Environmental Toxicology and Chemistry—Volume 40, Number 7—pp. 1948–1961, 2021
Received: 15 July 2020 | Revised: 30 November 2020 | Accepted: 19 March 2021

Environmental Toxicology

Bioavailability and Ecotoxicity of Lead in Soil: Implications for Setting Ecological Soil Quality Standards

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Abstract: Ecological soil quality standards for lead (Pb) that account for soil Pb bioavailability have not yet been derived. We derived such standards based on specific studies of the long-term bioavailability and toxicity of Pb to soil organisms and a compilation of field data on the bioaccumulation of Pb in earthworms. Toxicity thresholds of Pb to plants, invertebrates, or microorganisms vary over more than 2 orders of magnitude, and the lowest values overlap with the range in natural Pb background concentrations in soil. Soils freshly spiked with Pb^{2+} salts exhibit higher Pb bioavailability and lower toxic thresholds than long-term aged and leached equivalents. Comparative toxicity tests on leaching and aging effects suggest using a soil Pb threshold that is 4.0 higher, to correct thresholds of freshly spiked soils. Toxicity to plants and earthworms, and microbial N-transformation and bioaccumulation of Pb in earthworms increase with decreasing effective cation exchange capacity (eCEC) of the soil, and models were derived to normalize data for variation of the eCEC among soils. Suggested ecological quality standards for soil expressed as total soil Pb concentration are lower for Pb toxicity to wildlife via secondary poisoning compared with direct Pb toxicity to soil organisms. Standards for both types of receptors vary by factors of approximately 4 depending on soil eCEC. The data and models we have collated can be used for setting ecological soil quality criteria for Pb in different regulatory frameworks. *Environ Toxicol Chem* 2021;40:1948–1961. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Lead; soil ecotoxicity; Metal bioavailability; Bioaccumulation; Soil quality standards

INTRODUCTION

Lead (Pb) is a natural constituent of the earth's crust and has been extensively used by humans since antiquity because of its ease of extraction and workability. This has resulted in extensive contamination of surface soils, mainly associated with mining and smelting activities, emissions from leaded gasoline, the application of sewage sludge, and the use of lead shot at shooting ranges (Steinnes 2013). The natural Pb concentration in soil ranges from 10 to 40 mg Pb/kg dry soil, with a median of approximately 20 mg Pb/kg dry soil (Smith et al. 2013; Reimann et al. 2014). Soil Pb concentrations of >10 000 mg Pb/kg can be found in highly contaminated soils (Steinnes 2013).

Lead in soil may present a risk to humans (mainly via dust ingestion), to wildlife via dust and food chain transfer, and to soil organisms. A large number of studies are available on the toxicity of Pb to soil organisms (plants, invertebrates, and microorganisms). The variability that researchers found in the levels of Pb causing toxicity is strikingly large: some studies found incipient toxicity at Pb levels approaching natural background concentrations (Chang and Broadbent 1981; Aery and Jagetiya 1997; Saviozzi et al. 1997; Hamon et al. 2003; An 2006; Lanno et al. 2019), whereas other studies failed to identify Pb-related effects at concentrations of >1000 mg Pb/kg (Ma 1982; Doelman and Haanstra 1984; Spurgeon et al. 1994; Speir et al. 1999; Langdon et al. 2005; Cheyns et al. 2012). Accordingly, setting soil quality standards that are conservatively based on the lower thresholds yields values that are within the natural background range and hence are considered overprotective of some soils. On the other hand, the highest thresholds may be underprotective when they are extrapolated

This article includes online-only Supplemental Data.

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Published online 23 March 2021 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5051

to other soil types. Neglecting bioavailability considerations may therefore result in soil quality standards that are either under- or overconservative, depending on the relevance of the treatments and the properties of the soils used for derivation of toxicity data for the soils to be protected. Soil quality standards for Pb have been proposed by different regulatory frameworks (Table 1). The US Environmental Protection Agency (2005) has derived an ecological soil screening level for plants of 120 mg total Pb/kg soil and for invertebrates of 1700 mg Pb/kg soil. In Canada, a soil quality guideline between 300 and 600 mg Pb/kg was derived for soil contact to plants and invertebrates depending on land use (Canadian Council of Ministers of the Environment 1999). Screening values for Pb in some European countries range from 25 to 700 mg Pb/kg (Carlon et al. 2007). However, these screening values are not solely based on ecotoxicological effects. Ecotoxicity-related soil clean-up standards for Pb in residential land range from 100 to 300 mg/kg in The Netherlands, Sweden, and Norway (Provoost et al. 2006).

The wide range of toxicity thresholds is largely related to differences in sensitivity of the species and endpoints tested and to differences in soil Pb bioavailability. The variation in bioavailability may be attributed to different contamination (spiking) treatments prior to toxicity testing and differences in bioavailability of Pb in the different soil types tested (Smolders et al. 2009; Organisation for Economic Co-operation and Development 2016). Comparative studies on the toxicity of Pb to soil organisms in different soils illustrate that differences in toxicity were mainly correlated to differences in pH or cation exchange capacity (CEC) of the soils (Cheyns et al. 2012; Romero-Freire et al. 2015; Li et al. 2016; Lanno et al. 2019; Zhang et al. 2019). The soil spiking protocol (Pb source, percolation, and equilibration after spiking) has also been shown to significantly affect differences in toxicity between laboratory and field conditions. Lead toxicity in soils contaminated under field conditions generally yields lower toxicity values compared with corresponding soils freshly spiked with soluble Pb salts (Lock et al. 2006). The difference can be related to the higher solubility of the metal in soil spiked with salts than in soil contaminated by "environmental" metal. The higher solubility of Pb in soils freshly spiked with soluble Pb salts is attributed to higher ionic strength and lower pH (Speir et al. 1999; Stevens et al. 2003; Bongers et al. 2004; Smolders et al. 2015; Zhang and Van Gestel 2019), longer equilibration period in field-contaminated soils ("aged" metal; Degryse et al. 2007; Ming et al. 2012; Smolders et al. 2015), and different sources of metals (Khan and Frankland 1983, 1984; Davies et al. 2003; Zhang and Van Gestel 2017). Under field conditions, potential excess ions are removed from the soil through leaching by percolating rain water, and the total Pb content in the soil is the result of the accumulation of small annual doses, allowing for much longer equilibration times (Speir et al. 1999; Hamon et al. 2003; Stevens et al. 2003; Bongers et al. 2004; Waegeneers et al. 2004; Lock et al. 2006; Smolders et al. 2015). When available, toxicity data derived in field-contaminated soils or soils that were leached and aged after artificial contamination are therefore preferred for the effects assessment over data derived in freshly spiked soils. However, an assessment based solely on data for leached and aged or field-contaminated soils would neglect a large quantity of the available toxicity data. This shortcoming highlights the importance of applying a correction factor to toxicity data from freshly spiked soils to achieve the toxicity level such as may be seen in a realistic field situation.

Apart from causing direct toxicity to soil-dwelling organisms, increased Pb concentrations in soils can also result in secondary poisoning of mammals and birds via food chain transfer of Pb. The food chain soil–earthworms–earthworm-eating predators is generally considered the most critical for the assessment of secondary poisoning (US Environmental Protection Agency 2005; European Chemicals Agency 2008). Therefore, a correct estimate of the bioaccumulation of Pb in earthworms is critical. Similar to Pb toxicity, Pb bioaccumulation in earthworms varies among different soils and experimental designs, and this variation should be accounted for when one is selecting

TABLE 1: Soil quality standards as defined under different regulatory frameworks[a]

Legislation	Limit (mg Pb/kg dry wt)	Note	Reference
Europe	25–85	Screening values for negligible risks	Carlon et al. 2007
	40–700	Screening values for warning risk for metals and metalloids (residential use)	
Europe (EU Directive 86/278/EEC)	50–300	Limit values for Pb in soil	European Commission 1986
	750–1200	Limit values for Pb in sludge for use in agriculture	
United States	120 (plants)	Ecological soil screening level (ECO SSL); no correction for soil properties	US Environmental Protection Agency 2005
	1700 (invertebrates)		
	11 (birds)		
	56 (mammals)		
Australia	110–440 (fresh)	Added contaminant level (i.e., natural background concentration not included); no correction for soil properties; values for natural, urban, and commercial land use	National Environment Protection Council 2011
	470–1800 (aged)		
Canada	75 (soil and food ingestion)	Soil quality guidelines for 4 land uses (agricultural, residential, commercial, and industrial)	Canadian Council of Ministers of the Environment 1999
	300–600 (soil contact)		

[a]Unless noted, all values are total lead (Pb) in soils (mg/kg dry wt).

bioaccumulation factors for the assessment of Pb toxicity to wildlife via secondary poisoning (Luo et al. 2014; Lanno et al. 2019; Richardson et al. 2020).

The objective of the present study was to incorporate bioavailability considerations into the derivation of ecological soil quality standards for Pb. This was based on a collation of the existing toxicity data from publications in international peer-reviewed journals and from research project reports combined with studies and models on soil Pb bioavailability, including the effects of leaching and aging on toxicity. Results are presented for the derivation of predicted no-effect concentrations (PNECs) according to the European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) Regulation (European Commission 2006), but the toxicity data and bioavailability corrections can also be used for other regulatory purposes.

MATERIALS AND METHODS

Ecological soil quality standards were derived after bioavailability normalizations of the toxicity data as described in the following sections. These normalizations included corrections for differences in toxicity between laboratory-spiked soils and field-contaminated soils and for differences in Pb bioavailability among soils differing in physical and chemical characteristics (Smolders et al. 2009; Organisation for Economic Co-operation and Development 2016).

Correction for discrepancies in toxicity between laboratory-spiked soils and field-contaminated soils

Comparative toxicity tests showed that soils freshly spiked with Pb^{2+} salts exhibited higher Pb bioavailability and lower toxic thresholds. Therefore, chronic toxicity data were corrected for this discrepancy by the application of correction factors to the toxicity data, to convert these to relevant field conditions, that is, considering leaching by percolating rainwater and sufficient aging time. Available toxicity data for soil organisms were first classified into 3 groups according to the soil Pb-spiking protocol: 1) soils freshly spiked with a soluble Pb salt and no leaching or aging, 2) soils artificially leached after spiking with a soluble Pb salt and no aging, and 3) soils artificially leached and aged after contamination or field-contaminated soils. The minimal aging time for the third protocol was 180 d after spiking. If data were available for the same soil-endpoint combination tested after different treatments after spiking, only the result for the most field relevant condition was selected, that is, the leached/aged or leached treatments, not the freshly spiked.

On these 3 groups of data, different factors were applied to account for the differences in toxicity between field-contaminated soils and spiked soils. These factors were based on a compilation of different studies that compared Pb toxicity for different contamination protocols. Briefly, 2 studies compared experimentally the effects of leaching and aging in spiked soils on the subsequent toxicity (Hamon et al., 2003; Smolders et al. 2015), and an additional study compared toxicity in 3 field contamination gradients from past industrial activities with toxicity in corresponding control soils that were freshly spiked with Pb nitrate (Waegeneers et al. 2004). Because leaching and aging do not affect the bioavailability of the natural background Pb concentration in soil, all factors were derived based on the difference in toxicity of the added dose only without consideration of the background Pb concentration in the soils, expressed as *x*% effect dose (ED*x*). Three different correction factors were derived: 1) Leaching factors were defined as the ratio of the ED*x* value of the spiked + leached soil and the ED*x* value of the freshly spiked equivalent, reflecting changes in toxicity due to reduced ionic strength and reduced acidification; 2) aging factors were calculated as the ratio of the ED*x* value of the spiked + leached + aged soil and the ED*x* value of the spiked + leached equivalent, reflecting the net effect of aging reactions; this factor was applied to toxicity data from leached soils without aging treatment; and 3) leaching/aging factors were calculated as the ratio of the ED*x* value of the spiked + leached + aged soil and the ED*x* value of the freshly spiked equivalent, reflecting the changes in toxicity due to the combined effects of reduced ionic strength (leaching) and aging reactions. This factor was applied to toxicity data from freshly spiked soils with no leaching or aging.

All the ED*x* values for aged and leached soils were based on measured concentrations to correct for the potential decrease in total Pb concentration due to leaching of Pb. The quantification of differences in toxicity due to leaching or aging depends on the effect levels observed in the aged soils. Because median effective dose (ED50) values are statistically a more robust estimate compared with lower effect levels (e.g., effective dose, 10% [ED10]), the ratio of ED50 values was preferred when bounded ED50 values were observed in both series of soils. When no reliable ED50 could be derived in one of the soil treatments, the ratio of ED10 values was selected. If the response curve after leaching or aging was not significant and no reliable ED*x* values could be derived for these soil treatments, a conservatively low factor difference in toxicity was derived assuming the largest tested dose as the ED10 in the leached or aged soils.

Correction for differences in toxicity among soils due to different soil properties

The second bioavailability correction refers to the effects of physicochemical soil properties. This was based on a specific set of tests (Smolders et al. 2011; Cheyns et al. 2012; Lanno et al. 2019). Briefly, the toxicity of $PbCl_2$ for 2 plants species (tomato and barley growth; International Organization for Standardization 2012), 2 invertebrate species (*Eisenia fetida;* Organisation for Economic Co-operation and Development 2004), *Folsomia candida;* International Organization for Standardization 1999), and 2 microbial processes (nitrification; International Organization for Standardization 1997; and respiration; Organisation for Economic Co-operation and

TABLE 2: Selected properties of the uncontaminated soils used in bioassays testing the effect of soil properties on the bioavailability and toxicity of lead (Pb)[a]

Soil	Country	Soil type[b]	Land use	pH	Total Pb (mg/kg)	Organic carbon (%)	Clay (%)	eCEC[c] ($cmol_c$/kg)
Barcelona	Spain	Calcic Luvisol	Arable land	7.4	137	1.2	16	14.3
Woburn	UK	Dystric Cambisol	Grassland	6.1	52	4.3	30	26.5
Leuven	Belgium	Haplic Luvisol	Arable land	6.2	21	1.0	12	8.4
De Meern	The Netherlands		Grassland	5.3	52	5.0	60	42.0
Borris	Denmark	Cambisol	Arable Land	5.7	15	1.5	3	4.2
Hygum	Denmark		Grassland	5.2	18	2.1	13	7.6
Kasterlee	Belgium	Haplic Podzol	Arable land	4.7	24	2.3	2	4.0
Zegveld[d]	The Netherlands	Histosol	Grassland	4.7	100	31	59	41.7

[a]Expressed on a soil dry weight basis.
[b]Soil classification according to the World Reference Base (Food and Agricultural Organization, International Soil Reference and Information Center, International Union of Soil Sciences 1998).
[c]eCEC = effective cation exchange capacity, that is, CEC at pH of the soil.
[d]Zegveld soil was used instead of De Meern soil for the plant assays.

Development 2000) was tested in 7 uncontaminated topsoils collected throughout Europe and comprising a wide range of soil properties (Table 2). All soils were spiked with $PbCl_2$, leached, and pH corrected prior to toxicity testing to remove confounding factors of increasing ionic strength and acidification with increasing Pb dose. The effects of soil physical–chemical characteristics on Pb toxicity were assessed using simple linear regression between log-transformed median effect concentration (EC50) values including the background Pb concentration in the soil; (ECx = EDx + background concentration) and log-transformed soil properties (except pH was not log transformed). Regressions were conducted using EC50 values because they are more precise (smaller confidence interval) and less affected by experimental error than no-observed-effect concentration (NOEC) or low effect levels (e.g., EC10 values).

Correction for variation in Pb bioaccumulation in earthworms among soils

The effect of soil properties on the bioaccumulation of Pb in earthworms was assessed based on a compilation of literature data whereby the bioaccumulation factor, calculated as the ratio of the Pb concentration in earthworm over the Pb concentration in the soil, was based on measured Pb concentrations in soil and biota from field observations when earthworms were exposed during their entire life (Sample et al. 1999; Richardson et al. 2020). Several data qualification criteria were applied in the assessment. The Pb concentration in soil had to be expressed as "total" soil Pb (e.g., Pb measured after aqua regia destruction), and results based on extractable Pb fractions (e.g., water-extractable Pb) were not considered reliable. Earthworms must have been rinsed and soil voided from the intestinal tract prior to analysis. Whether the bioaccumulation factors were expressed on a dry or wet weight basis had to be reported. Data were only considered relevant and reliable if the data came from field studies or laboratory studies using soil and biota collected at the same field site. This was to ensure that biota Pb burdens were at steady state with soil Pb concentrations, and it avoided the need for correcting for differences in Pb availability between laboratory-spiked soils and field-contaminated soils. Data from laboratory studies in which Pb was added to the soil as a Pb salt were hence excluded. The effects of the soil properties on Pb bioaccumulation factors were assessed by linear correlation.

Derivation of ecological soil standards

The implementation of bioavailability into the derivation of ecological quality standards concentrations for direct toxicity of Pb to soil organisms follows the general approach developed for metals (Smolders et al. 2009; Organisation for Economic Co-operation and Development 2016). Briefly, the following steps can be distinguished.

First, the relevant and reliable toxicity data must be selected, and a choice made for the effect level (x in ECx) to be used as the basis for the soil quality standards (e.g., EC10 or NOEC for PNEC under the European REACH Regulation; European Commission 2006).

In a second step, all selected toxicity data are corrected for the discrepancy in toxicity between laboratory-spiked soils and field conditions by multiplying the toxicity data, expressed as added doses (EDx, = x% effect dose, corrected for natural background concentration of Pb in the soils tested) with the appropriate leaching and or aging factors. The background Pb concentration from each individual test soil are then added back to calculate the "field" ECx or NOEC values expressed as total metal concentrations including the Pb background concentration.

$$\text{EC}x \text{ "field"} = \text{ED}x \times \text{correction factor} + C_b \quad (1)$$

where C_b is the Pb background concentration of the soil tested.

In a following step, the toxicity values are corrected for differences in metal availability among soils by normalizing each total "field" ECx or NOEC value to the soil properties of a specific target soil, using the slope of the respective regression function (log–log based) and information on soil properties of the test soils and the reference soil according to the following equation:

$$ECx_{reference} = ECx_{test} \times \left[\frac{eCEC_{reference}}{eCEC_{test}}\right]^{slope} \quad (2)$$

where reference is the soil for which the soil standard must be derived, test is the tested soil, and slope is the slope of the selected regression model with the effective CEC (eCEC) of the soil, as will be given in the *Results and Discussion* section. Because regressions based on EC50 values provide the best estimate of the effect of soil properties on Pb toxicity, these slopes are used for correction of all toxicity thresholds (e.g., NOEC or EC10) for varying soil properties. When no specific regression model was available for an organism, a model from similar species within the same trophic level was selected after an assessment of its applicability for the organism (Organisation for Economic Co-operation and Development 2016).

Data on the ecotoxicological effects of Pb on soil organisms (plants, invertebrates, and microorganisms) were selected from original studies published in international peer-reviewed journals and from research project reports. The relevance and reliability of all data were evaluated according to the assessment criteria specified for metals (Arche Consulting, Eurometaux, International Council of Mining and Metals 2016). In summary, only tests in natural or artificial (Organisation for Economic Co-operation and Development) soil media were considered relevant, and tests performed in substrates such as nutrient solutions, agar, pure quartz sand, or farmyard manure were excluded from further analyses. Soluble Pb compounds (e.g., $PbCl_2$, $Pb(NO_3)_2$, Pb acetate) must have been used as a source of Pb contamination in the soil, to ensure maximal bioavailability of the added Pb. The Pb compounds must have been mixed thoroughly in the test soil and allowed adequate time (i.e., ≥24 h) between mixing the metal/metal compounds into the test medium and the introducing of biota (plants or soil invertebrates) at the start of the test, thus ensuring initial partitioning of Pb between the solid and solution phases in the soil. The thresholds were preferentially based on actual measured soil Pb concentrations. Only chronic exposure toxicity data for endpoints with direct effects at the population level for plants and soil invertebrates (mortality, growth, reproduction) and for soil microbial functional endpoints (respiration, nitrification, mineralization) were considered. Soil enzymatic processes were not included as relevant endpoints (Kuperman et al. 2014). If various endpoints were derived from one test (e.g., reproduction, growth, mortality), only the most sensitive endpoint was included. Exposure durations were related to recommendations from standard ecotoxicity protocols (e.g., International Organization for Standardization, Organisation for Economic Co-operation and Development, ASTM International), whenever available.

The ECx (*x*% effect concentrations including background concentrations) values as calculated from the concentration–effect relationships were preferred over the NOEC (i.e., the highest test concentration showing no statistically significant effect compared with the control; Chapman et al. 1996; Organisation for Economic Co-operation and Development 1998; European Chemicals Agency 2008). When ECx values were not reported in the original study, it was calculated using a logistic (sigmoidal) dose–response model from the original data if available. In some cases, no reliable ECx could be derived because the data could not be described using a logistic model or the ECx was outside the concentration range tested (Organisation for Economic Co-operation and Development 2006). When EC10 values were selected, these were considered not reliable when the corresponding ED10 (i.e., 10% effect concentration based on added or background corrected dose) was more than a factor of 2 below the lowest added dose. In these cases, if a bounded NOEC could be derived, this NOEC value was used instead of the EC10. Unbounded NOEC (i.e., no effect at highest dose tested) or unbounded lowest-observed-effect concentration (LOEC; i.e., significant effect at lowest dose tested) values were not used for derivation of the soil standards, except for unbounded LOEC values with <20% inhibition. In the latter case, a NOEC was defined as LOEC/2.

A robust and large data set was available for evaluating the toxicity of Pb to soil organisms and processes, including plants, invertebrates, and microbial processes. Therefore, the use of the statistical extrapolation method was preferred for the derivation of ecological soil standards rather than a deterministic method that uses an assessment factor on the lowest ECx or NOEC values (European Chemicals Agency 2008; Arche Consulting, Eurometaux, International Council of Mining and Metals 2016). Values for PNECs according to the European REACH Regulation (European Commission 2006) were determined as the median hazardous concentration for 5% of the tests (HC5–50; i.e., the median 95% protection level) derived from the cumulative distribution of EC10 or NOEC values after bioavailability corrections.

The quality standard for secondary poisoning is based on literature data for oral toxicity of Pb for mammals and birds and the soil-specific bioaccumulation factor for Pb in earthworms. Data on oral toxicity were only considered relevant and reliable when they were based on subchronic and chronic studies (≥21 d) and the endpoint was ecologically relevant (e.g., growth, reproduction) and not merely a biomarker for Pb exposure. At least 2 Pb concentrations above the control must have been applied. Mixed-metal feeding studies, studies in which Pb was injected in test animals, and tests in which Pb was administered through drinking water or as lead shot pellets were all considered not relevant and were excluded. When low doses of Pb had been added to the diet (≤10 mg Pb/kg), or if there was a NOEC ≤10 mg Pb/kg diet, the Pb concentration in the diet of the control animals must have been measured and quality control of these measurements reported. Unbounded toxicity data (i.e., significant effects observed at the smallest dose or no significant effect observed at the largest dose tested) were not considered reliable and not taken forward. Following this approach, relevant and reliable threshold values for oral toxicity to mammals and birds were identified for 8 mammal and 5 bird species (Supplemental Data, Table S1), resulting in a PNEC for oral toxicity ($PNEC_{oral}$) of 10.9 and 16.9 mg Pb/kg diet (fresh wt) for mammals and birds,

respectively, under the European REACH Regulation (European Commission 2006). Following a different approach, a toxicity reference value of 4.70 and 1.63 mg Pb/$kg_{body\ wt}$/d was derived for mammals and birds, respectively for the derivation of soil screening levels in the United States (US Environmental Protection Agency 2005). Quality standards for Pb concentrations in soil were calculated based either on the critical Pb concentration in food (in mg Pb/kg diet) and the bioaccumulation factor for Pb in earthworms or on the critical Pb intake rate (mg Pb/$kg_{body\ wt}$/d) and assumptions on food intake rate and the bioaccumulation factor for Pb in earthworms.

RESULTS AND DISCUSSION

Bioavailability corrections

Derivation of laboratory-to-field correction factors. The factor difference in toxicity due to aging processes ranged between 0.2 and >65.6, with a median of 3.2 (Figure 1 and Supplemental Data, Table S2). When the effects of both leaching and aging processes were taken into account, the factor difference in toxicity ranged between >1.1 and >530, with a median of 6.0. Although some aging factors were <1, none of these values were significantly different from 1 ($p > 0.05$), that is, toxicity never increased significantly after aging. In contrast, most (>75%) of the factors for the effects of aging and leaching+aging were significantly >1, that is, EDx values increased significantly and toxicity decreased significantly after leaching and aging of soils. It must be noted that no significant toxic effects were observed in most of the leached and aged soils and therefore, most of the aging and leaching/aging factors were unbounded (12 of 27 and 16 of 26, respectively). These unbounded factors were conservative, lower estimates for the value of these aging or leaching/aging factors. The overall distribution was therefore biased toward lower (conservative) values.

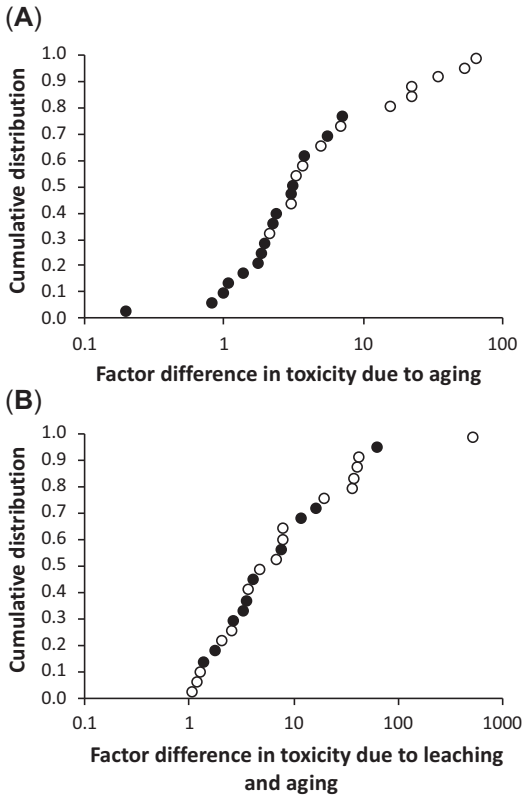

FIGURE 1: Distribution of factor differences in toxicity due to (**A**) aging and (**B**) leaching + aging. The difference in toxicity is quantified as the ratio of *x*% effect added dose values (EDx) from soils with realistic exposure for field conditions, that is, removal of excess ions (leaching) and long-term equilibration (aging), to corresponding values from soil (**A**) spiked with soluble lead (Pb) salts and leached or (**B**) soils spiked with soluble Pb salts without leaching or aging. Closed symbols refer to bounded values, and open symbols indicate unbounded values, that is, lower estimate of real leaching/aging factor because no toxicity was observed in leached and aged soils. All data are reported in the Supplemental Data, Table S2.

The distribution of aging and leaching/aging factors with and without data based on field-contaminated sites (Waegeneers et al. 2004) did not show large differences (Supplemental Data, Table S2). Hence, bioavailability of Pb in these sites contaminated by industrial activities was not significantly affected by the Pb source, and including these data did not create a bias toward larger correction factors. Therefore, all data were included in the overall analysis of the effects of leaching and aging and the derivation of laboratory-to-field correction factors (Figure 1).

Neither soil properties (pH, organic matter content, clay content, eCEC) nor species and endpoints tested had a significant effect on the laboratory-to-field correction factors for Pb (details not shown). Therefore, an empirical generic constant correction factor was selected for the effect of aging or of leaching + aging and the resulting difference in Pb toxicity between laboratory and field exposure conditions. Based on the overall weight of evidence, a value of 2.0 was chosen for the laboratory-to-field correction factor for the effect of aging. The aging factor of 2.0 corresponds to approximately the 27th percentile of the distribution of observed factor differences in toxicity due to aging reactions. This factor 2 matches a mean isotopically exchangeable fraction of 58% for Pb in field-contaminated soils, that is, that fraction is approximately 2.0-fold lower than the total concentrations (Degryse et al. 2007). The fraction of metals that is isotopically exchangeable is an accurate predictor of the leaching/aging factor in field contaminated soils for Zn and copper (Cu; Hamels et al. 2014). A value of 4.0 was selected for the laboratory-to field correction factors covering effects of both leaching and aging. This larger factor highlights the importance of salt stress for Pb toxicity in freshly spiked soils (Stevens et al. 2003; Bongers et al. 2004; Smolders et al. 2015; Li et al. 2016). This factor of 4.0 corresponds to approximately the 40th percentile of the mainly unbounded factor differences in toxicity of Pb in soils due to the combined effect of leaching and aging after spiking with a soluble Pb source. This laboratory-to-field factor for Pb is larger than observed for other metals (Smolders et al. 2009; Organisation for Economic Co-operation and Development 2016), which can be mainly attributed to the pronounced acidification and salt effect at high Pb doses required to elicit significant effects in soil

organisms because Pb ions are easily hydrolyzed in solution and are strongly adsorbed by soils (Speir et al. 1999; Degryse et al. 2009).

There is no guidance on which percentile of these factors should be used in risk assessment, and the choice uses a weight-of-evidence approach. Earlier correction factors chosen for risk assessment of other metals in Europe were based on a reasonable worst-case scenario, complete with additional evidence such as soil chemical information and absolute toxicity thresholds in field-contaminated soils (Smolders et al. 2009; Organisation for Economic Co-operation and Development 2016).

Influence of soil properties on Pb toxicity to soil organisms. Variation in Pb toxicity among soils was smaller than generally observed for other metals (Cheyns et al. 2012).The key soil property identified to affect Pb toxicity in soils was the eCEC (i.e., CEC at pH of the soil; Table 3). This is in line with observations for other metal cations in soil such as cobalt (Co^{2+}), nickel (Ni^{2+}), Cu^{2+} and zinc (Zn^{2+}) (Smolders et al. 2009; Organisation for Economic Co-operation and Development 2016), and the eCEC of the soil can be regarded as an adequate measure for the binding of cations to soil, combining the effects of clay, organic matter, and pH on the bioavailability of cations in soil. Because calciumions (Ca^{2+}) dominate the exchange sites of most temperate soils, the eCEC can also be regarded as a measure for the hardness of the soil porewater, which is also known to affect metal toxicity in aqueous environments (Organisation for Economic Co-operation and Development 2016). However, the regression analysis indicated that not all endpoints show a significant effect of soil properties on the bioavailability and toxicity of Pb. Highly significant single linear regressions between log EC50 and log eCEC were observed for the potential nitrification rate (PNR) and *E. fetida* reproduction assays (Figure 2), with toxicity of Pb on *E. fetida* reproduction most impacted by differences in eCEC (largest slope of regression equations). Insignificant or unreliable regressions were observed for plants (tomato or barley shoot yield separately), the respiration assay, and *F. candida* reproduction, which could be partly explained by the small number of soils tested (Table 3) and the limited variation in EC50 values among the soils (<a factor of 5). The precipitation of Pb-phosphates after amendment of soluble Pb salts to a soil and the resulting phosphorus deficiency may be an additional explanation for the lack of a significant effect of soil properties

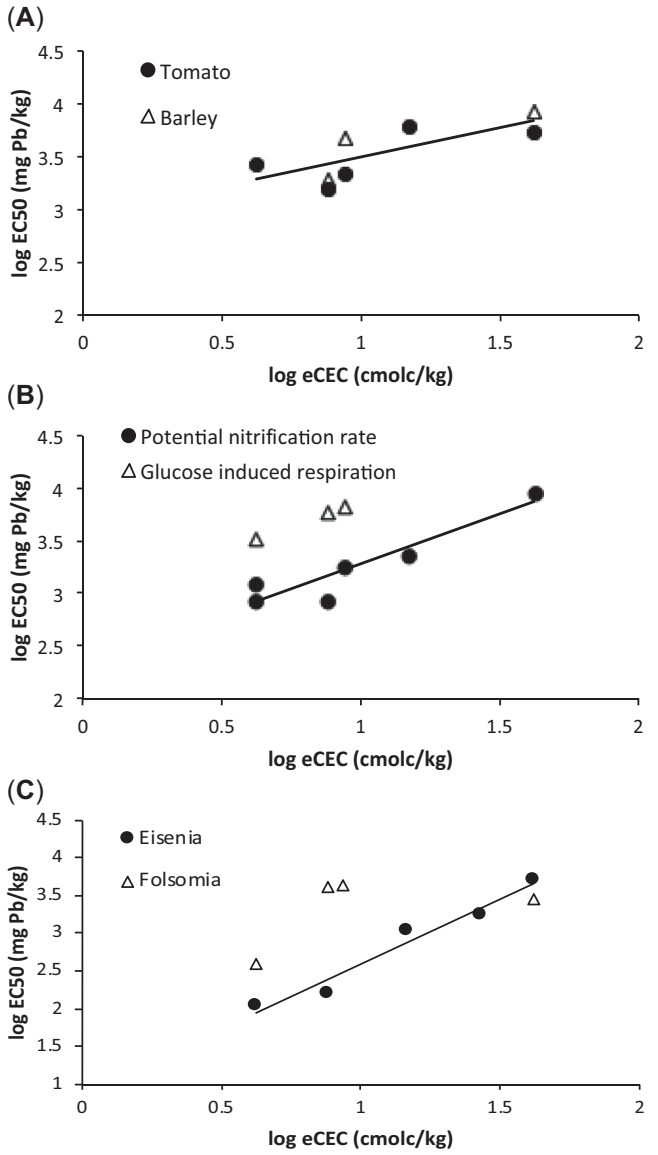

FIGURE 2: Correlation between log-transformed median effect concentration (EC50) for (**A**) tomato and barley shoot yield, (**B**) nitrification (potential nitrification rate) and respiration, and (**C**) *Eisenia fetida* and *Folsomia candida* reproduction and the log-transformed effective cation exchange capacity (eCEC) of the soils. The regression line for plants is based on the combined dataset for tomato and barley.

TABLE 3: Simple linear regression between log-transformed EC50 values for the various endpoints and the log-transformed effective cation exchange capacity (eCEC) of the soils[a]

Endpoint	Regression equation	R^2	No.	*p*
Tomato shoot yield	log EC50 = 3.00 + 0.47 × log eCEC	0.48	5	0.19
Barley shoot yield	log EC50 = 2.89 + 0.65 × log eCEC	0.68	3	0.38
Tomato + barley	**log EC50 = 2.94 + 0.55 × log eCEC**	0.56	8	0.03
Potential nitrification rate	**log EC50 = 2.33 + 0.95* × log eCEC**	0.86	6	<0.01
Substrate- induced respiration	log EC50 = 2.94 + 0.95 × log eCEC	0.99	3	0.02
Eisenia fetida reproduction	**log EC50 = 0.89 + 1.70 × log eCEC**	0.96	5	<0.01
Folsomia candida reproduction	log EC50 = 2.77 + 0.54 × log eCEC	0.22	4	0.53

[a]Selected regressions in bold.

on Pb toxicity to plants (Cheyns et al. 2012). The EC50 values for barley and tomato largely overlapped, and intercepts and slopes from the individual regression equations did not differ significantly between the plant species. It was therefore concluded that there was no significant difference in sensitivity of the 2 plant species to Pb toxicity. When data for both plants were combined, a significant regression with eCEC was observed (Table 3 and Figure 2). The slope of this combined regression was selected for normalization of toxicity data for plants. The highly significant regression between EC50 values for the glucose-induced respiration assay and eCEC proved to be not reliable because it was only based on 3 data points with a narrow range in eCEC values (4.2–8.7 $cmol_c$/kg). Hence this regression was considered not representative and was not used for normalization of toxicity data on microbial carbon respiration processes to reference soil properties.

Influence of soil properties on Pb bioaccumulation in earthworms. In total, 248 reliable bioaccumulation factors for earthworms were identified, ranging from 0.01 to 22.05 $kg_{dry\ wt\ soil}/kg_{dry\ wt\ worm}$ on a dry weight basis. The median bioaccumulation factor for earthworms was 0.23 $kg_{dry\ wt\ soil}/kg_{dry\ wt\ worm}$ (10th and 90th percentiles of 0.06 and 1.19, respectively).

Results are available for several earthworm species belonging to different ecological groups of earthworms: anecic, endogeic, and epigeic. No distinct differences in bioaccumulation factors across these groups could be identified. Soil properties were not reported for all studies, but based on the reported data, it can be concluded that the bioaccumulation factors were derived in a wide range of soils and the data available can be considered as representative for soils in Europe (pH: 3.0–8.4, $n = 217$; organic carbon content: 1.1–24.6%, $n = 186$; clay content: 4–53%, $n = 111$; CEC: 5.3–78.8 $cmol_c$/kg, $n = 114$; and total Pb content in soil: 9.4–16 700 mg/kg, $n = 231$).

Correlation of the bioaccumulation data for earthworms with soil properties showed that only CEC was significantly correlated with bioaccumulation values. No significant correlation of bioaccumulation factors with Pb content, pH, organic carbon content, or clay content was observed. Because of the lack of any effect of Pb level in soil on the bioaccumulation factor for Pb in earthworms, data from Pb-contaminated soils could also be included in the analysis.

Four field studies reported CEC data for the soils from which the earthworms were sampled (Beyer et al. 1982; Ma 1982; Ernst et al. 2008; Nannoni et al. 2011). Correlations between bioaccumulation factors in earthworms and soil properties for individual studies were either nonsignificant or contradictory. The combined data set showed a significant decrease in bioaccumulation factors for Pb with increasing eCEC of the soil (Figure 3):

$$\text{Log bioaccumulation factor for Pb } (kg_{dry\ wt\ soil}/kg_{dry\ wt\ worm}) = -0.89 \times \log \text{eCEC}(cmol_c/kg) + 0.55 \quad R^2 = 0.16,\ p < 0.01 \tag{3}$$

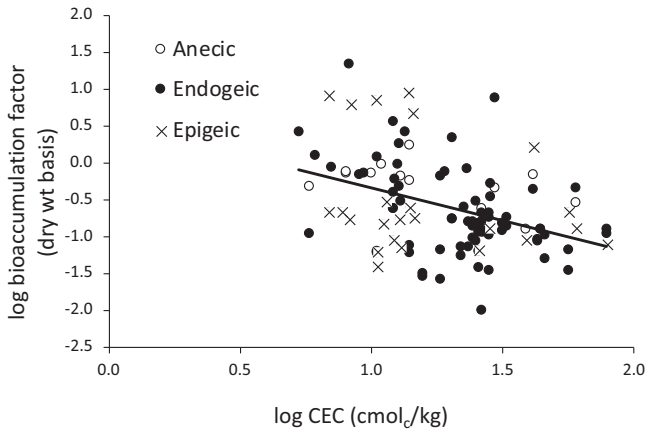

FIGURE 3: Correlation of field bioaccumulation factors for lead (Pb) in earthworms with cation exchange capacity (CEC) of the soil. Data from Beyer et al. (1982), Ernst et al. (2008), Ma (1982) and Nannoni et al. (2011).

This regression is based on data from different studies for 9 different earthworm species (anecic: *Aporrectodea longa*, *Lumbricus terrestris*; endogeic: *Aporrectodea caliginosa*, *Aporrectodea rosea*, *Aporrectodea tuberculate*, *Octolasion cyaneum*, *Octolasion tyrtaeum*; epigeic: *Lumbricus rubellus*, *Dendrodrilus rubidus*) and for a wide range of Pb levels and forms in soil (from natural and various anthropogenic sources). No clear distinction could be noticed between different ecological groups of earthworms, indicating that feeding strategy does not significantly affect Pb accumulation and that Pb bioaccumulation is mainly affected by the direct exposure to the soil and hence by the availability of Pb in the soil (Figure 3).

The slope of this regression between bioaccumulation of Pb in earthworms and the CEC of the soil corresponded well with the results of a recent laboratory study on the effects of soil type on the bioavailability and toxicity of Pb salts to the earthworm *E. fetida* exposed for 28 d to Pb in 6 different soils spiked with $PbCl_2$ and leached with a dilute salt solution (Lanno et al. 2019). This finding points to a similar effect of CEC on Pb accumulation in earthworms in controlled laboratory conditions and in field conditions. The significant regression between bioaccumulation of Pb in earthworms and CEC was also consistent with the regression observed between toxicity of Pb to *E. fetida* reproduction and eCEC of the soil. Equation 3 is therefore used to calculated soil-specific bioaccumulation factors for Pb in earthworms.

This yielded a generic bioaccumulation factor for Pb in earthworms of 0.30 ($kg_{dry\ wt\ soil}/kg_{dry\ wt\ worm}$) for the median eCEC value of 16 $cmol_c$/kg soil for European natural soils (Reimann et al. 2014). Based on an average dry matter of 16% in earthworms (Jager 1998), this corresponded to a fresh weight–based bioaccumulation factor for Pb in earthworms of 0.048 $kg_{dry\ wt\ soil}/kg_{fresh\ wt\ worm}$. Fresh weight–based bioaccumulation factors for a soil with an eCEC of 8 and 30 $cmol_c$/kg soil, corresponding to the 10th and 90th percentiles of eCEC in European arable soils are 0.089 and 0.028, respectively (Table 4).

TABLE 4: Bioaccumulation factors for lead (Pb) in earthworms and critical soil limits for secondary poisoning of Pb to mammals and birds[a]

Scenario[b]	Bioaccumulation factor ($kg_{dw\ soil}/kg_{fw\ worm}$)	Soil limit for mammals (mg Pb/kg)	Soil limit for birds (mg Pb/kg)
eCEC = 8 $cmol_c$/kg	0.089	122	189
eCEC = 16 $cmol_c$/kg	0.048	226	350
eCEC = 30 $cmol_c$/kg	0.028	394	611

[a]Based on predicted no-effect concentrations for oral toxicity to mammals and birds of 10.9 and 16.9 mg/kg diet (fresh wt), respectively (European Chemicals Agency 2021) and the worst-case assumption that the diet consists of 100% earthworms).
[b]Effective cation exchange capacity (eCEC) values corresponding to the 10th, 50th, and 90th percentiles of eCEC in arable land across Europe (Reimann et al. 2014).

Toxicity data and derivation of ecological soil standards

All toxicity data and soil standards are expressed on a dry weight basis. A total of 105 relevant and reliable EC10 or NOEC values that met the selection criteria for the chronic effects assessment of Pb^{2+} to soil organisms were identified in the scientific literature and selected for the risk assessment of Pb in soil under the European REACH Regulation (European Commission 2006; Supplemental Data, Table S3). Among the 105 toxicity values selected, 14 values were derived from tests with soils that were leached and aged after spiking, 35 were from tests in which soils were spiked and leached, and 56 were derived from tests with soils immediately after spiking. For plants, 44 individual high-quality EC10 or NOEC values were identified. These data include various endpoints (shoot and root yield or net photosynthesis) for 14 different species from 6 different families and range from 57 mg Pb/kg for *Hordeum vulgare* (oat) to 6774 mg Pb/kg for *Triticum aestivum* (wheat). Fourteen reliable EC10 or NOEC values for 5 annelid worm species were found with values ranging from 64 mg Pb/kg for reproduction of *E. fetida* to 2445 mg Pb/kg for mortality of *A. caliginosa*. In addition, 17 high-quality EC10 or NOEC values for 3 arthropod species were identified, ranging from 34 to 2306 mg Pb/kg for reproduction of the springtail *F. candida*. In total, 30 individual high-quality EC10 or NOEC toxicity values for soil microbial processes were selected, evaluating 5 functional parameters representing the N-cycle (i.e., denitrification, N-mineralization, and nitrification) and the C-cycle (basal respiration and substrate-induced respiration). Toxicity values for microorganisms varied from 97 (for basal respiration) to 7880 mg Pb/kg (for the PNR).

For 7 EC10 or NOEC values, no data on eCEC were available and the eCEC could also not be calculated based on pH, organic matter, or clay content. Because this precludes normalization of the toxicity data for variation in soil properties, these data were removed from the final database, which resulted in the loss of 3 plant species (*Avena sativa*, *Picea rubens*, and *Pinus taeda*) and 1 earthworm species (*A. caliginosa*) from the database.

In case more than one toxicity value was available for a particular species and endpoint, this is reduced to one single value (e.g., geometric mean for the most sensitive endpoint) per species or microbial process to reflect the species sensitivity distribution. This would be feasible if the variation in toxicity due to abiotic factors was negligible or could be fully eliminated and the intraspecies variation in sensitivity could be assumed as the main source of variation remaining for a given species or microbial process. However, significant bioavailability models could not be derived to account for the large variation in toxicity thresholds for arthropods and microbial respiration. Moreover, even after normalization, the variation in toxicity values for the other species was significant compared with the overall variation in sensitivity among species (Figure 4). It was found that the median 5% hazardous concentration (HC5–50) based on a distribution of species geomean values of EC10 or NOEC values (normalized to reference soil conditions, except for arthropods and microbial respiration) was always higher than the HC5–50 value based on the distribution of all individual EC10 or NOEC values, and the more conservative approach was selected for setting soil quality standards for Pb (Table 5).

Different distributions were evaluated for fitting the species sensitivity distributions. The final distribution function was selected on the basis of the Anderson–Darling goodness-of-fit test because this test focuses on the differences between the tail of the distribution (lower tail is the region of interest) and the input data. There was no consistent best-fitting distribution for the various soil scenarios tested. The log-normal distribution was accepted for all soil scenarios tested according to the Anderson–Darling test at $\alpha = 0.01$. Comparison of the uncertainty around the HC5–50 showed that there was no consistent difference between results of the log-normal and best-fitting distributions. Therefore, the consistent application of the log-normal distribution was selected for derivation of quality standards for direct toxicity of Pb to soil organisms.

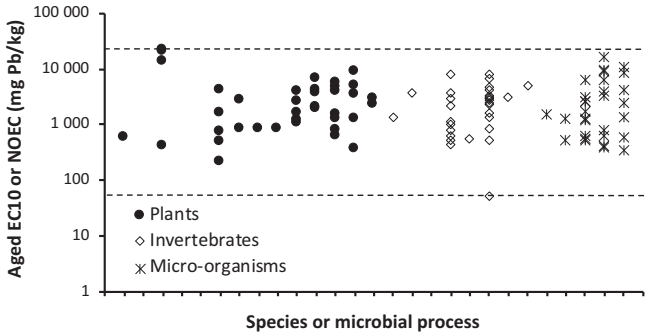

FIGURE 4: Variation in chronic toxicity thresholds 10% effect concentration (EC10) and no-observed-effect concentration (NOEC) values for the toxicity of lead to soil organisms, after correction for leaching and aging and normalization to reference soil properties (effective cation exchange capacity = 16 $cmol_c$/kg). Each column presents toxicity thresholds for one single species or microbial process, and the values of the toxicity thresholds are shown on the *y*-axis. The broken lines indicate the lowest and highest toxicity thresholds observed in the complete dataset.

TABLE 5: Median hazardous concentration for 5% of the tests (HC5–50), that is, median 95% protection level, with 5% and 95% (HC5–5 and HC5–95) confidence limits (total soil Pb concentrations, mg Pb/kg dry soil) for the terrestrial environment after various bioavailability correction scenarios[a]

	All individual data			Species mean values		
Scenario	No. of data	HC5–50	HC5–5 to HC5–95	No. of data	HC5–50	HC5–5 to HC5–95
No laboratory-to-field corrections, no normalization	105	90	65–118	27	135	83–191
Laboratory-to-field corrections, no normalization	105	212	152–282	27	402	246–576
Laboratory-to-field corrections + normalization to eCEC = 8 $cmol_c/kg$[b]	98	170	121–227	23	240	125–379
Laboratory-to-field corrections + normalization to eCEC = 16 $cmol_c/kg$	98	308	226–399	23	466	241–741
Laboratory-to-field corrections + normalization to eCEC = 30 $cmol_c/kg$	98	440	324–571	23	718	345–1204

[a]All fits according to log-normal distribution (Aldenberg and Jaworska 2000).
[b] Effective cation exchange capacity (eCEC) values corresponding to the 10th, 50th, and 90th percentiles of eCEC in arable land across Europe (Reimann et al. 2014).

Without bioavailability correction for leaching and aging or normalization for the effect of soil properties, the HC5–50 of all selected reliable EC10 or NOEC values was 90 mg Pb/kg soil. After correction for differences between laboratory and field conditions due to leaching and aging reactions but without normalization for effects of soil properties on Pb bioavailability and toxicity to soil organisms, an HC5–50 of 212 mg Pb/kg soil was derived based on a log-normal distribution of all individual reliable EC10 or NOEC values (Table 5 and Figure 5). There was no distinction in sensitivity of the 3 trophic levels of soil organisms (plants, invertebrates, and microorganisms) to Pb because data for all 3 trophic levels were scattered over the entire distribution curve. The effect of the normalization on the HC5–50 was studied for 3 different soils with eCEC values corresponding to the 10th, 50th, and 90th percentiles of eCEC in arable land across Europe (Reimann et al. 2014). The HC5–50 values for Pb varied by approximately a factor of 2.5 between soils representing the 10th and 90th percentile of eCEC in European arable land (Table 5). The generic HC5–50 of 212 mg Pb/kg was only slightly higher than the HC5–50 derived for the reasonable worst-case eCEC (i.e., 10th percentile) in European soils (170 mg Pb/kg) and can therefore be considered conservative for the majority of soils in Europe.

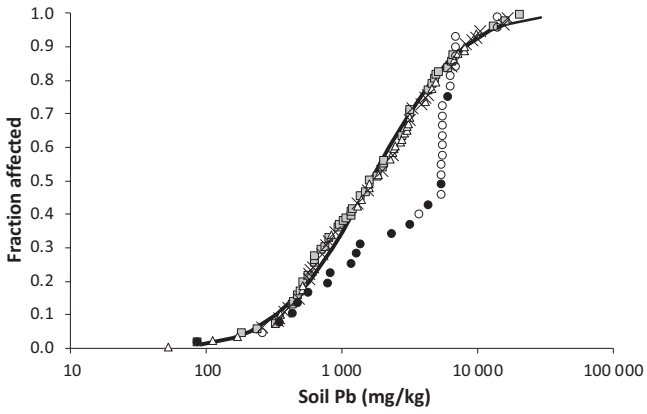

FIGURE 5: Cumulative distribution of high-quality chronic 10% effective concentration (EC10) or no-observed-effect concentration (NOEC) values after correction for leaching and aging (aging factor = 2.0 and leaching/aging factor = 4.0) and without normalization for effects of soil properties on lead (Pb) toxicity to soil organisms. The squares denote data for plants, triangles for invertebrate data, and crosses for microorganism data. The solid line shows the fitted log-normal distribution. The predicted median 95% protection level (HC5–50) is 212 mg Pb/kg. The EC10 or NOEC values observed in field contaminated or leached and aged soils ($n = 34$; data from Wilke 1989; Kádár 1995; Hamon et al. 2003; Waegeneers et al. 2004; Lofts et al. 2007; Smolders et al. 2011; Lanno 2012) are included as a reference (circles, closed cricles refer to bounded values and open symbols refer to unbounded values, that is, no toxicity observed at highest dose tested).

Evaluation of derived soil quality standards

In the risk assessments of metals under the European REACH Regulation (European Commission 2006), additional assessment factors have been applied to the HC5–50 value to obtain environmental quality standards (European Chemicals Agency 2008). These factors have no scientific basis but are discussed on a case-by-case study. Applying an assessment factor is outside the scope of the present study, but the following discussion attempts to assess the robustness of the HC5–50 value, that is, taking into account the evaluation of chronic toxicity data below the HC5–50 value and a comparison with field data based on the European framework (European Chemicals Agency 2008). The selected endpoints are all relevant for potential effects at the population level: shoot and root yield or net photosynthesis for terrestrial plants; mortality, growth, and reproduction for invertebrates; and nitrogen and carbon transformation processes for microbes. Data are either from tests focusing on sensitive life stages (e.g., root elongation) or from "chronic exposure" (e.g., growth, reproduction). The reliable chronic EC10 and NOEC values for each trophic level were based on tests performed in natural and artificial soils, covering a wide range of the soil characteristics in Europe (pH, organic carbon, clay, and eCEC; Table 2). The available database largely fulfils the requirements for the statistical extrapolation approach of a minimum of 10 species belonging to the major taxonomic groups of soil organisms

TABLE 6: Individual no-observed-effect concentration or effect concentration values below the generic median 95% protection level (HC5–50) of 212 mg lead (Pb)/kg for effects of Pb on soil organisms

Species (endpoint)	Aged EC10 (mg Pb/kg)	eCEC ($cmol_c$/kg)	Normalized HC5–50 (mg Pb/kg)	Reference
Lactuca sativa (shoot yield)	87	3.3	64	Hamon et al. 2003
Hordeum vulgare (root yield)	184	8.3[a]	176	Aery and Jagetiya 1997
Eisenia fetida (reproduction)	113	4.2	85	Lanno et al. 2019
Eisenia fetida (reproduction)	172	7.6	162	Lanno et al. 2019
Folsomia candida (reproduction)	53	4.2	85	Lanno et al. 2019

[a]Effective cation exchange capacity (eCEC) predicted based on pH 7.8, 0.5% organic carbon, and estimated clay content for sandy loam soil of 10% (Helling et al. 1964).
EC10 = effect concentration, 10%; HC5–50 = median 95% protection level.

(European Chemicals Agency 2008; Arche Consulting, Eurometaux, International Council of Mining and Metals 2016).

After laboratory-to-field correction, 5 of 105 individual toxicity data values fell below the generic HC5–50 value of 212 mg Pb/kg (Table 6). These results were obtained in soils with an eCEC below 8 $cmol_c$/kg (10th percentile of eCEC in European arable soils), except for one result for *H. vulgare* root yield (184 mg/Pb/kg) that was derived in a sandy loam soil with unreported eCEC. If a clay content of 10% is assumed for this sandy loam soil, the eCEC was predicted to be 8.3 $cmol_c$/kg (based on pH, organic carbon content, and clay content; Helling et al. 1964). Normalizing the toxicity data to the eCEC of these soils yielded HC5–50 values below the corresponding EC10 values for 4 of 5 cases. Only the EC10 for *F. candida* in a sandy soil was still below the corresponding HC5–50. This value is an exception because laboratory-to-field–corrected NOEC or EC10 values for *F. candida* in other soils were all in the range of 500 to 8000 mg Pb/kg (geometric mean: 1950 mg Pb/kg), and no clear relationship between Pb toxicity to Collembola and soil properties was observed.

The data set further contains unbounded NOEC values, that is, concentrations of Pb in the soil that were the highest tested and for which no toxic effect was observed. These unbounded NOECs, after correction for aging and leaching processes, ranged from ≥2230 to ≥46 500 mg Pb/kg and were all well above the HC5–50 value. Finally, several results are available for Pb toxicity to soil organisms measured in either field-contaminated soils or soils spiked in laboratory conditions and subsequently leached and equilibrated for >1 yr. In total, 34 reliable EC10 or NOEC values were identified: 15 results for 5 plant species, 2 results for the worm *E. fetida*, 6 results for the collembolan *F. candida*, and 11 results for 3 microbial processes. These data ranged from 87 to >14 436 mg Pb/kg soil and included 19 unbounded NOEC values (Figure 5). Only one EC10 value of 87 mg Pb/kg soil for shoot yield of *L. sativa*, tested in a sandy soil with eCEC of 3.3 $cmol_c$/kg (Hamon et al. 2003), fell below the generic HC5–50 value of 212 mg Pb/kg. As just explained, this eCEC is low compared with the representative range of eCEC in European soils, and it was shown that after normalization of the toxicity data toward this eCEC, the HC5–50 is also conservative for these types of soils (Table 6). For all other available field or aged data (>264 to >14 436 mg Pb/kg; bounded values: 355–6150 mg Pb/kg), it was not possible to demonstrate toxicity below the generic HC5–50. Hence, based on the preceding uncertainty analysis, and in particular the quality of the database, the diversity of species and soils covered, the goodness-of-fit of the log-normal distribution, and extensive field validation, it can be concluded that the available data and bioavailability correction models allow for the derivation of a robust HC5–50 that is protective for direct toxicity of Pb to soil organisms.

Although the dissolved Pb fraction in porewater is presumed to be the more bioavailable fraction, methods expressing Pb toxicity in soil based on total soil solution Pb concentration or free Pb^{2+} activity generally increase the variability in toxicity thresholds among soils and hence do not explain differences in bioavailability (Cheyns et al. 2012; Lanno et al. 2019). This is consistent with observations for other metals such as Cu, for example (Smolders et al. 2009). This observation that free Pb^{2+} activities do not explain variability in toxicity does not invalidate the concept that the free metal ion in solution is the directly available and toxic metal species because interactions with competing ions should also be accounted for when toxicity is expressed based on free Pb^{2+} activities, according to the concept of the biotic ligand model (Thakali et al. 2006); in addition, porewater composition can strongly vary among soil types.

In addition, several soil extraction techniques have been used to predict metal bioavailability and toxicity in soils. A 0.01 M $CaCl_2$ extract accurately predicted variation in the toxicity of Pb to the invertebrate *Enchytraeus crypticus* (Zhang et al. 2019; Zhang and Van Gestel 2019). Results for one species can, however, not be extrapolated to other soil organisms because the extract best explaining metal bioavailability often varies among organisms and metals. Similar to Pb concentrations in porewater, Pb toxicity thresholds based on extracts neglect salt effects and interactions with competing ions. Moreover, expressing soil quality standards as extractable concentrations to obtain a better estimation of the bioavailable fraction requires a full recalibration exercise of the available toxicity data expressed as total metal concentrations into concentrations for the selected extract. In view of the large data set available for Pb toxicity to soil organisms, this would be a huge effort.

Comparison of soil quality standards for direct toxicity to soil organisms and secondary poisoning (Tables 4 and 5) shows that secondary poisoning is predicted to be the critical pathway for toxicity of Pb in soil. The proposed soil quality standards are

all above the natural background concentrations of Pb in soil and are within the range of soil quality standards defined under different regulatory frameworks (Table 1).

In conclusion, a large number of reliable chronic toxicity data for Pb ($n = 105$) covering a wide range of species or microbial processes ($n = 27$) are available for the derivation of a soil quality standard protecting soil-dwelling organisms. Comparative data sets on the effect of spiking protocol or soil properties on the toxicity of Pb to soil organisms indicate that the spiking protocol (presence or absence of leaching or aging treatments) prior to toxicity testing is of paramount importance to obtaining toxicity thresholds. Toxicity of Pb to soil organisms and Pb bioaccumulation in earthworms both vary significantly with the CEC of the soil, resulting in an approximate factor 4 variation of the predicted standards between the 10th and 90th percentile of CEC in soils from temperate regions. The soil quality standards derived for direct toxicity to soil organisms and secondary poisoning to wildlife are above natural background concentrations, especially when the standards account for bioavailability. Including bioavailability considerations in setting soil quality standards is important to increase the relevancy of the quality standards for local field conditions and to avoid over- or underprotective standards depending on type of toxicity data available and the site-specific conditions. The information in the present study can be used for national and international regulatory purposes in which other conventions apply for the derivation of soil limits.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.5051.

Acknowledgment—The authors thank the Lead REACH Consortium and the International Lead Association for funding the present study.

Data Availability Statement—All toxicity data and bioavailability corrections discussed are included in a freely available spreadsheet allowing calculation of soil type–specific ecotoxicological standards for Pb and other metals in soil for different regulatory purposes (https://www.arche-consulting.be/tools/threshold-calculator-for-metals-in-soil/). Depending on the protection goals, selections can be made on the organisms to be considered, the effect level (ECx) for the toxicity thresholds, the protection level in the species sensitivity distribution, soil properties of interest, etc. for the derivation of ecological soil quality standard concentrations for Pb. Data, associated metadata, and calculation tools are also available from the corresponding author (koen.oorts@arche-consulting.be).

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