calculations were applied to the number of juveniles and to the avoidance values (expressed as percentage) for each concentration for the collembolan tests and for the earthworm tests, respectively.

For wood preservatives applied to the soil using an organic solvent as carrier (i.e. IPBC and Propiconazole), significant differences ($p \leq 0.05$) between the negative control and solvent control were determined using a Wilcoxon signed rank test. For the collembolan tests, as no significant differences were detected, the negative control and solvent control were pooled. For earthworm tests, as a significant effect ($p \leq 0.05$) of the solvent was observed in both experiments, all treatments were compared with the solvent control.

Significant differences ($p \leq 0.05$) between the control and the concentrations as well as the highest no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) values were determined using a One-way Analysis of Variance (ANOVA) followed by

Dunnett's multiple comparisons test. Intermediate statistically significant effects were not taken into account when determining the NOEC or LOEC. The GraphPad Prism 5 software was used for calculation.

4 Results and Discussion

4.1 Validity and performance of the tests

According to the OECD guideline n° 232 for collembolan testing, the following 3 criteria should be satisfied at the end of the test in the negative (untreated) control for test result to be considered as valid: 1) the mean adult mortality should not exceed 20%, 2) the mean number of juveniles per vessel should reach a minimum of 100 instars, 3) the coefficient of variation (CV) calculated for reproduction should not exceed 30%. The 3 validity criteria were fulfilled in all cases except for IPBC and Propiconazole were the CV in the negative control was of 40.25% and 32.51%, respectively. As the 2 other validity criteria were respected for IPBC and Propiconazole and as the limit value of the CV was only slightly exceeded, dose-response curves, effects and no-effect concentrations were calculated anyway for these two substances. It should be noted that for other soil ecotoxicity tests like for example the reproduction test with the enchytraeid *E. Crypticus*, the CV for reproduction is set to ≤ 50% within the control replicates (Castro-Ferreira et al. 2012).

For earthworms, 2 validity criteria have to be fulfilled according to ISO 17512-1. The test is valid if: 1) the number of dead or missing worms is ≤ 10 % per treatment and 2) the worm ratio should be within the range 60 % : 40 % between the two section of the test container containing the same soil type (i.e. in our case, for the negative control).The 2 validity criteria were respected in all cases except for Quats were the recommended criterion for worm distribution was of 38.8% : 61.3% (the worm ratio was not respected for only 2 out of 8 replicates). As the validity criterion was respected for the 3 other tested wood preservatives in LUFA 2.2 soil and also for other avoidance tests conducted in LUFA 2.2 at the Ecotox Centre in the past, the avoidance dose-response curve, effect and no effect concentrations were calculated for Quats anyway.

The H_3BO_3 positive control used to ensure the performance of both collembolan reproduction and earthworm avoidance tests indicated that the organisms responded positively and within the expected range of toxicity (EC50s concentration were tested).

4.2 Effects of IPBC

Effects of IPBC on collembolans and earthworms are summarized in Table 10.

The reproduction dose-response curve for collembolans exposed to IPBC contaminated soil is shown in Figure 2. Concentrations ≥ 107.37 mg a.i./kg lead to over 95% mortality of the adult collembolans (LC50 = 41.93 mg a.i./kg; graph not shown) and consequently to a 100% inhibition of the reproduction. Effect on the reproduction were observed at the same order of magnitude as the effect on adults survival and the EC50 was calculated at 39.84 mg a.i./kg. An hormesis response occurs at lower concentrations and a significant ($p \leq 0.05$) increase in the number of juveniles produced is observed at 1.02 and 3.28 mg a.i./kg.

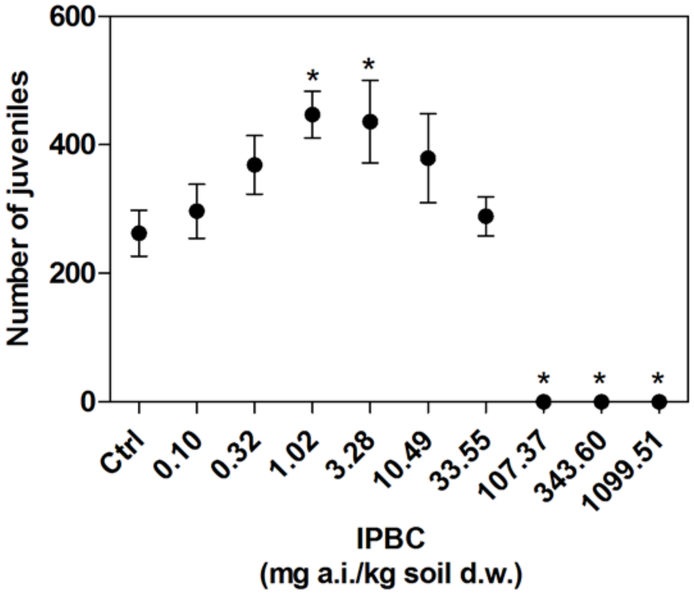

*Figure 2: Juvenile production (mean ± SD) of Folsomia fimetaria exposed to IPBC in LUFA 2.2 soil for 21 days (*statistically different from the control (Ctrl), ANOVA Dunnett's post hoc test p < 0.05); a.i.= active ingredient.*

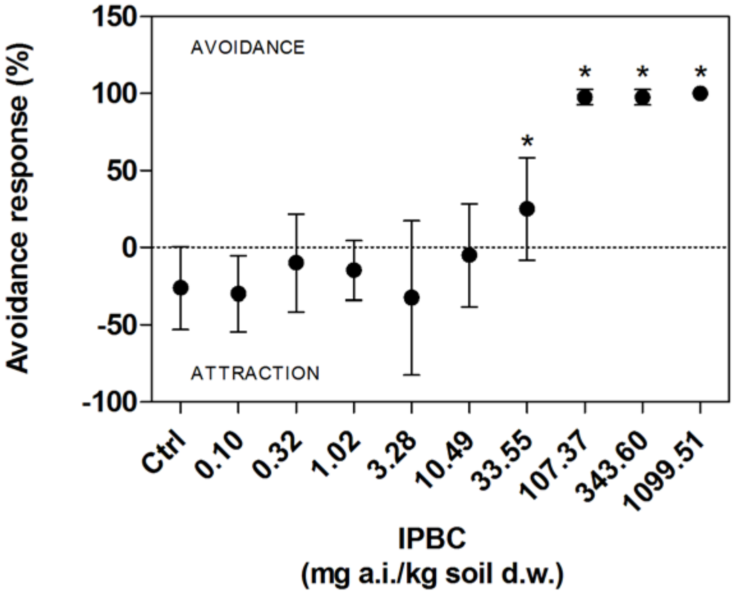

*Figure 3: Avoidance or attraction response (mean ± SD) of Eisenia andrei exposed to IPBC in LUFA 2.2. soil for 48h (*statistically different from the control (Ctrl), ANOVA Dunett's post hoc test p≤0.05); a.i.= active ingredient.*

For earthworms, a significant ($p \leq 0.05$) avoidance behaviour was observed for the four last tested concentrations (≥ 33.55 mg a.i/kg) (Figure 3) and an EC50 of 32.82 mg a.i./kg was calculated (Table 10).

In regard to the EC50 values resulting from our experiments (Table 10), a similar range of toxicity for collembolans and earthworms towards IPBC is observed. Effect concentrations are also in the range of toxicity induced by other carbamate compounds for collembolans and earthworms (Table 4; Section 2.5.1).

Table 10: Effect and no effect concentrations with 95% confidence intervals of IPBC (mg a.i./kg d.w. soil) on F. fimetaria reproduction and E. andrei behaviour.

Test Organisms	EC50 (95% CI)	LC50 (95% CI)	LOEC	NOEC
Collembolans *F. fimetaria*	39.84 (28.13 - 56.42)	41.93 (1.92 - 915.10)	107.37	33.55
Earthworms *E. andrei*	32.82 (21.70 - 49.63)	-	33.55	10.49

Only few data were found in the literature on IPBC toxicity for soil organisms (i.e. for earthworms, microorganisms and terrestrial plants) and are reported in Table 3 (Section 2.5.1). An LC50 > 1000 mg/kg d.w. soil is reported for earthworm acute toxicity (14 days; *E. fetida;* artificial soil) in the EU assessment reports for IPBC (EU 2008, 2013). In our case and using LUFA 2.2 soil for testing, concentration of 1099 mg a.i/kg d.w. soil induced a total avoidance of earthworms to IPBC contaminated soil and 100% mortality of adult collembolans at the end of the tests. Collembolans and earthworms seem to be more sensitive to IPBC than soil microorganisms (EC50 = 312.5 mg/kg d.w. soil) but less sensitive than terrestrial plants (EC50 = 4.92 mg/kg d.w. soil for *Avena sativa*) when comparing the EC50s. According to our knowledge, our results represent the first data obtained for collembolans.

Data on measured soil concentrations of IPBC originating from treated wood were not found. In 2008, the Swedish Environmental Research Institute performed a screening study to determine the concentration of IPBC in different matrices in the environment (i.e. water, air, sediment and soil) (Norström et al. 2009). Possible sources were identified based on the use and the distribution of the compound in the environment. For soil, diffuse sources from urban area (2 soil samples) and point sources from paper and pulp industry (2 soil samples) were considered. No IPBC was found in the analyzed samples. IPBC emissions from wood treated surfaces do not seem to have been considered.

In the EU assessment report on active substance evaluation for IPBC in PT8 (EU 2008), predicted environmental concentration (PECs) for soil are calculated for different life cycle stages of the wood preservatives, i.e. "applications and storage" and "outdoor service life"[4]. Data are summarized in the following table (Table 11). The receiving soil volume was based on a default compartment assuming 10 cm distance and depth from the treated wood.

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[4] Emission Scenario for PT8 estimate the emissions of wood preservatives from two stages of their life cycle: 1) during the wood preservative application processes and storage of treated wood prior to shipment and 2) from treated wood-in-service (OECD 2003).

Table 11: Predicted environmental concentrations (PECs) calculated according to OECD models for different situations.

Wood preservative life-cycle stage		Service time after application	PEC in soil (mg/kg wet soil)
Application and storage	Outdoor storage	30 days	0.03 to 0.24
		20 years	0.03 to 0.24
	In-situ treatment - professionals (3% emission)	Initial (just after application)	2.8 (water based formulation) 9.7 (solvent based formulation)
		30 days	Negligible (for both water and solvent based formulation)
	In-situ treatment - amateurs (5% emissions)	Initial (just after application)	4.6 (water based formulation) 16.2 (solvent based formulation)
		30 days	Negligible (for both water and solvent based formulation)
Outdoor service life (class 3)	"timber house" scenario	30 days	0.1 to 1.1
		Relevant time period taking into account half-life of IPBC in soil	0.001 to 0.03

PEC calculated for the outdoor *in-situ* treatment just after application are relatively close to the effect concentrations found for collembolan reproduction and earthworm avoidance. However, emission and distribution into the soil have to be considered for an exposure scenario.

4.3 Effects of Propiconazole

Effects of Propiconazole on collembolans and earthworms are summarized in Table 12.

A steep dose-response curve was observed for collembolan reproduction for Propiconazole (Figure 4). Concentrations ≥ 343.60 mg a.i./kg lead to over 95% adult mortality (LC50 = 199 mg a.i./kg; graph not shown) and consequently to a 100% inhibition of the reproduction. For concentrations <343.60 mg a.i./kg, no significant differences in the number of juveniles were observed in comparison to the control. An EC50 of 191.70 mg a.i./kg was obtained for the reproduction.

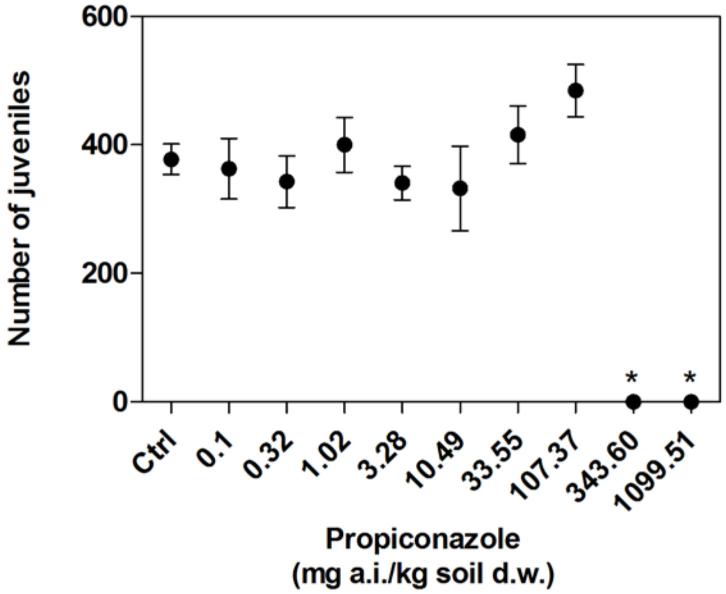

*Figure 4: Juvenile production (mean ± SD) of Folsomia fimetaria exposed to Propiconazole in LUFA 2.2 soil for 21 days (*statistically different from the control (Ctrl), ANOVA Dunnett's post hoc test p < 0.05); a.i.= active ingredient.*

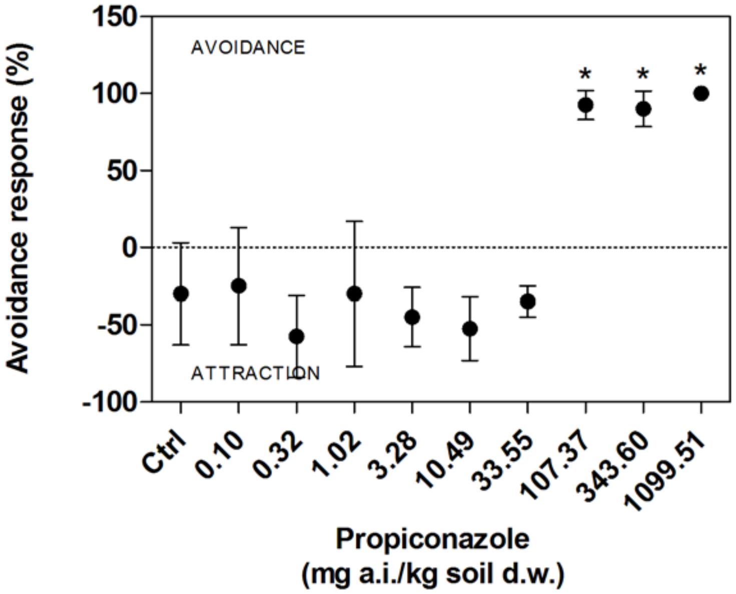

*Figure 5: Avoidance or attraction response (mean ± SD) of Eisenia andrei exposed to Propiconazole in LUFA 2.2. soil for 48h (*statistically different from the control (Ctrl), ANOVA Dunett's post hoc test p≤ 0.05); a.i.= active ingredient.*

Earthworms avoided significantly (p ≤ 0.05) soils contaminated with Propiconazole at concentrations ≥ 107.37 mg a.i/kg) (Figure 5) and an EC50 of 52.02 mg a.i./kg was calculated (Table 12).

Based on the EC50 values, earthworms are slightly more sensitive to Propiconazole than collembolans.

The toxicity data obtained from the earthworm avoidance test are in accordance with toxicity data (LC50 = 686 mg a.i./kg; NOEC of 0.998 mg a.i./kg) reported for *E. fetida* in several evaluation dossier and assessment reports for Propiconazole and listed in Table 5 (Section 2.5.2). Reproduction and behaviour are generally considered to be more sensitive parameters than survival in earthworms (Pelosi et al. 2014).

Table 12: Effect and no effect concentrations with 95% confidence intervals of Propiconazole (mg a.i./kg d.w. soil) on F. fimetaria reproduction and E. andrei behaviour.

Test Organisms	**EC50 (95% CI)**	**LC50 (95% CI)**	**LOEC**	**NOEC**
Collembolans *F. fimetaria*	191.70 (0.0 - infinity)	199.00 (93.49 - 423.40)	343.60	107.37
Earthworms *E. andrei*	52.02 (34.75 - 77.86)	-	107.37	33.55

No measured environmental soil concentrations were found for Propiconazole originating from treated wood. Data on leaching under semi-field conditions were also unavailable.

4.4 Effects of CuCrB

Effects of CuCrB on collembolans and earthworms are summarized in Table 13. The corresponding concentrations of H_3BO_3[5] and Cu are given in Table 14.

Mortality of the adult collembolans reached 60% at 1500 mg/kg, 87% at 4800 mg/kg and was more than 96% for the two last tested concentrations of CuCrB (LC50 = 1325 mg/kg; graph not shown). Consequently, reproduction was totally inhibited for CuCrB concentrations $\geq$ 4800 mg/kg and a significant reduction ($p \leq 0.05$) of the number of juveniles was observed at 470 mg/kg and 1500 mg/kg of CuCrB (Figure 6). An EC50 of 240 mg/kg was calculated for CuCrB, corresponding to 9.12 mg/kg H_3BO_3 and 23.39 mg/kg Cu.

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[5] Corresponding concentration is given for Boric acid (H3BO3) and not for Boron (B) as most of the toxic effect data for soil organisms are given for H3BO3.

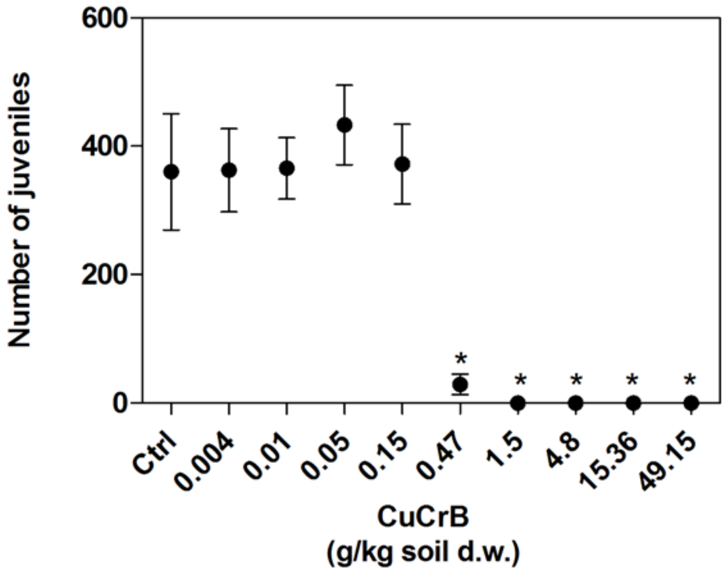

Figure 6: Juvenile production (mean ± SD) of Folsomia fimetaria exposed to CuCrB in LUFA 2.2 soil for 21 days (*statistically different from the control (Ctrl), ANOVA Dunnett's post hoc test p < 0.05).

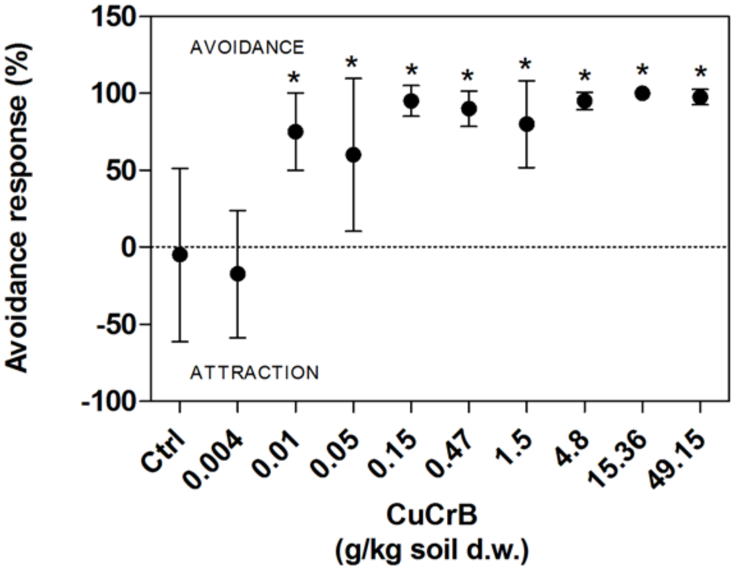

Figure 7: Avoidance or attraction response (mean ± SD) of Eisenia andrei exposed to CuCrB in LUFA 2.2. soil for 48h (*statistically different from the control (Ctrl), ANOVA Dunett's post hoc test p≤ 0.05).

A significant ($p \leq 0.05$) avoidance behaviour was observed for *E. andrei* at concentrations of CuCrB ≥ 10 mg/kg (Figure 7), which corresponds to a concentration of 0.38 mg/kg H_3BO_3 and 0.97 mg/kg Cu (Table 14). The EC50 was calculated at 11 mg/kg for CuCrB (i.e. 0.42 mg/kg H_3BO_3 and 1.07 mg/kg Cu).

In the conducted experiments, earthworm reaction towards CuCrB occurred at quite low concentrations, if referring to individual active ingredients. Based on EC50s, earthworms seem to be more sensitive to this wood preservative than collembolans.

As already mentioned in Section 2.5.6, Cr(VI) is used as fixing agent in the CuCrB wood preservative and reduced to Cr(III) after wood impregnation. In our study, as ecotoxicity tests were conducted with soils spiked with the CuCrB formulation and not with leachates, a toxicity of Cr(VI) rather than Cr(III) is potentially expected. However, a chemical analysis should be conducted to confirm which oxidation state is present as it is shown that important reduction of Cr(VI) to Cr(III) can occur for soils containing a high level of organic matter (Bigorgne et al. 2010). Concentration of Cr(VI) in the tested CuCrB wood preservative is expected to account for approximately 23% of the product (personal communication C. Ganne-Chedeville and T. Volkmer, Berner Fachhochschule). Regarding the conducted experiments, the corresponding Cr(VI) concentrations derived from the EC50 obtained for CuCrB would be of 55.20 mg/kg for collembolans and of 2.53 mg/kg for earthworms.

Table 13: Effect and no effect concentrations with 95% confidence intervals of CuCrB (mg/kg d.w. soil) on F. fimetaria reproduction and E. andrei behaviour.

Test Organisms	**EC50 (95% CI)**	**LC50 (95% CI)**	**LOEC**	**NOEC**
Collembolans *F. fimetaria*	238.80 (187.80 - 303.80)	1325.00 (1012 - 1734)	470.00	150.00
Earthworms *E. andrei*	10.65 (5.31 - 21.38)	-	10.00	4.00

Table 14: Concentrations of H_3BO_3 (4% of the CuCrB formulation) and Cu (15% of the CuCrB formulation) in mg/kg d.w. soil, corresponding to the CuCrB toxic effects obtained for collembolans and earthworms.

Test Organisms	**EC50 (95% CI)**		**LOEC**		**NOEC**	
	H_3BO_3	**Cu**	**H_3BO_3**	**Cu**	**H_3BO_3**	**Cu**
Collembolans *F. fimetaria*	9.12 (7.22 - 11.40)	23.39 (18.51 - 29.23)	17.86	45.80	5.70	14.62
Earthworms *E. andrei*	0.42 (0.19 - 0.80)	1.07 (0.49 - 2.05)	0.38	0.97	0.15	0.39

No data were found in the literature regarding the toxicity of the CuCrB wood preservative to soil organisms. However, the toxicity of H_3BO_3, Cu and Cr as single substance for collembolans, earthworms and other soil organisms is well documented (see Table 6, Table 7 and Table 8, Section 2.5). Although the difference in soil types between our study and the toxicity data found in the literature for Boric acid, Copper and Chromium do not allowed a direct comparison, it is clear that the effects observed for CuCrB in our study are not of the same order of magnitude than effects found for the substances taken individually (Section 2.5). A combined effects of the 3 active ingredients can be observed. Further studies on mixture effects should be considered.

Measured environmental soil concentrations were not found for CuCrB. However, a few studies are available regarding leaching of Copper-Chromium-Borate active ingredients from treated wood under semi-field conditions. In the study performed by Garcia-Valcarcel and Tadeo (2006), leaching of Cu, Cr and B from a CrCuB-treated timber wood exposed aboveground was evaluated under field conditions during one year. Metal leaching was assessed for horizontal (i.e. decks) and vertical (i.e. fences) timber orientations and compared to emission rates obtained in a laboratory study performed previously. The results showed leaching rates of 227 and 87 mg/m^2 for Cu, 200 and 42 mg/m^2 for Cr and 110 and 33 mg/m^2 for B from decks and fences, respectively, after one year. Comparison of the emission rates from the laboratory study extrapolated to a one year period underestimated metal leaching from decks but overestimated emissions from fences. This underline the importance of also performing field assays to determine emissions of active ingredients from treated wood placed outdoor and exposed to real environmental conditions. In another study, emissions of active ingredients from wood (*Pinus silvestris*) treated with Copper-amine based preservatives (i.e. impralit-KDS) were evaluated in a semi-field study over a 2 year period (Pfabigan et al. 2014) (see also Section 4.5). CCB salt (i.e impralit CCO flüssig, a wood preservative containing a mix of Cu, Cr and B active ingredients) was used as reference product. After 2 years, results of the semi-filed test showed an accumulated leaching of 178 mg/m^2 for Cu and of 226 mg/m^2 for B. Chromium leaching was not evaluated.

4.5 Effects of Quats

Effects of Quats on collembolans and earthworms are summarized in Table 15. The corresponding concentrations of Didecylpolyoxethylammoniumborate (DPAB), Copper and Boric acid are given in Table 16.

For the collembolan reproduction test, an EC50 of 873 mg/kg was found for Quats (table), which correspond to a concentration of 87.30 mg/kg DPAB, 104.46 mg/kg Cu and 69.84 mg/kg H_3BO_3 (Table 16). Significant effects ($p \leq 0.05$) were observed on the reproduction for concentrations of Quats ≥ 1300 mg/kg (Figure 8). The two last tested concentrations of Quats induced mortality (> 99.5%) of adult collembolans (LC50 = 5781 mg/kg; graph not shown).

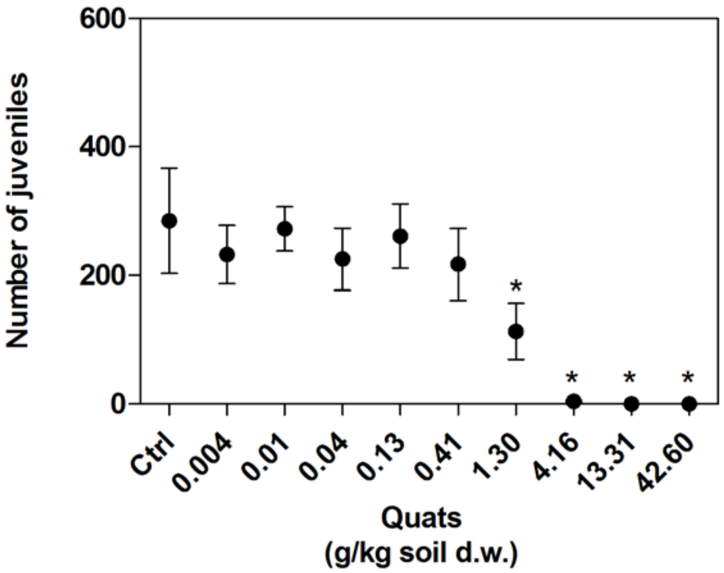

Figure 8: Juvenile production (mean ± SD) of Folsomia fimetaria exposed to Quats in LUFA 2.2 soil for 21 days (*statistically different from the control (Ctrl), ANOVA Dunnett's post hoc test $p < 0.05$).

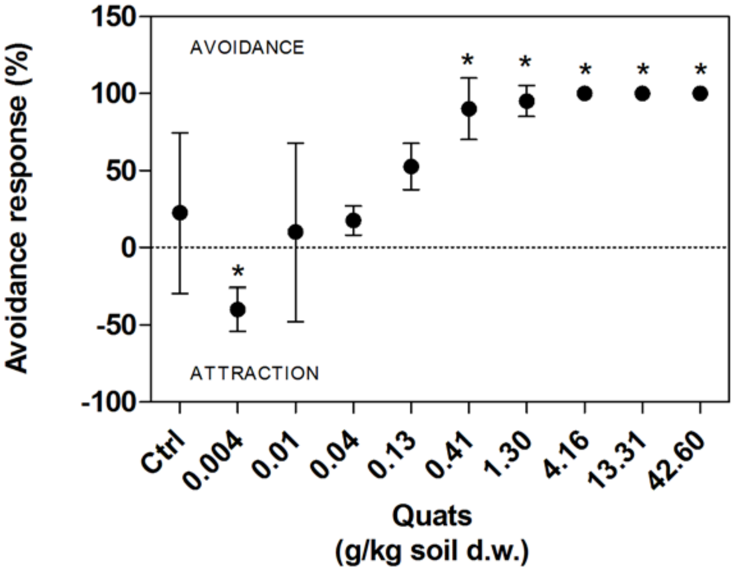

Figure 9: Avoidance or attraction response (mean ± SD) of Eisenia andrei exposed to Quats in LUFA 2.2. soil for 48h (*statistically different from the control (Ctrl), ANOVA Dunett's post hoc test p≤ 0.05).

Significant earthworm avoidance behaviour started at Quats concentrations ≥ 410 mg/kg (Figure 9). A significant attraction was observed at the lowest tested concentration (i.e. 4 mg/kg). The EC50 for earthworm avoidance was determined at 47 mg/kg of Quats, which correspond to a concentration of 4.7 mg/kg DPAB, 5.53 mg/kg Cu and 3.76 mg/kg H_3BO_3 (Table 15 and Table 16).

As for the CuCrB wood preservative, earthworms reacted more sensitively to Quats than collembolans when comparing the EC50s values. Moreover, the avoidance reaction of the earthworms was stronger towards CuCrB than for Quats although amount of Copper salts and Boric acid is between 1.4 to 2.1 time larger in the Quats wood preservative. The Chromium present as fixing agent in the CuCrB wood preservative (see Section 4.4) could explain this difference of sensitivity. DPAB do not seems to be very toxic for soil organisms, but it should be tested as a single substance to confirm this hypothesis. As for CuCrB, combined effects of the active ingredients present in the biocide formulation are also observed for the Quats wood preservative.

It must be noted that a significant pH increase was measured for the soil of the highest Quats tested concentration (i.e. 42.6 g/kg d.w. soil) for both collembolans and earthworms. This was not the case for the 3 other wood preservatives tested. A mean pH value of 9.3 was found for this concentration whereas for the control and lowest tested concentration, the mean pH value of the soil was of 5. *Eisenia andrei* is tolerant to a wide range of pH values (i.e. 4 to 8) (Environment_Canada 2004) as well as collembolans (pH 2 to 9 for *F. candida*). It seems then unlikely that the elevated pH had an impact on the earthworms or collembolans. However, it might change metals availability (increase of cationic metal retention) (McLean and Bledsoe 1992) in comparison to the soil with a pH 5 from the lowest Quats tested concentration. This should be kept in mind in case complementary studies are performed.

Table 15: Effect and no effect concentrations with 95% confidence intervals of Quats (mg/kg d.w. soil) on F. fimetaria reproduction and E. andrei behaviour.

Test Organisms	EC50 (95% CI)	LC50 (95% CI)	LOEC	NOEC
Collembolans *F. fimetaria*	873.00 (617.20 - 1235.00)	5781.00 (4096.00 - 8160.00)	1300.00	410.00
Earthworms *E. andrei*	47.03 (29.50 - 74.96)	-	410.00	130.00

Table 16: Concentrations of Cu (21% of the Quats formulation), DPAB (10% of the Quats formulation), H_3BO_3 (8% of the Quats formulation) in mg/kg d.w. soil, corresponding to the Quats toxic effects obtained for collembolans and earthworms.

Test Organisms	Active ingredient	EC50 (95% CI)	LOEC	NOEC
Collembolans *F. fimetaria*	DPAB	87.30 (61.72 - 123.50)	130.00	41.00
	Cu	104.46 (73.85 - 147.77)	155.55	49.06
	H_3BO_3	69.84 (49.38 - 98.80)	104.00	32.80
Earthworms *E. andrei*	DPAB	4.70 (2.95 - 7.50)	41.00	13.00
	Cu	5.63 (3.53 - 8.97)	49.06	15.56
	H_3BO_3	3.76 (2.36 - 6.00)	32.80	10.40

No data on the toxicity of Quats preparation for soil species were found in the literature. However, as for CuCrB, toxicity data are available for the majority of the single substance composing the Quats wood preservative, except for DPAB. Effects of Boric acid and Copper on soil species are described in details in Section 2.5.

A semi-field test was conducted over a 2 year period in Austria (Pfabigan et al. 2014) to study the emissions of active ingredients from wood (*Pinus silvestris*) treated with Copper-amine based preservatives (i.e. impralit-KDS, a wood preservative containing Copper salts, Quaternary ammonium salt and Boric acid). After 2 years, results of the semi-filed test showed an accumulated leaching of 457 mg/m^2 for Cu and of 506 mg/m^2 for B. Leaching of the quaternary ammonium salt was not assessed. No other data were found in the literature regarding emission or environmental soil concentrations for Quats released from treated wood.

5 Conclusions and Outlook

The amount and type of biocidal active ingredients available on the market and used to protect wood material against fungi and insects is in constant evolution. For 2014 and for service situation of wood in use class 3, IPBC, Propiconazole, Permethrin and Tebuconazole remain the most relevant active ingredients in term of number of entry for the registered active ingredients in wood preservatives for Switzerland. Boric acid and Quats are the most representative of the use class 4. In most of case, a mix of two or more active ingredients is employed in the wood preservative in order to increase the efficacy of the product formulation.

Toxicity data for soil species are scarce for substances such as IPBC or DPAB which are mainly used in wood preservatives and not likely to result in a direct exposure to the soil compartment or where only limited exposure is expected in space. For substances with a broader range of use such as Propiconazole (e.g. also used in PPP) or where major environmental contamination have occurred like for example for Copper and Chromium, more information is available regarding their toxicity towards soil organisms.

Results of the ecotoxicity tests conducted showed that IPBC induced toxic effects in the same order of magnitude for both collembolans and earthworms. These effects are also in the same range of toxicity than effects reported in the literature for other carbamate compounds. The EC50 value found in our study for *E. andrei* avoidance behaviour is far lower than the acute toxicity value reported for earthworms in the PT8 assessment report for IPBC. This result underlines the importance of considering other and more sensitive endpoints than earthworm survival in risk assessment. Earthworms reacted to concentrations of Propiconazole that are in the same order of magnitude than effect concentrations found for IPBC. However, they showed to be slightly more sensitive than collembolans to this compound. Our toxicity values are in the range of toxicity reported for soil organisms for Propiconazole in the PT8 assessment report. Both CuCrB and Quats wood preservatives contained a mix of active ingredients and therefore showed a quite high toxicity for collembolans but even more important for earthworms. The toxicity induced by the mixture of active ingredients is far below the toxic effects observed for the individual substance itself. Chromium seems to participate to a large extend of the toxicity of CuCrB to earthworms and collembolans. It is however not considered as an active ingredient in the CuCrB products and not always taken into account in leaching studies. Different toxicity may arise depending on its oxidation state and have to be considered. Soil chemical analysis should be run in parallel to the conducted ecotoxicity tests to define the type of chromium present, but also to inform on the available fraction of the active ingredients present in the soil matrix during the assays. DPAB do not seem to be highly toxic neither for collembolans nor for earthworms. Based on the obtained results and in regards to the available earthworm toxicity data found in the literature, earthworm avoidance behaviour can be considered as an appropriate and sensitive endpoint to assess toxicity of biocidal substances.

Most of the biocidal active ingredients employed in PT8 are known to leach after a certain time in the environment. Leaching studies are usually performed in the laboratory over a short time and extrapolated to a longer time period. A few studies are also available for leaching under semi-field conditions. To our knowledge, data for measured environmental soil concentrations are inexistent for the active ingredients contained in the selected wood preservatives.

In light of these conclusions, it appears that even if few or no literature data were available regarding the toxicity to soil organisms of the selected wood preservative active ingredients, the risk posed by these substances considered individually seems to have been safely encompassed. However, the risk that active ingredient mixtures may pose for the soil organisms should be further investigated, as well as the issue regarding Chromium leaching and its toxicity. As leaching is generally a continuous process over a long period of time, long time and repeated exposure for soil organisms should be assessed. Studies aiming at characterizing the impact of wood preservative active ingredients on soil organisms in field conditions or in microcosms should be performed and concentrations of wood preservatives in soil under realistic environmental exposure conditions should be measured.

6 Indices

6.1 List of Figures

6.2 List of Tables

7 References

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