Soil pollution by nonylphenol and nonylphenol ethoxylates and their effects to plants and invertebrates

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Abstract

Background, aim and scope Nonylphenol polyethoxylates (NPEOs) are a widely used class of nonionic surfactants known to be toxic and endocrine-disrupting contaminants. Their use and production have been banned in the European Union and substituted by other surfactants, considered as environmentally safer. However, their use continues in many countries without any legal control. Discharges of effluents from wastewater treatment plants and the application of sewage sludge application, landfilling and accidental spillage to soils are the major sources of NPEOs in the environment. The biodegradation of these surfactants is relatively easy, leading to the accumulation of the simplest chemical forms of nonylphenol ethoxylates (NP, NP1EO and NP2EO) and nonylphenol carboxy acids (NP2EC or NP1EC). However, these are also the most toxic end-products and have a higher environmental persistence. Compared to aquatic ecosystems, not much is known about the effects of NPEOs in terrestrial organisms, with few studies mainly centred on the effects on plants and soil microorganisms.
The main aim of this study is to provide the range of concentrations of NPEOs with ecotoxicological effects on different plants and soil invertebrate species. In addition, we aim to identify the main soil properties influencing their toxicity.
Materials and methods Two natural soils collected and OECD artificial soil were used in toxicity bioassays. Two different NPEO formulations were tested. On the one hand, a technical mixture of NPEOs containing chain isomers and oligomers with an average of 8 ethoxy units was used for the experiments and is referred to herein as NP8EO. On the other hand, technical-grade 4-nonylphenol 95% purity was also used and called NP in this study. The chemicals were applied and mixed with soil as an acetone solution. The toxicity of NP8EO and NP was assessed in different taxonomical groups (plants, earthworms, enchytraeids and collembolans) according to their respective standardised methods. The effect on lethal and sublethal endpoints was assessed, and by means of linear and non-linear regression models, the NPEO concentration causing 10 and 50% inhibition were estimated. The influence of soil properties on the toxicity was assessed using generalized linear models.
Results The chemicals tested showed contrasting toxicities, NP being clearly more toxic than NP8EO. There were also substantial differences in the sensitivity of the species and endpoints, together with clearly different toxicities in different soils. Plants were the least affected group compared to soil invertebrates, since plant endpoints were unaffected or only slightly inhibited. In soil invertebrates, reproduction was the most affected endpoint compared to growth or survival. Toxicity was the lowest in OECD artificial soil in comparison to natural soils, with a lower organic matter content.
Discussion The higher toxicity of NP, both in plant and soil invertebrate bioassays, is consistent with previously published studies and its relatively high persistence in soil. The low of phytoxicity of NP8EO and NP, unaffected at concentrations over 1 g NP kg^{-1}, also accords with the known low uptake in plants. The effects on soil invertebrates appeared at lower concentrations than observed in plants, enchytraeids being less affected by NP8EO than earthworms and collembolans. Drastic inhibition in the invertebrate's endpoints generally appeared over 1 g kg^{-1} for NP8EO and below 1 g kg^{-1} for NP. The range of concentrations with effects is in agreement with the few similar studies published to date. Generally, the lowest toxicity values were obtained in OECD soil, with the highest organic matter content while the highest toxicity was found in the PRA soil, with the lowest content. However, few of the models developed by GLM identified organic carbon as a significant factor in decreasing the bioavailability and toxicity of NPEO. The probable explanation for this is the simultaneous contribution of other soil properties and in particular the limited number of soils used in the bioassays.
Conclusions A low ecotoxicological risk of NPEOs might be expected for plants and soil invertebrates, since the usual concentrations in soils (below 2.6 mg kg^{-1}) are clearly less than the lowest concentrations reported to be toxic in our study.
Recommendations and perspectives Although the apparent risk of NPEOs for soil ecosystems is limited, such risks should not be neglected since significant concentrations in soil could be reached with elevated application rates or when highly polluted sludges are used. More importantly, NPEO concentrations in soils should be maintained low given the extremely high toxicity for aquatic organisms. Despite the reduced leaching of NPEOs, runoff events might transport NP attached to soil particles and affect adjacent aquatic ecosystems.

Keywords nonylphenol ethoxylates, soil toxicity, plants, earthworms, enchytraeids, collembolans

1 Background, aim and scope

Nonylphenol polyethoxylates (NPEOs) are an important class of nonionic surfactants known to be toxic and endocrine-disrupting contaminants, which are widely used in industrial, agricultural, commercial and household applications such as detergents, emulsifiers, wetting and dispersing agents, antistatic agents, demulsifiers and solubilisers (**Staples et al. 2004, Soares et al 2008**). NPEO structure corresponds to a phenol nucleus that is o, m, or p substituted with one of a variety of hydrophobic, branched, isomeric nonyl (C9) moieties, and with an hydrophilic polyethylene glycol (ethoxylate) chain ether linked at the phenolic oxygen (**John and White 1998**).

Due to the potential harmful effects of the degradation products of NPEOs, their use and production have been banned in the European Union (**Directive 2003/53/EC**) and substituted by other surfactants (mainly alcohol ethoxylates), considered to be environmentally safer as they degrade more rapidly (**Campbell 2002**). However, the environmental risk of their degradation products is unknown (**Soares et al. 2008**). In contrast, NPEOs are strictly monitored in many other countries such as Canada and Japan. Interest in their environmental risk is still high, since despite the European prohibition and the decreasing environmental concentrations these compounds are still present in environmental samples (**Soares et al. 2008**). More importantly, widespread use of NPEOs continues in many countries without any legal control (**Sjöström et al. 2008**).

The main source of NPEOs in the environment is the discharge of effluents from wastewater treatment plants to aquatic environments (**Ahel et al. 1994a**). In soils, sewage sludge application, landfilling and accidental spillage are the main sources of NPEOs (**Soares et al. 2008**). Despite the fact that NPEOs are considerably biodegraded during wastewater treatment and sludge treatment (**Hernández-Raquet et al. 2007**), the concentrations are higher in effluents and sewage sludges from industrial or highly populated areas (**Langford and Lester 2002**). It has been reported that wastewater treatment plant removal efficiencies average 59% for all NPEOs based on the evaluation of 11 treatment plants (**Ahel et al. 1994b**). Significant biodegradation has also been reported with sewage sludge composting, due to the aerobic nature of the process (**Domene et al. 2007, Das and Xia 2008, Pakou et al. 2009**). Nonylphenols may be also present in animal manures which are routinely spread to land (**Domene et al. 2007**). To a lesser extent, the occurrence of NP in soil is also linked to other anthropogenic activities such as landfilling and accidental spillage (**Soares et al. 2008**).

The biodegradation of NPEOs leads to the accumulation of the simplest chemical forms of nonylphenol ethoxylates (NP, NP1EO and NP2EO) and nonylphenol carboxy acids (NP2EC or NP1EC) (**Ahel et al. 1994b, 1994c, Staples et al. 2001**). The accumulation is due to the degradation process, which advances decreasing the length of the ethoxy chain, resulting in higher hydrophobicity and lower degradation rates (**Gejlsberg et al. 2003**). This is why NP is the nonylphenol with the highest persistence in soil and is the NPEO associated with sewage sludge (90%) (**Soares et al. 2008**). In addition, the most prevalent NPEOs are also the most toxic intermediates, as the NPEOs (NP1EO, NP2EO and especially NP) are more toxic compared to NPECs (**Staples et al. 2004**).

The fate of nonylphenol in different environmental compartments (surface water, sediment, groundwater, soil and air) relies on their physico-chemical properties, which in turn determine its degradation (**Soares et al. 2008**). In soil, the biodegradation of NP is also possible, but it is limited by the oxygen supply (**Topp and Starratt 2000**) and bioavailability (**Kelsey et al. 1997**). As an example, a mineralization half-life of 4.5 to 16.7 has been reported in soil (**Topp and Starratt 2000**). **Sjöström et al. (2008)** reported half-lifes in different soils of around 7-20 days for NP and 5-30 days for NP12EO. **Jacobsen et al. (2004)**, in a outdoor lysimeter experiment, reported a 45% decline in NP concentration following amendment with sludge after only 10 days, while NP was undetectable after 110 days. **Petersen et al. (2003)** in agricultural amendments with sewage sludge with low levels of NP, failed to find detectable concentrations of NP 200 days after the application. However, in other studies (**Sjöström et al. 2008, Mortensen and Kure 2003**), a fraction between 8-36% of recalcitrant NP was found to remain in soil after 1-3 months. This recalcitrant fraction is thought to be bound to organic matter but also within soil aggregates, due to the less aerobic conditions (**Sjöström et al. 2008**). An additional explanation for this recalcitrance might be the fact that some NP isomers are more prone to breakdown than others (**Trocmé et al. 1988**). Anaerobic biodegradation has been also demonstrated (**Corvini et al. 2006**) but is slower, and as example, a half-life of more than 60 years has been estimated in sediments (**Shang et al. 1999**). However, the repeated application of sludge as amendment can lead to persistent levels of NPEOs in soil. **Vilkesøe et al. (2002)**, studying levels of NPEOs (NP and NPE2O) in different agricultural soils amended with sludge (0.7 to 17 tons dw/ha/year) reported increased concentrations of NPEOs (3.1 µg/kg to 2.62 mg/kg) compared to unamended soils and soils amended with manure or chemical fertilizers (3.4-3.8 µg/kg). It has also been pointed out that degradation half times change substantially in different soils, mainly related to soil properties (**Sjöström et al. 2008**). On the other hand, the stimulation of microbial communities mediated by plant roots in soil, has been shown to increase the biodegradation and removal of NP (**Mortensen and Kure 2003, Sjöström et al. 2008**).

In soil, NP generally tends to bind to organic material, decreasing its concentration in porewater, and hence reducing its bioavailability. Accordingly, when applied with organic wastes, NP should be even less bioavailable. Nevertheless **Scott-Fordsmand and Krogh (2004)** did not find that sludge addition significantly altered NP bioavailability. The adsorption degree depends on the quality of the organic matter, and it has been shown that more hydrophobic materials (lignin, humic acids) permit higher adsorption, resulting in a lower toxicity than more hydrophilic materials (cellulose, chitin) (**Burgess et al. 2005**). **Vogel et al. (2003)** showed that NP leaching is extremely limited, since most NP was found within the first 30

cm of the surface. **Jacobsen et al. (2004)** also indicated that downward transport of NP was negligible in a lysimeter experiment. However, the slight solubility of NP might allow some leaching and some bioavailability of this chemical (**Brix et al. 2001**). For instance, **Oman and Hynning (1993)** detected nonylphenols in landfill leachates (<10 to 170 μg/l). In addition, **Vilkesøe et al. (2002)** showed NPEO pollution in a meadow soil receiving runoff from a sewage sludge storage facility (0.15 mg/kg) which might be mainly due to transport attached to solid particles.

In the air, since NP is theoretically semi-volatile (vapour pressure of 2.07×10^{-2} Pa and a Henry's law constant of 8.39×10^{-9} Pa m^3/mol) water-air exchange might be possible (**Ney 1990**). However, in soil, given its high affinity for organic matter and its low solubility, volatilization should be limited. **Trocme et al. (1988)** demonstrated that the volatilization rate of nonylphenol from soils is not significant, since only 0.2% of the NP in a soil (1 mg/kg) was removed after 40 days. However, NP atmospheric transport can occur associated with aerosols produced by the aerators of the sewage sludge treatment plants and these aerosols can reach aquatic and terrestrial ecosystems by wet deposition (**Fries and Puttmann 2004**). In fact, NPEOs have been detected in rainwater in all the locations monitored in a study carried out in The Netherlands, while NP was detectable in 16 of the 50 locations studied (**Peters et al. 2008**). Given that most of the NPEOs found had a relatively high number of ethoxylated units, they concluded these NPEOs had a direct human origin and were not from other environmental compartments, where forms with few ethoxylated units predominate.

Despite the usual presence of NPEOs in soils, not much is known about their effects on terrestrial organisms compared to aquatic organisms, and studies have mainly been restricted to the effects on plants and microorganisms (**Scott-Fordsmand and Krogh 2004**). This is why the main aim of this work is to provide the range of concentrations with ecotoxicological effects on different plant and soil invertebrate species of a mixture of NPEOs with an average of 8 ethoxy units (NP8EO) and 4-nonylphenol (NP). In addition, as toxicity is reported in different soils, we aim to identify the main soil properties influencing the toxicity of these chemicals.

2 Materials and methods

2.1. Soils

The upper layer (20 cm) of two natural soils was collected for the soil bioassays. Soils differed mainly in terms of texture and organic matter concentration (**Table 1**). PRA soil was a loamy sand soil collected in a dry grassland in Serra de Prades (Tarragona, NE Spain). UAB soil was a loamy agricultural soil obtained from an experimental agricultural soil within the Universitat Autònoma de Barcelona campus (Cerdanyola del Vallès, NE Spain). UAB soil has been free of pesticides for at least the last five years. Soils were defaunated by alternating two consecutive freezing-thawing cycles, each consisting in placing soil at -20ºC for 4 days followed by a period of 4 days at 20ºC.

In addition, OECD artificial soil was used for the bioassays so that our results would be comparable with other similar studies. The artificial soil was prepared according to **OECD (1984)** by mixing *Sphagnum* peat (10%), kaoline (20%) and quartz sand. Soil pH was adjusted to 6±0.5 with the addition of calcium carbonate.

Soil properties were characterized by suitable methods: water holding capacity (WHC), water pH (1:2.5 w/w), electrical conductivity (EC, 1:5 w/w, 25ºC), texture (pipette method), organic carbon (Walkley-Black titration method), total nitrogen (Kjeldahl method), cation exchange capacity (**Chapman et al. 1965**), and heavy metals (Cd, Cu, Cr, Ni, Pb, Zn) (**ISO 1996**). No heavy metal analysis was carried out for OECD soil given its artificial nature.

Table1

The relatively high copper levels found in UAB soil are inherited from the prior use of copper sulphate in traditional vineyard cultures of this area, and were not expected to affect the results of the bioassays. In this soil, copper was expected to be mainly in non available forms through aging processes (**Lock and Janssen 2003**). This is consistent with the outcomes of the test species in this soil, which do not suggested any potential toxic effect.

2.2. Chemicals

Two different nonylphenol ethoxylate formulations were used for the experiments. On the one hand, a technical mixture of NPEOs containing chain isomers and oligomers with an average of 8 ethoxy units was used for the experiments (FINDET 9Q/20 Kao Corporation, Barcelona, Spain) and is referred to herein as NP8EO. On the other hand, technical-grade 4-nonylphenol of 95% purity (Kao Corporation, Barcelona, Spain) was also used and called NP in this study.

Both chemicals were applied to soils dissolved in acetone (95%, Panreac, Barcelona, Spain) given the relative hydrophobicity of these compounds. For each bioassay and soil, a preliminary assay was carried out in order to find the range of concentrations with effects. The preliminary assay consisted of 0, 1, 10, 100, 1000 and 10000 mg of NP or NPEO kg^{-1}. After that, the range of concentrations showing an inhibition between 10 and 90% were selected for the definitive assay.

To prepare the concentrations, the amount of soil to be used for each concentration was moistened with a fixed volume of acetone solution containing the appropriate quantity of NP or NP8EO. Two control soils were prepared, one to which the

same volume of acetone was added and another one to which acetone was not added and that served to detect any noxius effect directly linked to the acetone addition. Acetone was then left to evaporate for 24 h in a fume hood. Prior to the addition of acetone, and for each concentration prepared, a small portion of the untreated soil (20%) was kept aside in order to reinoculate the polluted soil once the acetone had evaporated.

2.3. Test organisms

The toxicity of NP and NP8EO for soils was assessed by different bioassays based on species representative of the main taxonomical groups present in soil ecosystems. Namely, we assessed the effects on germination and biomass production of a monocot plant (*Lolium perenne*) and a dicot (*Brassica rapa*), and also the effects on survival and reproduction of an earthworm (*Eisenia andrei*), an enchytraeid (*Enchytraeus crypticus*), and a collembolan (*Folsomia candida*).

Plant toxicity was assessed in accordance with OECD Guideline 208 (**OECD 2006**). Water content of soil was adjusted to 60% of maximum water-holding capacity. Each replicate consisted of a 250 ml polyethylene cup filled with 100 g of soil (dry weight). Five replicates per concentration were prepared and incubated in a growth chamber at 21°C, 16/8 h (light/dark) and 70% air humidity. Ten seeds per replicate were sown uniformly in each pot (1.5 cm depth in *L. perenne*, 0.5 cm depth in *B. rapa*). When half of the seeds in the controls germinated, the germination percentage was determined. Then, five seedlings were retained per replicate and the remaining plants were removed. After that, replicates were incubated for 15 days and the aerial part of the seedlings was removed and weighed as fresh weight.

Earthworm toxicity was assessed as described in ISO Guideline 11268-2 (**ISO 1998**). Soil water content was adjusted to 60% of the maximum water holding capacity. Each replicate consisted of a 1000-ml polyethylene container covered with a perforated lid that allowed aeration filled with 500 g (dry weight) of moist soil. Four replicates per concentration were prepared. Ten clitellated individuals of synchronized age (4 weeks difference at most), were placed in each container. Animals were fed with 5 g cooked oat flakes at the start and weekly thereafter. Replicates were maintained in a 16:8 light/dark photoperiod and a constant temperature of 21 °C for 28 days. The moisture loss of each replicate was checked weekly by weight, and restored if necessary with distilled water.
After 28 days of exposure, adults were removed from the test substrate, counted and weighed and the replicates were incubated 28 more days in order to allow juveniles to emerge and grow. This enabled assessment of the survival rates and total earthworm biomass (sum of weights of the surviving adults). After this period, each replicate was placed in a water bath at a temperature of 60 °C. After 20 min, juveniles appeared at the substrate surface and were collected and counted.

Enchytraeid toxicity was assessed in accordance with ISO Guideline 16387 (**ISO 2003**). The assay was also performed in soil moistened to 60% of its water-holding capacity. Five replicates per concentration were prepared, each in 150-ml polyethylene flasks filled with 30 g of the test substrate. Ten adults (clearly identified by the clitella) were introduced into each flask. The animals were fed with 25 mg of ground oats at the start of the assays, and weekly thereafter. Replicates were aerated twice a week and maintained in the dark at 21°C. Some methodological variations were carried out compared to the ISO protocol. Specifically, the adults were maintained in test vessels during all the experimental period (and not removed after 3 weeks). In addition the test period was also shorted to 4 weeks compared to the 6 weeks indicated in the protocol. These changes were in accordance to **Kuperman et al. (2006)** and due to the species used, *E. crypticus*, which is more easily damaged and has a shorter generation time than *E. albidus,* the species usually used and for which the protocol was initially designed. At the end of the test period, adults and juveniles were fixed by direct addition of ethanol (80%, v/v) to each flask. Then a few drops of Bengal red (1% solution in ethanol) were added to colour the individuals. After two hours, individuals were separated from the more fine soil particles by rinsing soil with tap water in a sieve (0.2 mm) and then were transferred to a Petri dish for counting. All the individuals counted were assumed to be alive given the quick degradation of individuals once dead.

Collembolan toxicity was assessed in accordance with ISO Guideline 11267 (**ISO 1999**). Five replicates per concentration were prepared consisting of sealed 100 ml plastic cups filled with 30 g of soil (dry weight) moistened to 50% of their maximum water-holding capacity. Ten individuals aged 10 to 12 days were added to each replicate, and fed with 3 mg of yeast at the start of the bioassay and after 14 days. After 28 days, the number of surviving adults and juveniles was determined. The procedure for this involved flooding with water the replicate content in a 500 ml beaker followed by the addition of a dark dye to allow the taking of a picture of the individuals floating on the surface of the water surface. Adults and juveniles were differentiated by their size. Again, all the individuals counted were assumed to be alive given the quick degradation of individuals once dead.
The collembolan lethal concentration (LC50) and effective concentrations for plant germination, shoot length, biomass or collembolan reproduction (EC50) together with their 95% confidence intervals were estimated using Statistica 6.0 (StatSoft Inc.) by suitable regression models (linear and non-linear), which were selected based on their best fit to the data.

2.4. Statistics

The suitability of the soils used in the experiments was assessed by the observation of outcomes in controls, and based on the validity criteria indicated in the corresponding protocols.

The inhibition on the endpoints assessed for each test organisms was assessed by means of linear and non-linear regression models using Statistica 6.0 (Statsoft Inc., Tulsa, OK, USA). From these models, and for each endpoint and species, the concentration causing 10 and 50% inhibition was estimated together with the 95% confidence intervals by iteration processes.

In addition, the influence of soil properties in the toxicity results in the soils studied was assessed by Generalized Linear Models (GLM) (Brodgar 2.0, Highland Statistics Ltd., Newburgh, UK). This influence was studied for each species and endpoint by the construction of matrix containing data of replicates as rows and the different variables as columns. A first column contained the outcome of the endpoint (response variable), and the remainder contained the chemical concentration and each of the soil properties (explanatory variables). Not all soil properties were included in the analysis, since those showing high autocorrelation (r>0.8) were discarded. More precisely, only total carbon, C/N ratio and cation exchange capacity (CEC) were included.

Soil properties influence the biological outcomes of test species directly and indirectly. The direct influence is due to the established fact that different soil properties determine different outcomes in the test species based on their ecological preferences (**Jänsch et al. 2005**). The indirect influence of soil properties is mediated by their role in chemical bioavailability (**Smit and van Gestel 1998**). In order to only take into account the indirect effect of soil properties in the bioavailability of the chemicals, the response variable was introduced in the model as a percentage with respect to the outcome observed in controls. Doing this, only the chemical concentration and the effects on bioavailability of soil properties were taken into account. A Poisson distribution of data was assumed from the GLM, with the logarithm as link function, since the data resembled more counting data than percentage data, as some replicates showed values over 100 due to hormetic effects.

3 Results

3.1. Soil and species performance suitability

No differences in the outcomes between controls with and without addition of acetone were found in any of the assays. The combination of the outcomes in both controls is presented in **Table 2**, where the suitability of the soils to sustain the test species is presented as mean performance values together with the standard deviation (**Table 2**).

The germination rates in OECD soil for *B. rapa* and *L. perenne* were over 95%. The fresh weight of their seedlings was 854±119 and 219±22 mg respectively, with no signs of nutrient deficiency. No tests were carried out in natural soils because in plants NP and NPEO generally showed inhibitory effects over 1 g kg^{-1} in OECD soil in the preliminary test. This is the concentration above which a pollutant is not considered as toxic according to the test protocol used in this study. Concerning assays with soil fauna, the outcomes fulfilled the validity criteria in all the soils for all the species. Adult weight of *E. andrei* at the beginning of the test was within the range indicated in the guideline (200-650 mg), survival was over 90%, and reproduction was over 30 juveniles with a coefficient of variation below 30%. In *E. crypticus*, survival was over 80% and more than 25 juveniles were produced, with a coefficient of variation below 50%. Finally, in *F. candida* survival was over 80% and more than 100 juveniles with 30% maximum coefficient of variation were produced. The only exception is enchytraeid survival in some tests with PRA soil. Their mean survival in this soil was below 80%. However, we decided not to discard the test results of this species in PRA soil for two reasons. First, the reproduction values in this soil fulfilled the validity criteria. Secondly, the difficulty to assess adult survival of this species due to its short generation time (**Kuperman et al. 2006**), which impairs the assessment of survival rates due to natural death of adults and the achievement of sexual maturity of some juveniles.

A generalized higher reproduction was observed in UAB soil controls both in earthworms and enchytraeids, while collembolan reproduction was higher in OECD soil.

Table 2

3.2. Species and endpoint sensitivity

NP8EO and NP showed clearly different toxicities (IC10, IC50); NP being the most toxic, since significant inhibition was demonstrated for most of the endpoints and species tested. There were also clear differences between the sensitivity of the species and endpoints, together with clearly different toxicities in different soils.

NP8EO showed a low toxicity for plants (**Table 3**), at least in the short-term, since EC50 values were generally over 10 g kg^{-1}. Concerning EC10 values, no inhibition was found for germination neither in *B. rapa* and *L. perenne*, while inhibitory effects were found for fresh weight between 211 and 303 mg kg^{-1}. NP presented a higher toxicity for plants (**Table 4**), but it was still a moderate toxicity because EC50 values for the endpoints studied were always above 1 g kg^{-1}. However, if EC10 was assessed, values ranged from 696 and 1386 mg kg^{-1} for germination and between 575 and 739 mg kg^{-1} for fresh weight.

Concerning the effects on survival of the soil invertebrate species tested, NP8EO also showed a lower toxicity (LC10: 394 to 2060 mg kg^{-1}; LC50: 747 to >3000 mg kg^{-1}) compared to the effects observed for NP (LC10: 52 to 663 mg kg^{-1}; LC50: 69 to 615 mg kg^{-1}).

The endpoint inhibited at lower concentrations by NP8EO and NP was soil invertebrate reproduction. Again, N8PEO showed lower toxicity values (EC10: 72 to 440 mg kg-1; EC50: 356 to 1876 mg kg^{-1}) than those observed for NP (EC10: 43 to 197 mg kg^{-1}; EC50: 64 to 226 mg kg^{-1}). The only exception is enchytraeid reproduction in PRA soil, which presented similar values for NPEO (EC10: 438 mg kg^{-1}; EC50: 654mg kg^{-1}) as for NP (EC10: 455 mg kg^{-1}; EC50: 642 mg kg^{-1}).

Concerning the effects on earthworm's total biomass, again NP presented effects at lower concentrations (EC10: 88 to 289 mg kg^{-1}; EC50: 240 to 523 mg kg^{-1}) compared to NP8EO (EC10: 455 to >3000 mg kg^{-1}; EC50: 784 to >3000 mg kg^{-1}).

Table 3

Table 4

3.3. Influence of soil properties on NPEO toxicity

Clearly different toxicities were observed in the different soils used in the bioassays, since regardless of the species or endpoint assessed, toxicity was generally higher in PRA soil compared to the toxicity in OECD soil. More precisely, the IC10 values observed in PRA soil were similar or generally lower than those observed in the remaining soils, while IC50 values were generally lower compared to the values observed in OECD soil, and also compared to UAB soil.

The GLM analysis allowed the construction of models to estimate the contribution of soil properties on the outcomes of the tests (**Table 5**). Besides the obvious influence of NPEO concentration, in most of the assays, NP and NP8EO toxicity was lower, the higher the NPEOs concentration and CEC. In others, also higher carbon content and C/N ratio was associated with a decreased toxicity. However, there were some exceptions to this general trend, since in some of the assays (*E. andrei* reproduction and *E. crypticus* survival and reproduction), increasing carbon contents were associated with increased toxicity.

Table 5

4 Discussion

4.1. Chemical formulation toxicity

NP showed higher toxicity compared to NP8EO, both in plant and soil invertebrate bioassays, as was expected for this hydrophobic chemical with relatively high persistence in soil (**Gejlsberg et al. 2003, Staples et al. 2004**). The biodegradation of NPEOs should lead to the accumulation of the simplest forms of nonylphenols (NP, NP1EO, NP2EO) and nonylphenol carboxy acids (NP2EC or NP1EC) (**Ahel et al. 1994b, 1994c, Staples et al. 2001**). The fast and easy biodegradation of NPEOs in aerobic environments has been shown, for example in wastewaters (**Hernández-Raquet et al. 2007**) and sludge composting (**Domene et al. 2007**). However, we found lower toxicity in bioassays using NP8EO compared to those using NP, something that might be attributed to the short duration of the bioassays (1 month, 2 months in earthworm reproduction assay), which might not allow a full conversion of NP8EO to these more toxic forms.

4.2. Plant toxicity

The uptake and translocation of NP by plants should be moderate according to their physico-chemical properties, and should be mainly retained in root tissues (**Wild and Jones 1992, Collins et al. 2006, Dettenmaier and Doucette 2007**). This has been proved in *Vicia faba* (**Sjöström et al. 2008**), *Brassica napus* (**Mortensen and Kure 2003**), wheat (**Kirchmann and Tengsved 1991**) and tomato (**Harms 1992**).
This phenomenon may explain the low phytotoxicity of NP8EO and NP for the species used in our study (*B. rapa* and *L. perenne*), since strong inhibition effects on germination and fresh weight (EC50) were only observed at very high concentrations (>10 g kg^{-1} for NP8EO and >1 g kg^{-1} for NP), too high levels to consider these chemicals as phytotoxic according to **OECD (2006)** (**Table 3 and 4**). In addition, germination was unaffected by NP8EO, and NP only inhibited this endpoint below 1 g kg^{-1} in *B. rapa* (EC10=696 mg kg^1). Concerning fresh weight, both for NP8EO and NP, slight inhibitory effects were observed below 1 g kg^{-1} in both species (EC10 ranging from 211 to 739 mg kg^{-1}), and indicating the low toxicity of the chemicals in these species.
This low toxicity is apparently consistent with studies that have failed to find toxic effects of NPEOs for plants. However these studies are not strictly comparable to ours because longer test durations and lower concentrations were used, and because NPEOs were applied to soil spiked with organic wastes. **Mortensen and Kure (2003)** found no effects on *Brassica napus* biomass after 30 days at concentrations below 534 ppb of NP, and applying NP alone or spiked with

different organic wastes. **Dettenmaier and Doucette (2007)**, in soil-sludge mixtures, did not find effects on *Agropyron cristatum* shoot dry weight at concentrations below 47 mg kg^{-1} of NP, NP4EO and NP9EO in an assay that lasted around 150 days. On the contrary, our results differ from those of **Nowak et al.** (**2008**), who reported leaf and root biomass inhibition in *Poa pratensis* at NP soil concentrations around 15 mg kg^{-1}. However, again the results are not comparable, since NP was applied spiked in sludge and the assay lasted for 82 days. . In addition, the inconsistencies in the results of different works might be also attributed to the known differential uptake of NP in different plant species (**Sjöström 2004**).

4.3. Soil invertebrate toxicity

The effects of NPEOs on soil invertebrates have been also rarely addressed, and restricted to 4-NP. A collection of NP toxicity data for soil invertebrates from published papers is shown in **Table 6**. To date, the effects of NP have only been reported in three soil invertebrate taxa (earthworms, enchytraeids and collembolans), lacking mites, one of the most abundant groups in soil together with collembolans. In our study we report for the first time the effects of NPEOs on an earthworm (*E. andrei*) and an enchytraeid species (*E. crypticus*). In addition, we add new data on the toxicity of NPEOs on the collembolan *F. candida*.

Table 6

NP8EO generally showed a low toxicity for soil invertebrates, since IC50 values were generally close to or over 1 g kg^{-1}, the maximum concentration to consider a chemical as toxic according to the respective guidelines (**ISO 1998, ISO 1999, ISO 2003**). However, the toxicity was clearly higher for invertebrates than for plants, and especially in the case of invertebrate reproduction. In addition, different sensitivities were observed in the species tested. More precisely, earthworms were more sensitive to NP8EO than enchytraeids and collembolans, both for survival and reproduction. When slight inhibition values are used as endpoint (IC10), effects on reproduction appeared at relatively low concentrations, as low as 72 mg kg^{-1} in PRA soil for earthworm reproduction, indicating the potential ecotoxicity of NP8EO in some taxa and soils despite the general low toxicity.To the best of our knowledge no other toxicity studies on the effects of ethoxylated nonylphenols on soil invertebrates have been published to date.

Concerning the effects of NP, our results generally agree with the few similar studies published to date (**Table 6**). As found for plants, NP showed a clearly higher toxicity than NP8EO in soil invertebrates, since NP inhibited the endpoints assessed at concentrations below 1 g kg^{-1}. In addition, NP generally affected the invertebrate's reproduction at lower concentrations than those affecting growth or survival, as found for NP8EO. Earthworms and collembolans were more sensitive taxa compared to enchytraeids, which were affected at higher concentrations, usually with IC10 and IC50 values over 200 mg NP kg^{-1}.

Two similar studies using other earthworm species using mortality as endpoint failed in finding significant effects at concentrations below 40 mg kg^{-1} for *Aporrectodea calliginosa* (**Krogh et al. 1996**) and below 50 mg kg^{-1} for *Dendrobaena octaedra* (**Widarto et al. 2004**). In our study, LC10 for *E. andrei* ranged from 182 to 498 mg kg^{-1} in the different soils used in this study, clearly over the range suggested in the previously mentioned studies. On the contrary, the LC50 values, ranging from 291 to 625 mg kg^{-1}, are consistent with **Jensen et al. (2009)**, who reported a LC50 of 308 mg kg^{-1} for *D. octaedra*.

The effects on reproduction were slightly higher in this study than those reported for *D. octaedra* (EC50=53 mg kg^{-1}, **Jensen et al. 2009**), since EC10 values in this work were between 43 and 64 mg kg^{-1} and EC50 ranged from 70 to 124 mg kg^{-1}. In addition, our results for *E. andrei* seem to agree with those reported for *D. octaedra* by **Widarto et al. (2004)** and for *A. calliginosa* (**Krogh et al. 1996**), unaffected below 40 and 50 mg kg^{-1}, respectively.

E. andrei's growth was inhibited at higher concentrations than *A. calliginosa*, with EC10 ranging from 88 to 290 mg kg^{-1} and EC50 ranging from 240 to 523 mg kg^{-1}, compared to the EC50=24 mg kg^{-1} reported for *A. calliginosa* (**Krogh et al. 1996**). No data are available for *D. octaedra*, with inhibition values over 50 mg kg^{-1} (**Widarto et al. 2004).**

The effects on *E. crypticus* survival were in accordance to those reported in the related species *Enchytraeus albidus*, since LC10 and LC50 range values, 214-663 and 319-906 mg kg^{-1} respectively, resemble the results of **Gejslbjerg et al. (2001)**, namely LC10=171 mg kg^{-1} and LC50=420 mg kg^{-1}. Similarly, the effects on reproduction were also consistent, with EC10 and EC50 values in *E. crypticus*, ranging from 24-456 and 212-642 mg kg^{-1} respectively, compared to the EC10=98 mg kg^{-1} and EC50=437 mg kg^{-1} reported for *E. albidus* (**Gejslbjerg et al. 2001**).

Survival of *F. candida* was also affected at similar concentrations as those reported in studies that used the same species or the related species *Folsomia fimetaria*. More precisely, LC10 values, between 52-102 mg kg^{-1}, are in the same range found to affect survival in the same species (67 mg kg^{-1} in **Gejlsbjerg et al. 2001**, >40 mg kg^{-1} in **Widarto et al. 2007**), but also *F. fimetaria* (32-94 mg kg^{-1} in **Krogh et al. 1996**, **Scott-Fordsmand and Krogh 2004**). Furthermore LC50 values (70-139 mg kg^{-1}) are in agreement with the previous studies for this species (>30 mg kg^{-1} in **Madsen et al. 1998**, 133 mg kg^{-1} in **Gejlsbjerg et al. 2001**) and *F. fimetaria* (99-151 mg kg^{-1} in **Krogh et al. 1996**, **Scott-Fordsmand and Krogh 2004**).

Similarly, the effects on reproduction also appeared at similar concentration ranges. EC10 ranged from 45-63, while in the literature EC10 values ranged from >32 (**Widarto et al. 2007**) to 51 mg kg^{-1} (**Gejlsbjerg et al. 2001**). This also concurs

with values found in *F. fimetaria* (around 30 mg kg^{-1} both in **Krogh et al. 1996** and **Scott-Fordsmand and Krogh 2004**). EC50 values, ranging from 64 to 92 mg kg^{-1}, were also in accordance with the studies of **Gejlsbjerg et al. (2001)** (71 mg kg^{-1}), but slightly higher than those of **Madsen et al. (1998)** (16-30 mg kg^{-1}). EC50 values were also similar to the EC50 for *F. fimetaria,* 40-65 mg kg^{-1} (**Krogh et al. 1996**, **Scott-Fordsmand and Krogh 2004**).

4.3. Effect of soil properties on NPEO toxicity

Soil properties influenced the NPEO toxicity, since different toxicity values were obtained in different soils. In particular, toxicity was generally lower the higher the organic matter content in soil, given their high affinity for hydrophobic materials (**Burgess et al. 2005**). This is why, generally the lowest toxicity values were obtained in OECD soil, which presented the highest organic matter content (6.9%), and the highest toxicity was in the PRA soil, with the lowest organic content (1.9%). UAB soil, with intermediate organic matter content (4.5%), also presented intermediate NPEO toxicity. However, few of the models developed by GLM to explain the toxicity of NPEOs (**Table 5**) identified organic carbon as a significant factor decreasing the bioavailability and the toxicity of NPEOs. This might be related to the simultaneous contribution of other soil properties to the outcomes of the bioassays, but especially due to the low number of soils used, which prevents drawing strong conclusions.

5 Conclusions

A clear high toxicity of NP was found when compared to the branched NPEO mixture (NP8EO) used in this study. Our results are in agreement with the scarce literature available on the effects of NPEOs on plant and soil invertebrates. NP8EO presented a very low phytotoxicity, while a low toxicity was generally found for soil invertebrates with some exceptions. Conversely, NP presented low phytotoxicity, but moderate toxicity for invertebrates, reproduction being clearly the most sensitive endpoint. Enchytraeids were generally less sensitive than earthworms and collembolans to NPEO pollution. Organic matter content appeared to decrease the toxicity of NPEOs, probably through decreasing their bioavailability.
The ecotoxicological risk of NPEOs to soil communities might be limited given that the usual concentrations in soils receiving sludge and reported in the literature are usually below the concentrations reported to be toxic in this study.

6 Recommendations and perspectives

The main source of NPEOs in the soil is the application of raw sewage sludge to soils (**Ahel et al. 1994a**). However, given the significant biodegradation reported in the aerobic conditions present in soil, concentrations of NPEOs can decrease considerably(**Topp and Starratt 2000, Sjöström et al. 2008**).Notwithstanding, a recalcitrant fraction of NP remains in soil and can be detected a few months after the amendment (**Sjöström et al. 2008, Mortensen and Kure 2003**), bound to organic matter and within soil aggregates, due to the less aerobic conditions therein(**Sjöström et al. 2008**). In addition, the recurrent application of sludge as amendment can lead to persistent levels of NPEOs in soil as reported by **Vilkesøe et al. (2002)**, who found increased concentrations of NPEOs (3.1 µg kg^{-1} to 2.6 mg kg^{-1}) in agricultural soils amended with sludge in comparison to soils amended with manure or chemical fertilizers (3.4-3.8 µg kg^{-1}). Also **Petersen et al. (2003)**, studying agricultural soils amended with sludge for three years, found NPEO concentrations below 0.1 mg kg^{-1} at the end of this period. This was 90% of the initial NPEOs added to soil degraded after 200 days in one of the sludge treatments.

If these studies are representative of the usual levels in soils receiving polluted sludge, a low ecotoxicologial risk might be expected for plants and soil invertebrates, since the usual concentrations in soils (below 2.6 mg kg^{-1}) are below the lowest concentrations reported to have effects on the organisms in this study.

However, the risk of these pollutants should not be neglected since higher concentrations in soil might be reached with higher application rates or highly polluted sludges and hence affect soil organisms. More importantly, NPEOs in soil should be maintained at low levels, given the extremely high toxicity for aquatic organisms (**Staples et al. 2004**). Despite the higher adsorption of NP to hydrophobic materials and their reduced leaching (**Vogel et al. 2003, Jacobsen et al. 2004**), it has been shown that runoff events can transport NP attached to soil particles from the polluted soil to adjacent environments (**Vilkesøe et al. 2002**), and reach aquatic ecosystems.

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Table 1 Properties of the soils used in the bioassays. WHC = water holding capacity, EC = electrical conductivity, CEC = cation exchange capacity.

Soil	OECD	PRA	UAB
WHC, %	44.8	28.1	55.3
pH, 1:2.5 w/w	6.2	6.5	8.3
EC, dS/m, 25ºC 1:5 w/w	0.5	0.1	0.2
Sand, %	45.7	80.3	36.4
Silt, %	31.8	16.5	44.9
Clay, %	16.3	3.2	18.7
Organic matter, %	6.9	1.9	4.53
Organic carbon, %	3.45	1.10	2.63
Total N, %	0.04	0.10	0.18
C/N ratio	86.2	11.0	14.6
CEC, meq(+)/100g	8.94	7.3	13.9
Cd, mg kg^{-1}	-	0.9	<0.1
Cu, mg kg^{-1}	-	77.0	121
Cr, mg kg^{-1}	-	11.0	25
Ni, mg kg^{-1}	-	<10	19
Pb, mg kg^{-1}	-	48.0	35
Zn, mg kg^{-1}	-	204.0	104

Table 2 Mean ± standard deviation of the soil fauna outcomes in controls of the different test soils.

Species	Soil	Survival	Reproduction	Initial weight/worm	Final weight/worm	Germination	Fresh weight
		# adults	*# juveniles*	*mg*	*mg*	*%*	*mg*
B. rapa	OECD	-	-	-	-	97±5	854±119
L. perenne	OECD	-	-	-	-	95±5	219±22
E. andrei	OECD	9.8±0.8	53.4±14.6	417±114	381±77	-	-
	PRA	10.0±0.0	73.1±16.2	488±86	450±106	-	-
	UAB	10.0±0.0	104.2±26.7	472±90	433±87	-	-
E. crypticus	OECD	9.0±1.8	391.8±179.6	-	-	-	-
	PRA	7.8±3.0	702.0±322.8	-	-	-	-
	UAB	8.8±0.9	777.2±311.3	-	-	-	-
F. candida	OECD	9.1±0.9	1323.6±189.5	-	-	-	-
	PRA	9.4±0.8	957.5±315.5	-	-	-	-
	UAB	9.4±0.9	900.2±110.2	-	-	-	-

Table 3 Toxicity values of a nonylphenol ethoxylated mixture (NP8EO) on several soil organisms in different soils, corresponding to a 10 and 50% endpoint inhibition (IC50), expressed as mg kg^{-1}.

Species	Endpoint	Soil	IC10	IC50
B. rapa	fresh weight	OECD	210.8 (24.6, 1808.9)	>10000
	germination		>10000	>10000
L. perenne	fresh weight	OECD	303.0 (62.2, 1475.1)	>10000
	germination		>10000	>10000
E. andrei	survival	OECD	435.9 (111.1, 1709.7)	1181.2 (689.9, 2022.5)
		PRA	394.2 (215.0, 722.6)	747.5 (604.2, 924.9)
		UAB	479.3 (241.9, 949.7)	1047 (930.7, 1177.8)
	reproduction	OECD	321.1 (80.0, 1289.3)	1181.2 (829.9, 1681.3)
		PRA	150.9 (71.6, 317.8)	356.2 (266.0, 477.1)
		UAB	71.8 (4.0, 1302.2)	943.8 (780.0, 1142.0)
	biomass	OECD	>3000	>3000
		PRA	455.2 (340.1, 609.1)	1593.2 (1136.3, 2234.0)
		UAB	968.7 (704.3, 1332.4)	1833.8 (1611.8, 2086.3)
E. crypticus	survival	OECD	2059.0 (638.1, 6643.9)	3041.7 (2446.7, 3781.3)
		PRA	>770	>770
		UAB	479.3 (241.9, 949.8)	1047.0 (930.7, 1177.8)
	reproduction	OECD	431.4 (117.5, 1583.6)	1876.3 (1104.0, 3188.9)
		PRA	438.2 (295.7, 649.5)	653.9 (566.4, 755.0)
		UAB	226.8 (45.0, 1144.5)	587.0 (206.1, 1672.1)
F. candida	survival	OECD	1864.0 (1357.0, 2560.4)	>3000
		PRA	520.2 (279.5, 968.2)	1689.5 (1326.6, 2151.7)
		UAB	1853.7 (1052.7, 3264.0)	>3000
	reproduction	OECD	281.6 (132.6, 597.9)	1450.1 (1139.0, 1846.2)
		PRA	338.7 (217.0, 528.5)	650.0 (560.6, 753.7)
		UAB	107.7 (20.8, 556.7)	745.0 (343.0, 1618.3)

Table 4 Toxicity values of 4-nonylphenol (NP) for several soil organisms in different soils, corresponding to a 10 and 50% endpoint inhibition (IC50), expressed as mg kg^{-1}.

Species	Endpoint	Soil	IC10	IC50
B. rapa	fresh weight	OECD	574.8 (279.9, 1180.4)	1449.1 (785.0, 2674.7)
	germination		695.9 (286.3, 1691.6)	8159.2 (5904.0, 11276.0)
L. perenne	fresh weight	OECD	738.9 (49.6, 11011.2)	4011.6 (862.2, 18664.5)
	germination		1385.8 (696.5, 2757.3)	7500.7 (6031.2, 9328.3)
E. andrei	survival	OECD	343.9 (160.6, 736.4)	625.5 (427.9, 914.1)
		PRA	182.4 (157.6, 211.2)	290.7 (272.0, 310.7)
		UAB	498.2 (439.2, 565.2)	622.0 (592.3, 653.2)
	reproduction	OECD	55.8 (13.7, 227.7)	82.0 (47.6, 141.1)
		PRA	43.1 (26.9, 69.0)	69.6 (57.7, 83.9)
		UAB	63.6 (46.5, 87.1)	124.3 (110.5, 139.9)
	biomass	OECD	88.3 (50.0, 155.8)	309.1 (240.4, 397.3)
		PRA	121.2 (93.6, 156.9)	240.2 (218.0, 264.8)
		UAB	289.4 (219.1, 382.4)	523.2 (474.5, 576.9)
E. crypticus	survival	OECD	663.5 (444.9, 989.5)	906.7 (786.5, 1045.3)
		PRA	214.5 (171.3, 268.6)	316.2 (284.9, 350.9)
		UAB	227.0 (89.5, 575.3)	615.2 (431.9, 876.3)
	reproduction	OECD	24.0 (5.2, 110.7)	225.8 (134.8, 378.4)
		PRA	455.8 (311.2, 667.5)	641.7 (554.2, 742.9)
		UAB	197.2 (97.9, 397.3)	212.8 (194.6, 232.8)
F. candida	survival	OECD	102.0 (69.7, 149.3)	138.7 (129.4, 148.7)
		PRA	52.3 (47.0, 58.1)	69.8 (65.8, 74.1)
		UAB	89.1 (70.0, 113.5)	116.3 (104.2, 129.8)
	reproduction	OECD	63.2 (47.3, 84.5)	92.8 (83.8, 102.8)
		PRA	45.0 (27.5, 73.7)	64.4 (54.6, 75.9)
		UAB	54.8 (23.9, 125.7)	88.0 (79.9, 96.9)

Table 5 Model parameters and fit derived by GLM of the outcomes, in different soils and test organisms, when exposed to NP8EO and NP. Endpoint values are expressed as a percentage with respect to the controls, chemical concentration as mg kg^{-1}, total carbon as a percentage and CEC as meq(+)/100g. All the coefficient estimates are significant with p<0.001.

Species	Endpoint	Chemical	Intercept	Concentration	C	C/N	CEC	R^2
E. andrei	survival	NP	4.4230	-0.0019	0.1439			0.49
		NP8EO	4.3130	-0.0008			0.0406	0.61
	reproduction	NP	3.8886	-0.0119	-0.1271		0.1066	0.83
		NP8EO	4.4870	-0.0013	0.0772			0.67
	biomass	NP	4.4701	-0.0029			0.0302	0.72
		NP8EO	4.1112	-0.0004			0.2227	0.24
E. crypticus	survival	NP	4.4284	-0.0013	0.0703			0.25
		NP8EO	4.6474	-0.0005	-0.2068	0.0089		0.24
	reproduction	NP	4.1572	-0.0051			0.0473	0.63
		NP8EO	4.5767	-0.0008	-0.0799	0.0051		0.38
F. candida	survival	NP	4.2413	-0.0104	0.1240		0.0258	0.48
		NP8EO	4.3584	-0.0002	0.0987			0.51
	reproduction	NP	4.8936	-0.0152				0.61
		NP8EO	4.4719	-0.0008		0.0032		0.66

Table 6 Published toxicity values of nonylphenol (NP) for different soil invertebrates in different soils, expressed as mg kg-1; (*) NP added spiked in sludge.

Species	Endpoint	Concentration	Soil	Reference
Aporrectodea caliginosa (Lumbricidae)	Survival LC10	>40	LUFA, standard	Krogh et al. (1996)
	Growth EC50	23.9		
	Reproduction EC10	3.44		
	Reproduction EC50	13.7		
Dendrobaena octaedra (Lumbricidae)	Adult survival	>50	Sandy loam, agricultural	Widarto et al. (2004)
	Juvenile survival	>50		
	Growth	>50		
	Rate reproducing worms	>30		
	Survival LC50 (25ºC)	308	Loamy sand, agricultural	Jensen et al. (2009)
	Reproduction EC50 (15ºC)	53		
Enchytraeus albidus (Enchytraeidae)	Survival LC10	171	Sandy, agricultural	Gejlsbjerg et al. (2001)
	survival LC50	420		
	Reproduction EC10	98		
	Reproduction EC50	437		
Folsomia candida (Collembola)	Survival LC50*	>30	Loamy sand, agricultural	Madsen et al. (1998)
	Reproduction EC50*	16 to 30		
	Survival LC10	67	Sandy, agricultural	Gejlsbjerg et al. (2001)
	Survival LC50	133		
	Reproduction EC10	51		
	Reproduction EC50	71		
	Survival	>40	Sandy loam, agricultural	Widarto et al. (2007)
	Time to first moulting	>24		
	Reproduction	>32		
Folsomia fimetaria (Collembola)	Survival LC10	75.1	LUFA, standard	Krogh et al. (1996)
	Survival LC50	151.3		
	Reproduction EC10	23.6		
	Reproduction EC50	65.5		
	Survival LC10 (females/males)	32/94	Sandy loam	Scott-Fordsmand and Krogh (2004)
	Survival LC50 (females/males)	140/99		
	Reproduction EC10	23		
	Reproduction EC50	40		