

# Derivation of ecological standards for risk assessment of molybdate in soil

Koen Oorts,<sup>A,G</sup> Erik Smolders,<sup>B</sup> Steve P. McGrath,<sup>C</sup> Cornelis A.M. van Gestel,<sup>D</sup> Michael J. McLaughlin<sup>E</sup> and Sandra Carey<sup>F</sup>

<sup>A</sup>ARCHE, Liefkensstraat 35D, BE-9032 Ghent (Wondelgem), Belgium.

<sup>B</sup>Department of Earth and Environmental Sciences, Division of Soil and Water Management, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, BE-3001 Leuven, Belgium.

<sup>C</sup>Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK.

<sup>D</sup>Department of Ecological Science, Faculty of Earth and Life Science, VU University Amsterdam, De Boelelaan 1085, NL-1081 HV Amsterdam, Netherlands.

<sup>E</sup>CSIRO Land and Water, Contaminant Chemistry and Ecotoxicology Program, Waite Campus, Waite Road, Urrbrae, SA 5064, Australia.

<sup>F</sup>International Molybdenum Association, 4 Heathfield Terrace, London, W4 4JE, UK.

<sup>G</sup>Corresponding author. Email: [koen.oorts@arche-consulting.be](mailto:koen.oorts@arche-consulting.be)

**Environmental context.** In order to assess the potential risks of elevated molybdenum concentrations in soil due to anthropogenic activities, toxicity thresholds must be known and environmental criteria defined. Setting such criteria for metals is not straightforward because of varying natural background concentrations and differences in toxicity between typical laboratory and field conditions and across soil types. Toxicity data and models were derived that account for these parameters so that soil quality criteria can be derived based on total molybdenum concentrations in soil.

**Abstract.** An extensive testing programme on the toxicity of sodium molybdate dihydrate in soil was initiated to comply with the European REACH Regulation. The molybdate toxicity was assayed with 11 different bioassays, 10 different soils, soil chemical studies on aging reactions, and toxicity tests before and after 1-year equilibration in field conditions. Differences in molybdate toxicity among soils were best explained by soil pH and clay content. A correction factor of 2.0 was selected to account for the difference in molybdate toxicity between laboratory and field conditions due to leaching and aging processes. Toxicity thresholds were determined as the HC<sub>5-50</sub> (median hazardous concentration for 5% of the species, i.e. median 95% protection level) derived from the species sensitivity distribution of ecotoxicity data after bioavailability corrections. Uncertainty analysis illustrated that the HC<sub>5-50</sub> provides a robust and ecologically relevant predicted no-effect concentration (PNEC) for risk characterisation. The 10th and 90th percentiles for site-specific PNEC values in European agricultural soil are 10.7 and 168 mg Mo kg<sup>-1</sup> dry weight respectively based on a large survey of metal concentrations and soil properties in arable land soils. Total soil Mo concentrations in these soils are below corresponding PNEC values at most locations, suggesting no regional risks of molybdate to soil organisms at this scale. The information presented can be used in the EU risk-assessment framework as well as for national and international regulatory purposes for the setting of soil quality criteria based on total molybdenum concentrations, soil pH and clay content.

**Additional keywords:** bioavailability, soil ecotoxicity.

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## Introduction

Molybdenum (Mo) is a naturally occurring element, playing an essential role in biochemical processes in microorganisms, plants and animals.<sup>[1,2]</sup> The average concentration of Mo in the upper earth's crust is 1.2 mg kg<sup>-1</sup>, making it 56th in order of crustal abundance.<sup>[3]</sup> Natural background concentrations in soil range between 1 and 5 mg kg<sup>-1</sup>, whereas Mo-rich soils may contain 10–100 mg Mo kg<sup>-1</sup>.<sup>[4]</sup> Molybdenum is used mainly in steel and alloys, with other applications in catalysts, pigments,

lubricants, corrosion inhibitors, smoke suppressants and fertilisers ([http://www.imoa.info/molybdenum\\_uses/molybdenum\\_uses.php](http://www.imoa.info/molybdenum_uses/molybdenum_uses.php), accessed September 2015). The main emission pathways to the environment are through mining activities, the application of biosolids and fertilisers, and atmospheric deposition from smelters.<sup>[5]</sup> Although the speciation of Mo in environmental emissions is unclear, it is most likely that aerosol-bound Mo is present as the sparingly soluble hexavalent Mo oxide (MoO<sub>3</sub>).

Molybdenum is present as  $\text{Mo}^{\text{VI}}$  in solutions at redox potential ( $E_h$ )  $> 0$  V and  $\text{pH} > 5$ .<sup>[6]</sup> The dominant soluble species found in oxygenated environments is the tetrahedral oxoanion molybdate ( $\text{MoO}_4^{2-}$ ). The dominant sorbents of molybdate in soils are oxides (e.g. iron(III) oxide, clay minerals and organic matter).<sup>[7–9]</sup> Molybdate sorption onto soils and minerals has been shown to decrease with increasing  $\text{pH}$ .<sup>[7,10]</sup>

Until recently, few data were available concerning  $\text{MoO}_4^{2-}$  toxicity to soil organisms. However, according to the European Union (EU) Regulation number 1907/2006 concerning the Registration, Evaluation and Restriction of Chemical Substances (REACH), the registration dossier for high-volume chemicals (i.e.  $> 1000$  tonnes per year), such as  $\text{MoO}_3$ , Mo metal and  $\text{Na}_2\text{MoO}_4$ , must comply with the minimum data requirements outlined in Annexes VII–X of the REACH legislation.<sup>[11]</sup> In practice, these requirements include data from chronic toxicity studies for at least one organism belonging to each of the three trophic levels of soil organisms, i.e. microorganisms, plants and invertebrates.<sup>[12]</sup>

The natural occurrence of metal compounds in all environmental compartments, including organisms, together with the chemical processes that affect the bioavailability of metals in soils have important implications for the effects assessment of metals to soil organisms.<sup>[13]</sup> The bioavailability and toxicity of metals or metalloids in soils is influenced by several abiotic factors such as (i) soil properties,<sup>[14–16]</sup> (ii) aging processes of the metals added,<sup>[17–19]</sup> and (iii) form of metal added to the soil.<sup>[20,21]</sup> It is generally accepted that the total metal concentration in soil is a poor predictor of its bioavailability and toxicity. However, there is not yet a generally accepted method for measurement of the bioavailable fraction of metals in soil, and data for (pseudo-) total metal concentrations, based on concentrated acid digestions, are most commonly available and used in setting soil quality criteria. Protocols have been developed to take bioavailability considerations into account for the derivation of ecological soil standard values based on total metal concentrations.<sup>[22]</sup> However, such information was not available for Mo and hence a research programme was initiated to collect data and develop bioavailability correction models to facilitate a sound risk assessment of Mo for soil organisms. The  $\text{MoO}_4^{2-}$  ion is the prevailing form of Mo in the environment at relevant conditions of  $\text{pH}$  and redox potential<sup>[5,23]</sup> and hence is the relevant form for essentiality and potential toxicity to living organisms. The objective was therefore to evaluate the toxicity in soils for the  $\text{MoO}_4^{2-}$  ion by assessing bioassays carried out with a soluble  $\text{MoO}_4^{2-}$  salt (i.e. sodium molybdate,  $\text{Na}_2\text{MoO}_4$ ). Results from toxicity tests with sodium molybdate dihydrate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ) show that this form is the preferred Mo form for testing rather than  $\text{MoO}_3$ , which is the dominant Mo compound in emissions to the environment. This is because large doses of  $\text{MoO}_3$  induce acidification and results are strongly confounded.<sup>[5]</sup>

The research programme consisted of testing the toxicity of  $\text{Na}_2\text{MoO}_4$  in 11 different bioassays encompassing plants, invertebrates and microbial processes in 10 different natural soils covering a representative range in soil properties for Europe. In three soils, toxicity was also tested after 6 and 11 months' equilibration after spiking with  $\text{Na}_2\text{MoO}_4$  in order to assess changes in soil toxicity with time.<sup>[5,24–30]</sup>

The current paper presents the implementation of these data and bioavailability correction models in the effects assessment and the derivation of ecological standards for Mo in soil. This assessment covers direct toxicity only to soil organisms,

i.e. microorganisms, plants and soil-dwelling invertebrates; the assessment of secondary poisoning to vertebrates is not discussed. The approach is illustrated by an environmental risk assessment for Mo in arable land soils at the European scale.

## Materials and methods

### *Selection of ecotoxicological data*

The ecotoxicological data in the present paper are derived from original papers in international peer-reviewed journals and from the International Molybdenum Association (IMOA) soils research programme. All data were thoroughly screened for their relevance and quality (reliability). Preference was given to standardised tests with standard species, as prescribed by organisations such as International Organization for Standardization (ISO), Organization for Economic Cooperation and Development (OECD) and United States Environmental Protection Agency (US EPA), but non-standard tests were also allowed when they met the relevance and reliability criteria. Tests that did not comply with the criteria below were rated as not relevant or reliable and were not used in the effects assessment.

Only data from observations in natural and artificial (OECD) soil media were selected. Tests performed in substrates that do not represent soils (e.g. nutrient solution, agar, pure quartz sand and farmyard manure) were not included in this effects assessment. The Mo compounds had to have been mixed homogeneously in the test soil and adequate time (i.e.  $\geq 24$  h) had to have elapsed between mixing metals or metal compounds into the test medium, introducing biota (plants or soil invertebrates) and the start of the test. In addition, the data used in the effects assessment had to be based on actual measured or analytically confirmed nominal soil Mo concentrations.

The assessment was based on toxicity data to plants and soil-dwelling organisms (invertebrates and microorganisms) after chronic exposure only. The selected toxicity data on soil organisms were from ecotoxicity tests that studied relevant ecotoxicological parameters such as survival, growth, reproduction, litter breakdown, abundance. Relevant endpoints for soil microorganisms focussed on functional parameters such as respiration, nitrification and mineralisation. What comprises 'chronic exposure' is dependent on the life cycle of the test organisms. A priori fixed-exposure durations were therefore deemed not relevant. The duration had to be related to the typical life cycle and ideally encompass the entire life cycle or, for longer-lived species, the most sensitive life stage. Retained exposure durations were related to recommendations from standard ecotoxicity protocols (e.g. ISO, OECD, American Society for Testing and Materials (ASTM)). Typically, chronic test durations for the higher plants were within the range of 4 days (e.g. the barley root elongation test based on ISO 11269-1<sup>[31]</sup>) and 21 days (e.g. the tomato shoot yield test based on ISO 11269-2<sup>[32]</sup> or OECD 208<sup>[33]</sup>). Tests assessing the chronic effects of substances on sublethal endpoints of soil invertebrates had a typical exposure duration of 4 to 8 weeks for standard organisms.<sup>[34–36]</sup> Reported test durations using soil microorganisms varied between 24 h (glucose-induced respiration assay) and 28 days for a plant residue mineralisation assay and a nitrogen transformation test.<sup>[37,38]</sup>

If no statistical methodology was reported or if effects concentrations were derived 'visually', the data were considered unreliable. Effect levels derived from toxicity tests using a single test concentration always result in unbounded, unreliable

**Table 1. Soils used for molybdate toxicity studies and the derivation of bioavailability correction models for molybdenum**

Soil properties analysed as described in Van Gestel et al.<sup>[25]</sup> eCEC, effective cation exchange capacity, CEC at pH of the soil; Al<sub>ox</sub>, oxalate-extractable aluminum; Fe<sub>ox</sub>, oxalate-extractable iron; Mn<sub>ox</sub>, oxalate-extractable manganese. Soil type classification according to World Reference Base for Soil Resources<sup>[54]</sup>

| Location    | Country        | pH<br>0.01 M CaCl <sub>2</sub> | Organic C<br>(%) | Clay<br>(%) | eCEC<br>(cmol <sub>c</sub> kg <sup>-1</sup> ) | Al <sub>ox</sub><br>(g kg <sup>-1</sup> ) | Fe <sub>ox</sub><br>(g kg <sup>-1</sup> ) | Mn <sub>ox</sub><br>(g kg <sup>-1</sup> ) | Mo<br>(mg kg <sup>-1</sup> ) | Soil type        | Land use      |
|-------------|----------------|--------------------------------|------------------|-------------|---|---|---|---|------------------------------|------------------|---------------|
| Zegveld     | Netherlands    | 4.4                            | 30.7             | 58.8        | 41.7  | 3.5                                       | 11.7                                      | 0.09                                      | 3                            | Histosol         | Grassland     |
| Kövlinge    | Sweden         | 5.0                            | 2.0              | 3.3         | 4.2   | 2.1                                       | 1.9                                       | 0.04                                      | 1                            | Dystric Regosol  | Arable land   |
| Kasterlee   | Belgium        | 5.2                            | 2.8              | 2.2         | 6.3   | 0.6                                       | 1.5                                       | 0.06                                      | <1                           | Haplic Podzol    | Arable land   |
| Zwijnaarde  | Belgium        | 5.2                            | 1.8              | 2.2         | 4.1   | 1.2                                       | 1.0                                       | 0.06                                      | 1                            | Haplic Podzol    | Arable land   |
| Woburn      | United Kingdom | 6.3                            | 3.6              | 31.4        | 30.0  | 0.6                                       | 15.3                                      | 0.17                                      | 1                            | Dystric Cambisol | Grassland     |
| Ter Munck   | Belgium        | 6.7                            | 0.9              | 13.3        | 12.2  | 0.6                                       | 2.2                                       | 0.35                                      | 1                            | Haplic Luvisol   | Arable land   |
| Souli       | Greece         | 6.8                            | 0.6              | 34.7        | 14.2  | 0.7                                       | 0.7                                       | 0.47                                      | 2                            | Chromic Luvisol  | Arable land   |
| Rots        | France         | 7.3                            | 1.3              | 12.5        | 14.3  | 0.4                                       | 1.2                                       | 0.21                                      | 1                            | Haplic Luvisol   | Arable land   |
| Nagyhörcsök | Hungary        | 7.6                            | 2.1              | 21.2        | 24.8  | 1.5                                       | 0.5                                       | 0.45                                      | 1                            | Chernozem        | Arable land   |
| Guadalajara | Spain          | 7.8                            | 0.8              | 18.0        | 14.1  | 0.3                                       | 0.1                                       | 0.05                                      | 1                            | Calcic Cambisol  | Olive orchard |

**Table 2. Reliable EC<sub>10</sub> (10 % effective concentration) or NOEC (no observed effect concentration) values for the toxicity of molybdenum (tested as molybdate) to soil organisms**

All values are expressed as total Mo in soil (mg kg<sup>-1</sup>). Results are derived from the toxicity tests in a range of different soils (see Table 1). Range normalised is corrected for aging and normalised to a reference soil with pH=6 and 10 % clay

| Species and endpoint   | Range<br>(mg Mo kg <sup>-1</sup> soil) | Range normalised<br>(mg Mo kg <sup>-1</sup> soil) |
|--|--|---|
| <i>Brassica napus</i> , shoot yield <sup>[28]</sup>          | 5–2847                                 | 37–275  |
| <i>Trifolium pratense</i> , shoot yield <sup>[28]</sup>      | 5–1505                                 | 32–147  |
| <i>Lolium perenne</i> , shoot yield <sup>[28]</sup>          | 15–3479                                | 61–512  |
| <i>Lycopersicon esculentum</i> , shoot yield <sup>[28]</sup> | 9–1578                                 | 30–128  |
| <i>Hordeum vulgare</i> , root elongation <sup>[28]</sup>     | 28–871                                 | 109–1455  |
| <i>Enchytraeus crypticus</i> , reproduction <sup>[25]</sup>  | 67–1663                                | 398–1625  |
| <i>Eisenia andrei</i> , reproduction <sup>[25]</sup>         | 9–917                                  | 14–735  |
| <i>Folsomia candida</i> , reproduction <sup>[25]</sup>       | 39–1865                                | 170–11 046  |
| Native biomass, nitrification <sup>[30]</sup>                | 35–3841                                | 237–5464  |
| Native biomass, respiration <sup>[30]</sup>                  | 10–1822                                | 60–1472   |
| Native biomass, plant residue mineralisation <sup>[30]</sup> | 164–2163                               | 988–9694  |

data. Therefore, only the results from toxicity tests using a control and at least two Mo concentrations were used. EC<sub>10</sub> (10 % effective concentrations) values as calculated from the concentration–effect relationship were preferred.<sup>[39]</sup> In some cases, no reliable EC<sub>10</sub> could be derived because, for example, no significant dose–response curve could be fitted or the EC<sub>10</sub> was outside the concentration range tested. When in these cases a bounded no observed effect concentration (NOEC) value could be derived, this NOEC value was used instead of the EC<sub>10</sub>. 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 or EC<sub>10</sub> values extrapolated outside the concentration range tested were not used.

#### Bioavailability corrections

Bioavailability correction protocols were developed according to the procedures described by Smolders et al.<sup>[22]</sup> The toxicity of Na<sub>2</sub>MoO<sub>4</sub> was tested in 10 uncontaminated topsoils collected throughout Europe comprising a representative range of key soil properties (Table 1). These soils were sampled in eight EU member states, covering arable and grassland soils and belonging to six major soil groupings (Cambisol, Chernozem,

Histosol, Luvisol, Podzol, Regosol). The soils selected are representative of those in the EU, and sufficient to facilitate regression modelling of the toxicity of MoO<sub>4</sub><sup>2-</sup> v. soil properties. The toxicity of Na<sub>2</sub>MoO<sub>4</sub> was tested in each soil for five plant species, three invertebrate species and three microbial processes (Table 2).

No suitable Mo-contaminated soils could be identified in the field to account for long-term effects that mitigate MoO<sub>4</sub><sup>2-</sup> toxicity. As an alternative, an experiment was conducted in which the change in MoO<sub>4</sub><sup>2-</sup> toxicity was monitored 0–11 months after spiking.<sup>[27]</sup> Briefly, three soils were spiked in the laboratory and aged outside under prevailing climatic conditions in Leuven, Belgium. After 6 and 11 months had elapsed, the soils were sampled and the toxicity was measured with 10 bioassays (the same as mentioned above, except for the barley root elongation assay). The changes in toxicity were quantified by the ‘laboratory-to-field’ factor (also called ‘leaching–aging factor’), which is calculated as the relative change in 10 or 50 % effective doses (ED<sub>10</sub> or ED<sub>50</sub>, i.e. background-corrected effective concentrations) after leaching and aging. This factor was based on added or background-corrected Mo concentrations in soil because no changes in the bioavailability and toxicity of the natural Mo background concentration in soil were anticipated.

The  $EC_x$  values for aged soils were all based on actual, measured concentrations to correct for the decrease in total Mo concentration due to  $MoO_4^{2-}$  leaching by percolating rainwater. During the aging period, the soils were incubated in pots allowing free drainage of percolating rainwater. Therefore, the laboratory-to-field factor covers both the effect of decreased ionic strength due to leaching of excess ions and the effect of long-term equilibration in soil of the added  $MoO_4^{2-}$  ions (aging) on organism toxicity.

In addition to the toxicity assays on aged soils, the changes in lability of added soluble  $MoO_4^{2-}$  ions in soil were also studied to measure differences in bioavailability over time of the added  $MoO_4^{2-}$ .<sup>[40]</sup> In summary, 15 soils (ten soils selected for the development of bioavailability models, Table 1, supplemented with five other soils) were spiked with  $Na_2MoO_4$  at the  $EC_{10}$  and  $EC_{90}$  of a plant assay (barley root elongation) and were equilibrated at 25 °C and at 60% of their moisture content at pF 2.0 (suction of 100 cm of water). The isotopically exchangeable fraction (E value) was measured at 0, 0.5, 1, 3, 6, 12 and 18 months after spiking using the radioactive tracer,  $^{99}Mo$  (half-life  $t_{1/2}$  67 h). The change in the isotopically exchangeable fraction (%E) between 0.5 months (within a typical timeframe of a toxicity test) and 18 months (taken as a surrogate for long-term equilibration) was used as the basis to calculate the factor to explain  $MoO_4^{2-}$  aging in soils (fixation factor = %E<sub>0.5 month</sub> / %E<sub>18 months</sub>).

#### Regional risk assessment at European scale (GEMAS)

The predicted no-effect concentrations (PNECs or ecological soil standards) can be compared with prevailing exposure concentrations in soil to identify areas with potential risk. The GEMAS (Geochemical Mapping of Agricultural and Grazing Land Soils; <http://gemas.geolba.ac.at/>, accessed September 2015) project provides high quality and comparable data for both metal concentrations and soil properties known to influence metal bioavailability (pH, organic carbon content, clay content and effective Cation Exchange Capacity (eCEC)) in arable and grazing land at the European scale.<sup>[41]</sup> In total, 2108 samples of agricultural (arable) soil and 2024 samples of grazing land soil were collected at an average sampling density of 1 site per 2500 km<sup>2</sup> (grid of 50 × 50 km). The measured *aqua regia* (3 parts HCl + 1 part HNO<sub>3</sub>)-soluble metal concentrations from the GEMAS project can be considered as ambient background concentrations, i.e. the sum of the natural background of a metal with diffuse anthropogenic input in the past or present (due to, for example, agricultural inputs, combustion of fossil fuels or traffic). The influence of point sources (e.g. from local industrial activities) was avoided owing to the sampling strategy adopted. The consistent land-use and sampling depth (0–20 and 0–10 cm for agricultural (arable) land and grazing land respectively) ensured a comparable level of exposure for all samples within the same land-use.

In order to assess the potential risk to soil organisms at the prevailing Mo concentrations, the GEMAS results for *aqua regia*-soluble Mo concentrations for arable land and grassland soils were compared with the site-specific ecological soil standards expressed as total Mo concentrations and normalised for the soil properties measured for the individual sampling sites. The risk characterisation ratio (RCR) for each site was calculated as the ratio of measured *aqua regia*-extractable Mo concentration to the corresponding PNEC. Distributions of PNEC and RCR values were calculated non-parametrically. Because sites were sampled in a regular grid over Europe, there

was no bias due to spatial heterogeneity of sampling density, and it was appropriate to derive the distributions of PNEC and RCR values from the measured observations without the need for spatial interpolation to derive area-based distributions.

## Results and discussion

### Toxicity data

In total, 82 relevant and reliable  $EC_{10}$  values and four additional NOEC values passed the selection criteria for the effects assessment of  $MoO_4^{2-}$  to soil organisms (Table 2). All selected data were derived from toxicity tests carried out according to international guidelines in the framework of the research programme initiated by IMO. <sup>[25,28,30]</sup> Although some relevant studies with toxicity data for plants and microorganisms were identified in the scientific literature published before 2010, none of these studies contained reliable toxicity data useful for the effects assessment (see Supplementary material).

For plants, in total, 45 individual high-quality  $EC_{10}$  values were selected for the derivation of a soil threshold concentration for  $MoO_4^{2-}$ . These  $EC_{10}$  values covered five different plant species and ranged from 5 mg Mo kg<sup>-1</sup> dry weight (DW) (for oilseed rape and red clover) to 3479 mg Mo kg<sup>-1</sup> DW (for ryegrass).<sup>[28]</sup> For five dose–response curves, no reliable  $EC_{10}$  or NOEC could be derived because a significant effect was already observed at the lowest dose tested.

The invertebrate toxicity assays resulted in 23 individual high quality NOEC or  $EC_{10}$  values (for three different species), ranging from 8.9 mg Mo kg<sup>-1</sup> DW for *Eisenia andrei* to 1865 mg Mo kg<sup>-1</sup> DW for the springtail *Folsomia candida*.<sup>[25]</sup> No significant inhibition of reproduction was observed at the largest dose tested in eight and two soils for the *Folsomia candida* and *Enchytraeus crypticus* assays respectively (unbounded NOEC values between 2628 and 3396 mg Mo kg<sup>-1</sup> DW).

For microbial assays, 18 individual high-quality NOEC or  $EC_{10}$  values, derived for three different processes, were selected as reliable. These values ranged from 10 mg Mo kg<sup>-1</sup> DW for glucose-induced respiration to 3841 mg Mo kg<sup>-1</sup> DW for substrate-induced nitrification.<sup>[30]</sup> No toxic effect was observed at the largest dose tested (10 000 mg Mo kg<sup>-1</sup> DW) in two, four and six soils for the substrate-induced nitrification, glucose-induced respiration and plant residue mineralisation assays respectively.

All results are expressed based on total or *aqua regia*-extractable elemental Mo concentrations and can be applied to other Mo compounds ('read-across'). The justification for the read-across is that the concentrations of  $MoO_4^{2-}$  in soils and the outcome of the tests conducted on a soluble Mo compound reflect the concentrations and toxicity levels for all soluble Mo compounds and the thresholds are a conservative estimate for sparingly soluble Mo compounds.

### Bioavailability corrections – influence of soil properties on molybdate toxicity to soil organisms

The selected  $EC_{10}$  values for an endpoint varied from 13-fold to more than 500-fold among the soils tested (Table 2). A regression analysis (log–log basis) revealed that the variation in  $EC_{50}$  values among soils was, for all endpoints studied, significantly correlated with soil properties such as clay, ammonium oxalate-extractable iron oxides, pH (0.01 M CaCl<sub>2</sub>), eCEC and organic carbon (Table 3).<sup>[25,29,30]</sup> Regressions with soil properties were preferentially based on  $EC_{50}$  values, because  $EC_{50}$  is



**Table 3. Summary of regression models relating the toxicity (log EC<sub>50</sub> (50 % effective concentration) values) of molybdenum (as molybdate) to soil organisms with abiotic factors in soil**Selected models are in bold. Org C, organic carbon content; Fe<sub>ox</sub>, oxalate-extractable iron content

| Species or process           | Soil property   | <i>n</i> | Adj. <i>R</i> <sup>2</sup> | Slope                                       | 5 to 95 % confidence interval  |
|------------------------------|---|----------|----------------------------|---|--------------------------------|
| <b>Plants</b>                |   |          |                            |   |                                |
| Oilseed rape                 | log Org C   | 10       | 0.67                       | 1.36  | 0.64 to 2.07                   |
|                              | log Fe <sub>ox</sub>  | 10       | 0.65                       | 1.02  | 0.46 to 1.58                   |
|                              | pH  | 10       | 0.42                       | -0.45                                       | -0.83 to -0.07                 |
|                              | pH and log clay   | 10       | 0.91                       | <b>-0.61 (pH)</b><br><b>1.08 (log clay)</b> | -0.77 to -0.44<br>0.70 to 1.46 |
| Red clover                   | log Org C   | 10       | 0.77                       | 1.22  | 0.71 to 1.72                   |
|                              | log Fe <sub>ox</sub>  | 10       | 0.64                       | 0.86  | 0.38 to 1.34                   |
|                              | pH  | 10       | 0.45                       | -0.39                                       | -0.72 to -0.08                 |
|                              | pH and log clay   | 10       | 0.78                       | <b>-0.50 (pH)</b><br><b>0.77 (log clay)</b> | -0.72 to -0.29<br>0.27 to 1.28 |
| Ryegrass                     | log Org C   | 10       | 0.38                       | 0.76  | 0.07 to 1.45                   |
|                              | log Fe <sub>ox</sub>  | 10       | 0.24                       | 0.5   | -0.08 to 1.07                  |
|                              | pH  | 10       | 0.13                       | -0.22                                       | -0.54 to 0.11                  |
|                              | pH and log clay   | 10       | 0.81                       | <b>-0.35 (pH)</b><br><b>0.90 (log clay)</b> | -0.51 to -0.18<br>0.52 to 1.29 |
| Tomato                       | log Org C   | 10       | 0.65                       | 1.08  | 0.49 to 1.66                   |
|                              | log Fe <sub>ox</sub>  | 10       | 0.66                       | 0.82  | 0.38 to 1.26                   |
|                              | pH  | 10       | 0.31                       | -0.32                                       | -0.65 to 0.01                  |
|                              | pH and log clay   | 10       | 0.86                       | <b>-0.45 (pH)</b><br><b>0.93 (log clay)</b> | -0.61 to -0.29<br>0.55 to 1.30 |
| Barley                       | log Org C   | 9        | 0.78                       | 0.79  | 0.45 to 1.13                   |
|                              | log Fe <sub>ox</sub>  | 9        | 0.65                       | 0.52  | 0.21 to 0.83                   |
|                              | pH  | 9        | 0.33                       | -0.21                                       | -0.44 to 0.01                  |
|                              | pH and log clay   | 9        | 0.8                        | <b>-0.28 (pH)</b><br><b>0.56 (log clay)</b> | -0.41 to -0.15<br>0.24 to 0.88 |
| <b>Invertebrates</b>         |   |          |                            |   |                                |
| <i>Enchytraeus</i>           | pH  | 6        | 0.86                       | 0.37  | 0.19 to 0.55                   |
|                              | log clay  | 6        | 0.84                       | <b>0.72</b>                                 | 0.34 to 1.09                   |
| <i>Eisenia</i>               | log clay  | 10       | 0.67                       | <b>0.73</b>                                 | 0.35 to 1.12                   |
| <i>Folsomia</i>              | Insufficient reliable EC <sub>50</sub> values (3) for sound regression analysis |          |                            |   |                                |
| <b>Microorganisms</b>        |   |          |                            |   |                                |
| Nitrification                | log clay  | 8        | 0.64                       | <b>1.17</b>                                 | 0.39 to 1.96                   |
| Respiration                  | log clay  | 4        | 0.85                       | <b>0.73</b>                                 | 0.00 to 1.46                   |
| Plant residue mineralisation | Insufficient reliable EC <sub>50</sub> values (1) for regression analysis       |          |                            |   |                                |

a more robust estimate (smaller confidence interval) for effects and is less affected by experimental error compared with the NOEC or EC<sub>10</sub> values.

For plants, soil organic carbon content was for the various species tested the best single regressor. However, these regressions were strongly affected by one soil with high organic carbon content (30.7 %). Except for ryegrass (*Lolium perenne*), MoO<sub>4</sub><sup>2-</sup> toxicity was also significantly correlated with the oxalate-extractable iron (Fe<sub>ox</sub>) content of the soils. This is consistent with observations for arsenate, which also prevails in soil as an oxoanion.<sup>[15]</sup> Multiple regressions with pH and clay content or pH and eCEC significantly improved the regression fit compared with single linear regressions. In these regressions, clay content was most likely a good surrogate for the actual binding surfaces present in the soil, including clays, oxides and organic matter. A larger clay content results in more binding surfaces and therefore in lower availability and toxicity of the MoO<sub>4</sub><sup>2-</sup> anion in soil. Similarly, in the multiple regressions with pH and eCEC (not shown), the eCEC, which is theoretically a measure for binding of cations, is probably a measurement that integrates clay and organic matter in soil, which both have binding sites for molybdate. The negative regression of EC<sub>50</sub> with pH (higher EC<sub>50</sub> values and lower toxicity at low pH) is

explained by the larger number of positively charged sorption sites for anions on the soil constituents (clay, oxides, organic matter) at low pH, resulting in a lower bioavailability. This regression with pH is consistent with common agricultural experience: Mo deficiency in acidic soils due to low MoO<sub>4</sub><sup>2-</sup> bioavailability can be overcome by liming to make the soil less acidic, thereby releasing MoO<sub>4</sub><sup>2-</sup> from the sorption sites and increasing its bioavailability.<sup>[42]</sup> Because of the large *R*<sup>2</sup> values (0.78–0.91) for all five plant species, and the sound mechanistic explanation, the regression models with pH and clay content were selected for normalisation of the plant data.

For invertebrates, only models for *Enchytraeus crypticus* and *Eisenia andrei* could be developed. For both species, the clay content was the best single regressor for the EC<sub>50</sub> values and the slopes of both regression equations were very consistent. Multiple linear regression models, using stepwise addition, did not consistently improve the regression fit for invertebrates. Bounded EC<sub>50</sub> values for MoO<sub>4</sub><sup>2-</sup> toxicity to *Folsomia candida* were only obtained for three sandy soils with comparable soil properties. Therefore, no sound regression analysis with soil properties could be performed.

For the microbial processes studied, only models for substrate-induced nitrification and respiration could be

developed. No valid model could be derived for the microbial plant residue mineralisation (PRM) assay because only one bounded  $EC_{50}$  value was observed for this assay. Although only four bounded  $EC_{50}$  values were available for glucose-induced respiration, the results of the regression analysis were considered useful because the range in soil properties covered by these four soils was still sufficiently wide (pH: 5.2–7.3; organic carbon: 0.9–2.8 %; clay: 2–12 %; eCEC: 4.1–14.3  $cmol_c$  (charge)  $kg^{-1}$ ;  $Fe_{ox}$ : 1–2.2  $g\ kg^{-1}$ ) and can be considered representative for the soils in Europe with the highest bioavailability and toxicity of Mo (i.e. high pH, low organic carbon content, clay content and eCEC). For both the nitrification and respiration processes, a consistent significant linear regression of  $EC_{50}$  values with the soil clay content was observed ( $R^2$  0.64 and 0.85). Similarly to the invertebrates, multiple linear regression models did not consistently improve the regression fit for the microbial endpoints studied.

In summary, the key soil properties identified as governing  $MoO_4^{2-}$  toxicity in soils were the pH and clay content for plants and the clay content for invertebrates and microorganisms. Molybdate toxicity to soil organisms generally decreased (i.e. increasing  $EC_{50}$  values) with decreasing pH and increasing clay content. The best linear regression models (log–log basis) accounted for 64 to 91 % of the variability of the  $EC_{50}$  for the soil organisms studied (Table 3).

#### Bioavailability corrections – effect of leaching and aging processes

All toxicity data discussed above referred to soils tested 7 days after spiking with soluble  $Na_2MoO_4$  without removal of excess salts by leaching. The threshold values for  $MoO_4^{2-}$  in soil derived from these data, expressed as total soil Mo, could overestimate the toxicity of Mo in a field with soil contaminated over a longer time period at the same total soil Mo concentration. Previous findings reported the solubility or availability of  $MoO_4^{2-}$  to decrease in soils or mineral phases with increasing incubation time.<sup>[42–46]</sup>

Comparison of  $MoO_4^{2-}$  toxicity between freshly spiked soils and soils aged for 11 months indicated that long-term equilibration of  $MoO_4^{2-}$  in soils under field conditions generally decreased its toxicity to soil organisms. Laboratory-to-field factors were calculated as the ratio of  $ED_{50}$  values before and after 11-month equilibration (Table 4). In total, eight  $ED_{50}$ -based laboratory-to-field factors were significantly larger than one (i.e. significant decrease in toxicity with aging), whereas no laboratory-to-field factor was significantly smaller than one (i.e. increase in toxicity with aging). The median decrease in toxicity after leaching and aging processes was a factor of 5.4.

The  $MoO_4^{2-}$  toxicity data for aged soils did not allow strong conclusions to be drawn on the potential effect of soil properties on the laboratory-to-field factor. The sandy Zwijnaarde soil (pH 5.2) seemed to have limited aging (Table 4), but results for this soil were strongly biased because of the high degree of  $MoO_4^{2-}$  leaching (up to 99 % at the largest dose) and the low  $MoO_4^{2-}$  concentration remaining in the soil after long-term equilibration under field conditions (<80  $mg\ Mo\ kg^{-1}$  after 11 months with initial spiked concentrations up to 10 000  $mg\ kg^{-1}$ ). Molybdate loss from the Woburn and Ter Munck soils was more limited, with on average 62 and 53 % of the added Mo lost from the soils respectively after 11 months of equilibration. Both soils still contained >4000  $mg\ Mo\ kg^{-1}$  at the end of the 11-month equilibration period.

**Table 4. Laboratory-to-field factors in three soils tested**

The laboratory-to-field factors are quantified as the ratio of  $ED_{50}$  values (50 % effective dose, i.e. background-corrected 50 % effective concentration,  $mg\ Mo\ kg^{-1}$  soil) from 11-month equilibration treatments to corresponding values from freshly amended soils. ROL, response obliterated by leaching, i.e. there was so much loss of molybdenum by leaching during equilibration that final concentrations were below the  $ED_{50}$  measured in freshly spiked soil and no toxic effects were seen in 11-month equilibrated soils; n.s., laboratory-to-field factor not significantly different from 1.0, meaning that  $ED_{50}$  values before or after equilibration are not significantly different at  $P$  0.05 in a one-sided  $t$ -test. Data for plants and invertebrates reported by Van Gestel et al.<sup>[27]</sup>

| Species or process           | Laboratory-to-field factor in soil |                     |                     |
|------------------------------|------------------------------------|---------------------|---------------------|
|                              | Zwijnaarde                         | Woburn              | Ter Munck           |
| Oilseed rape                 | 0.5 (n.s.)                         | 12 ( $P < 0.001$ )  | 9.2 ( $P < 0.005$ ) |
| Red clover                   | – <sup>A</sup>                     | 11 ( $P < 0.001$ )  | – <sup>A</sup>      |
| Ryegrass                     | ROL                                | 5.5 ( $P < 0.001$ ) | 5.4 ( $P < 0.005$ ) |
| Tomato                       | 0.7 (n.s.)                         | 9.6 ( $P < 0.001$ ) | 9.3 <sup>B</sup>    |
| <i>Enchytraeus</i>           | ROL                                | 4.1 ( $P < 0.001$ ) | 1.0 (n.s.)          |
| <i>Eisenia</i>               | ROL                                | 8.8 <sup>B</sup>    | 4.8 ( $P < 0.05$ )  |
| <i>Folsomia</i>              | ROL                                | (2.1) <sup>C</sup>  | (1.7) <sup>C</sup>  |
| Nitrification                | ROL                                | – <sup>C</sup>      | – <sup>C</sup>      |
| Respiration                  | ROL                                | – <sup>C</sup>      | 0.3 (n.s.)          |
| Plant residue mineralisation | ROL                                | – <sup>C</sup>      | – <sup>C</sup>      |

<sup>A</sup>No dose–response curve could be fitted in case of equilibrated soils, so no comparison of  $ED_{50}$  values was possible.

<sup>B</sup>95 % confidence interval for  $ED_{50}$  in equilibrated soil was very wide, therefore no statistical test was possible.

<sup>C</sup>No reliable laboratory-to-field factor could be derived because  $ED_{50}$  values before or after equilibration were higher than the highest test concentration.

Van Gestel et al.<sup>[27]</sup> concluded that natural attenuation of  $MoO_4^{2-}$  ecotoxicity under field conditions is related to leaching of excess  $MoO_4^{2-}$  and other ions, as well as to slow aging reactions. The loss of  $MoO_4^{2-}$  through leaching during the equilibration period was taken into account by calculating all  $ED_{50}$  values based on actual measured Mo concentrations in soil, corrected for the background Mo concentration in the control soils. The laboratory-to-field factor is hence the result of combined effects of (i) decreased ionic strength after leaching and the corresponding alleviation of salt- or pH-related stress to soil organisms, and the change in  $MoO_4^{2-}$  availability through the decrease in concentration of competing ions in the soil solution; and (ii) stronger binding or fixation of  $MoO_4^{2-}$  onto or into soil solid phases (aging). Studies including NaCl testing with plants and microorganisms suggested direct salt stress to be of limited importance at a  $Na_2MoO_4$  dose below 1000  $mg$  added  $Mo\ kg^{-1}$ .<sup>[5,29]</sup> No literature or test data were available on change in molybdate availability and toxicity through the decrease in concentration of competing ions in the soil solution after leaching excess ions from the soil. However, data for Cu, Ni and Pb show a median leaching factor (i.e. ( $ED_x$ , leached)/( $ED_x$ , spiked)) between 1.3 and 2.0 across a broad range of soils.<sup>[18,47–49]</sup> As a first approximation, a similar level of effect may be anticipated for the influence of leaching of excess ions after application of  $Na_2MoO_4$  on  $MoO_4^{2-}$  toxicity. The isotopically exchangeable fraction (E value) for  $MoO_4^{2-}$  (expressed as percentage of total Mo added) was assessed with an isotopic dilution technique and was found to decrease with increasing incubation time.<sup>[40]</sup> The chemical fixation factor, calculated as the change in isotopically exchangeable Mo between 0.5 and

18 months, ranges between 1.0 and 2.8, with a median fixation factor of 1.4 for the 15 soils tested. The isotopically exchangeable fraction of Mo not only depended on time after spiking, but was also correlated with both clay content and pH of the soil. However, no significant correlation between the fixation factor (i.e. change of E value with time) and soil properties was found.

Because there were no significant correlations between the laboratory-to-field factors or fixation factors with the soil type or endpoint type, a generic constant correction factor was selected for the discrepancy in  $\text{MoO}_4^{2-}$  toxicity between laboratory and field conditions. A constant factor of 2.0 was chosen for the derivation of ecological soil thresholds and risk characterisation of  $\text{MoO}_4^{2-}$  in soil. This factor corresponds to the 32nd percentile of the individual laboratory-to-field factors values based on  $\text{ED}_{50}$  values (Table 4). The factor of 2.0 is approximately equal to the product of the median factor found for chemical fixation of  $\text{MoO}_4^{2-}$  in several soils (factor 1.4) and the median factor for the effects of leaching on the toxicity thresholds for other metals (factor 1.3–2.0). The factor 2.0 further corresponds to the 2.1- and 2.0-fold decrease in solution Mo concentrations between freshly spiked and 11-month equilibration observed for two of the three test soils respectively, at a total soil concentration of  $\sim 50 \text{ mg Mo kg}^{-1}$  dry soil.<sup>[27]</sup>

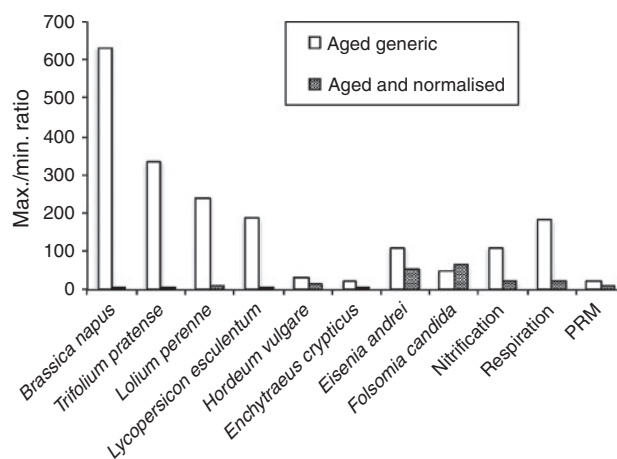
#### Derivation of ecological soil standards

The available ecotoxicity database for the effect of  $\text{MoO}_4^{2-}$  on soil organisms covers five plants species, three invertebrate species and three microbial processes and therefore fulfils the requirements of the REACH regulation for the use of the species sensitivity distribution (SSD) approach for derivation of ecological soil standards.<sup>[39]</sup>

The general framework for implementation of bioavailability into derivation of ecological threshold concentrations is reported by Smolders et al.<sup>[22]</sup> In summary, the following steps were followed. After selection of the reliable  $\text{EC}_{10}$  (or NOEC) values, the added  $\text{EC}_{10}$  values (=  $\text{ED}_{10}$  values) were derived by subtracting the background concentrations of Mo in the tested control soils from the  $\text{EC}_{10}$  values expressed as total measured concentrations. In a second step, toxicity thresholds derived from soils tested within 120 days after spiking with a soluble Mo salt were corrected for the discrepancy in toxicity between freshly spiked soils in laboratory conditions and field-contaminated soils by multiplying all individual added  $\text{EC}_{10}$  or NOEC values by the laboratory-to-field factor of 2.0 for  $\text{MoO}_4^{2-}$ . The background Mo concentration from each individual test soil was then added again to calculate the total ‘aged’  $\text{EC}_{10}$  or NOEC values. Because of the negligible contribution of the background concentration of Mo to the total NOEC or  $\text{EC}_{10}$  values, application of the laboratory-to-field factor has an almost proportional effect on the final toxicity thresholds.

In the following step, the toxicity data were corrected for differences in metal availability among soils. Normalising for the effect of soil properties allows the calculation of a specific threshold concentration for the effect of  $\text{MoO}_4^{2-}$  to soil organisms in the soil under investigation. Each total aged  $\text{EC}_{10}$  or NOEC value is normalised to the soil properties of a specific target soil, using the slope of the respective regression function (log–log based, Table 3) and the following equation:

$$\text{EC}_{10,\text{reference}} = \text{EC}_{10,\text{test}} \left[ \frac{\text{abiotic factor}_{\text{reference}}}{\text{abiotic factor}_{\text{test}}} \right]^{\text{slope}}$$



**Fig. 1.** Within species variability, expressed as maximum/minimum ratio of  $\text{EC}_{10}$  (10% effective concentration) and NOEC (no observed effect concentration) values for the toxicity of molybdenum (as molybdate) to soil organisms, before and after normalisation to reference soil properties (pH = 6, 10% clay). PRM, plant residue mineralisation.

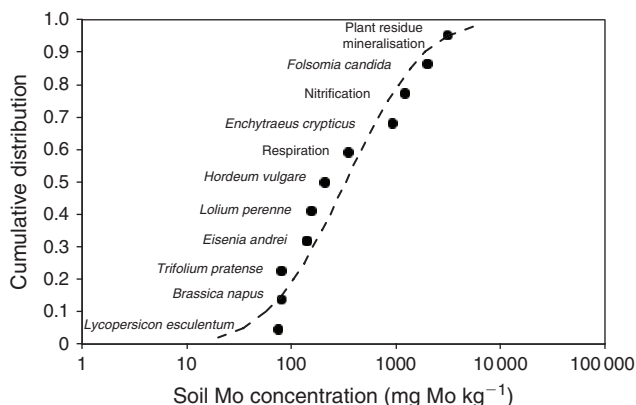
where ‘reference’ is the soil for which the soil standard must be derived, ‘test’ is the tested soil, and ‘abiotic factor’ is the soil property in the selected regression model. Both pH and clay content of the tested soils and target soil must be known to normalise toxicity data for  $\text{MoO}_4^{2-}$  for specific soil conditions.

In case no regression model is available for a specific species or endpoint, the bioavailability model of another similar species or endpoint within the same trophic level can be used. The model with the smallest slope observed for the invertebrate assays, i.e. the model for *Enchytraeus crypticus*, was selected for normalisation of *Folsomia candida*  $\text{EC}_{10}$  values for site-specific ecological soil standard derivation. The selection of the model with the smallest slope can be considered as a conservative approach because it minimises the risk of overcorrection. The slope derived for the substrate-induced respiration assay was selected for normalisation of  $\text{EC}_{10}$  values for the PRM assay because both assays are related to the carbon mineralisation process.

Normalisation of the individual  $\text{EC}_{10}$  or NOEC data towards specific soil properties strongly reduces the within species-variation in  $\text{EC}_{10}$  or NOEC values for most organisms covered by the database (Table 2, Fig. 1). This illustrates the adequacy of these models and the significance of soil properties in controlling  $\text{MoO}_4^{2-}$  bioavailability and toxicity to soil organisms, and further demonstrates the importance of normalising toxicity data and separating the biological variation from the variation in  $\text{MoO}_4^{2-}$  availability due to varying soil properties.

Where multiple data are available for the same species or microbial process, a species or process mean value is calculated as the geometric mean from all data for the most sensitive endpoint for each species or process. This species or process mean approach is preferred for normalised data, where the remaining variation among data for a given species or process can be mainly attributed to intraspecies variation in sensitivity. This is, however, not the case for non-normalised data, where variation between toxicity data is also caused by differences in bioavailability among soils.

Finally, a SSD was fitted to the normalised, aged species or process mean  $\text{EC}_{10}$  values, and the median hazardous concentration for 5% of the species ( $\text{HC}_{5-50}$ ), equivalent to a median



**Fig. 2.** Species sensitivity distribution (SSD) for the toxicity of molybdenum (tested as molybdate) to soil organisms. All data are normalised to reference soil properties (pH = 6, 10% clay). The  $HC_{5-50}$  calculated for this soil is  $32.6 \text{ mg Mo kg}^{-1}$ . SIN, substance-induced nitrification; SIR, substance-induced respiration; PRM, plant residue mineralisation.

95% protection level, was derived as the median 5th percentile of this distribution (Fig. 2). The SSD for the effect of  $\text{MoO}_4^{2-}$  on soil organisms illustrates that plants are generally the most sensitive to  $\text{MoO}_4^{2-}$  toxicity in soil and that microbial endpoints are the most tolerant.

Once an  $HC_{5-50}$  value is derived, its robustness and degree of protection for direct molybdate toxicity to soil organisms under field conditions should be evaluated. Therefore, the following criteria are usually used: the quality and representativeness of the data set, the statistical uncertainty on the  $HC_{5-50}$  value, the evaluation of chronic toxicity data below this  $HC_{5-50}$  value and a comparison with field data.<sup>[39]</sup>

Chronic toxicity data are available for  $\text{MoO}_4^{2-}$  for several plant species, invertebrate species and microbial processes. The selected endpoints were all relevant for potential effects at the population level: yield based on root elongation and shoot yield for the terrestrial plants, reproduction for the invertebrates, and nitrogen and carbon transformation for microbial processes. Data were either from tests focussing on sensitive life stages (e.g. root elongation, reproduction) or from 'chronic exposure' (e.g. growth, reproduction). All reliable chronic  $EC_{10}$  and NOEC data were extracted from tests performed in natural and artificial soils, covering a wide range of the soil characteristics in Europe (pH, organic carbon, clay and oxalate-extractable iron content, Table 1). The soils covered by the toxicity data properly reflected the variability in physicochemical conditions encountered in European soils (Table 5).

The toxicity data set was composed of plant, invertebrate and microbial data and includes the major taxonomic groups. In total, 86 individual chronic  $EC_{10}$  or NOEC values were selected covering 11 different species or microbial processes. Data were available for five different agricultural plant species belonging to five different families and covering both monocotyledonous and dicotyledonous plants. Toxicity data for invertebrates covered arthropods and *Annelida* with different exposure routes and feeding strategies, belonging to three different species from three different families. The data set further included three different microbial endpoints representing the C and N cycles. This largely fulfils the requirement of 10–15 different  $EC_{10}$  or NOEC values (preferably more than 15) from chronic studies for different species covering at least eight different taxonomic groups from three trophic levels for the use of the statistical extrapolation approach.<sup>[39]</sup> The overall quality, diversity and

**Table 5.** General soil characteristics, predicted no effect concentrations (PNEC) and risk characterisation ratios (RCR) for molybdenum in arable soils sampled across Europe (data from the GEMAS project<sup>[41]</sup>)

eCEC, effective cation exchange capacity, CEC at pH of the soil; P10, 10th percentile; P90, 90th percentile

| Parameter                                | Minimum | P10   | Median | P90  | Maximum |
|--|---------|-------|--------|------|---------|
| pH $\text{CaCl}_2$                       | 3.3     | 4.4   | 5.8    | 7.4  | 8.0     |
| Clay (%)                                 | 1.0     | 1.4   | 15.2   | 27.0 | 62.8    |
| Organic C (%)                            | 0.4     | 0.9   | 1.8    | 3.9  | 46.0    |
| eCEC ( $\text{cmol}_c \text{ kg}^{-1}$ ) | 1.8     | 8.0   | 16.4   | 30.3 | 48.3    |
| Mo ( $\text{mg kg}^{-1}$ DW)             | 0.03    | 0.15  | 0.42   | 1.24 | 13.9    |
| PNEC ( $\text{mg Mo kg}^{-1}$ DW)        | 0.6     | 10.7  | 34.1   | 168  | 458     |
| RCR                                      | 0.0002  | 0.002 | 0.01   | 0.06 | 1.06    |

representativeness of the taxonomic groups covered by the data were therefore considered adequate.

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 highlights 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 according to the Anderson–Darling test. Comparison of the uncertainty around the  $HC_{5-50}$  showed that there was no consistent difference between results of the log-normal and best-fitting distributions. Therefore, the uniform application of the log-normal distribution was preferred for derivation of the  $HC_{5-50}$  values.

A comparison of the  $HC_{5-50}$  values with the corresponding normalised species or process mean  $EC_{10}$  values for several EU soil scenarios shows that no species or process mean values fall below the  $HC_{5-50}$  derived by the log-normal distribution, except for acidic soils (pH < 4.5), where the normalised species-mean value for *Eisenia andrei* reproduction can be lower than the  $HC_{5-50}$  value fitted with the log-normal distribution.

The  $HC_{5-50}$  value was finally validated by  $\text{MoO}_4^{2-}$  toxicity data from field or microcosm studies. Only one field study was identified where the effect of  $\text{MoO}_4^{2-}$  applications at 0, 90, 270 and  $810 \text{ kg Mo ha}^{-1}$  (as  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ), corresponding to an added dose of ~0, 30, 90 and  $270 \text{ mg Mo kg}^{-1}$  in the plough layer (0–20 cm), on crop yield was studied.<sup>[50–53]</sup> The same Nagyhörsök soil (Hungary) was used in the present research programme (Table 1): a calcareous Chernozem soil with pH 7.3, 3% organic matter, 20% clay and 5%  $\text{CaCO}_3$ . Molybdate was applied in the spring of 1991 with two-fold replication and each plot had a total area of  $21 \text{ m}^2$ . Maize, carrot, potato, pea, red beet, spinach and wheat were grown in the first, second, third, fourth, fifth, sixth and seventh year respectively. Only in the first cropping season was a significant effect on maize dry matter yield (82% effect) and maize grain yield (44% effect) observed at the highest dose (Table 6). In subsequent seasons, no effects on carrot, potato, pea, red beet, spinach or wheat yield were observed. Measured total soil Mo concentrations are only reported for samples taken in 1994 and 22–42% of the  $\text{MoO}_4^{2-}$  added in 1991 was recovered. It can be expected that actual Mo concentrations during the first cropping season in 1991 were still significantly higher. Measured ammonium acetate–EDTA-extractable Mo-concentrations in soil showed

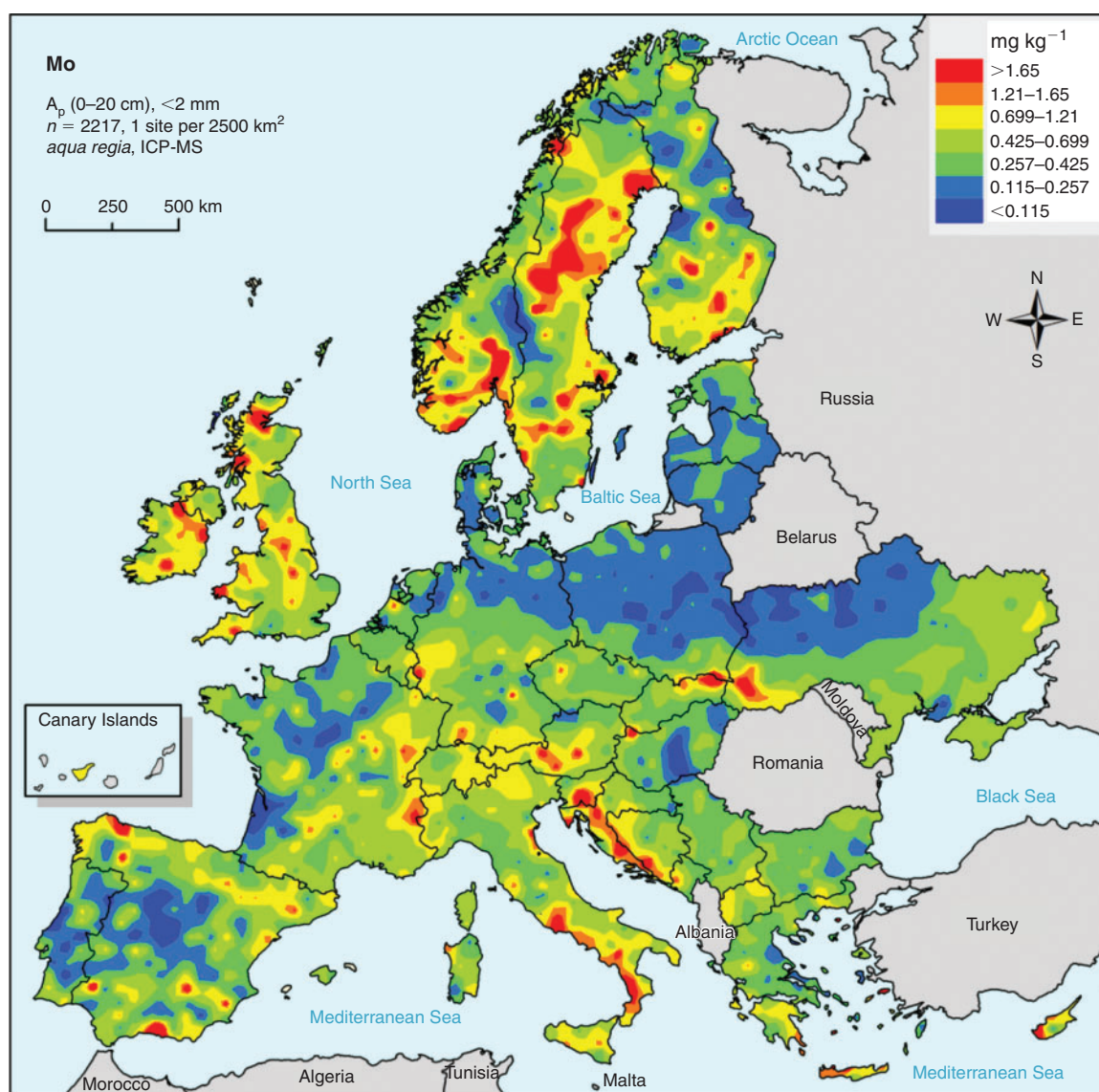


**Table 6. Phytotoxicity of molybdate in a field trial on the Nagyhörsök soil (Hungary)**  
Data from Kadar et al. [50–53]

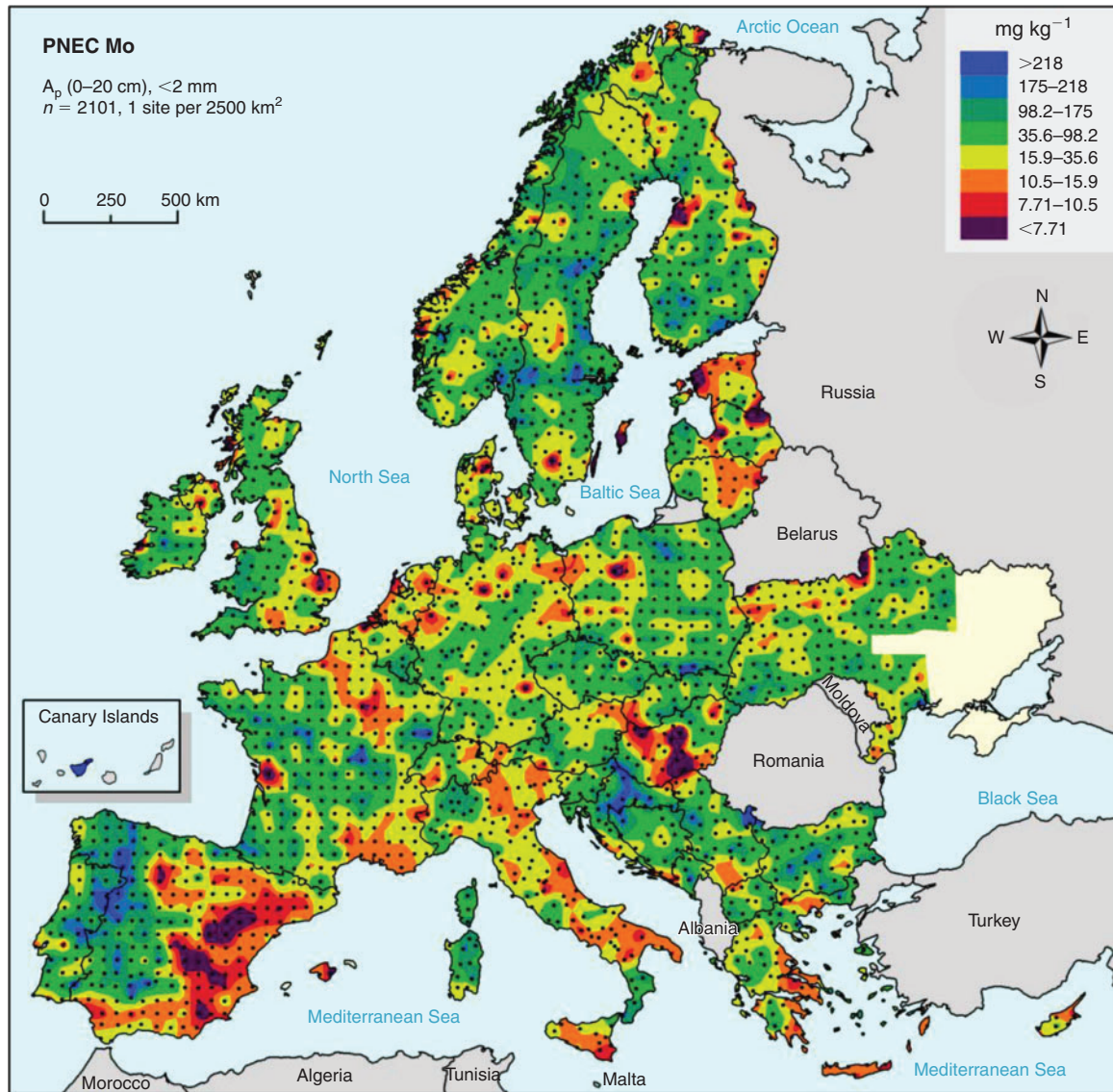
|   | Dose 0 | Dose 1 | Dose 2 | Dose 3           |
|---|--------|--------|--------|------------------|
| Mo application spring 1991 (mg Mo kg <sup>-1</sup> )                        | 0      | 30     | 90     | 270              |
| Total Mo, April 1994 (mg kg <sup>-1</sup> )                                 | 0      | 10     | 20     | 114              |
| Available <sup>A</sup> Mo, July 1991 (mg kg <sup>-1</sup> )                 | 1      | 21     | 26     | 104              |
| Available <sup>A</sup> Mo, October 1992 (mg kg <sup>-1</sup> )              | 0      | 12     | 22     | 43               |
| Available <sup>A</sup> Mo, April 1994 (mg kg <sup>-1</sup> )                | 0      | 7      | 8      | 25               |
| Maize dry-matter yield (plants with 4–6 leaves) 1991 (kg ha <sup>-1</sup> ) | 140    | 130    | 95     | 25 <sup>B</sup>  |
| Maize grain yield 1991 (t ha <sup>-1</sup> )                                | 8.5    | 8.4    | 7.4    | 4.7 <sup>B</sup> |
| Fresh carrot root yield 1992 (t ha <sup>-1</sup> )                          | 11.4   | 15.9   | 14.2   | 13.1             |
| Pea grain yield 1994 (t ha <sup>-1</sup> )                                  | 2.89   | 3.07   | 2.91   | 2.71             |

<sup>A</sup>Available Mo concentration measured in ammonium acetate–EDTA extract.

<sup>B</sup>Significantly different from control.



**Fig. 3.** Molybdenum concentrations in agricultural land soils across Europe. Data from the GEMAS project.<sup>[41]</sup> (ICP-MS, inductively coupled plasma mass spectrometry; A<sub>p</sub>, arable land.)



**Fig. 4.** Predicted no-effect concentrations (PNEC) for molybdenum in agricultural land soils in Europe. Dots represent sampling points. Areas between sample points were interpolated using the Kriging technique. Colour classes are based on 5th, 10th, 25th, 50th, 75th, 90th and 95th percentiles. Data from the GEMAS project.<sup>[41]</sup> ( $A_p$ , arable land.)

a clear decrease in extractability of Mo with time after application.

This field study in Hungary suggests that the toxicity in the first year after application (NOEC = 26 mg ammonium acetate–EDTA-extractable Mo kg<sup>-1</sup>) is more than a factor 2 above the HC<sub>5-50</sub> value calculated for this soil (11.4 mg total Mo kg<sup>-1</sup>) and hence the HC<sub>5-50</sub> can be considered as conservative. Moreover, the ammonium acetate–EDTA-extractable soil concentration underestimates the actual total Mo concentrations during the maize cropping season and therefore actual total effect concentrations were likely still higher. In this field study, maize yield was tested almost immediately after MoO<sub>4</sub><sup>2-</sup> application to the soil.<sup>[50,53]</sup> Therefore, significant aging may not have already taken place before the start of the maize growth and hence the data for maize yield can be considered as freshly spiked data, overestimating MoO<sub>4</sub><sup>2-</sup> toxicity after aging. Based on this field study, it is concluded that no negative effects are predicted at concentrations below the HC<sub>5-50</sub> derived from the laboratory

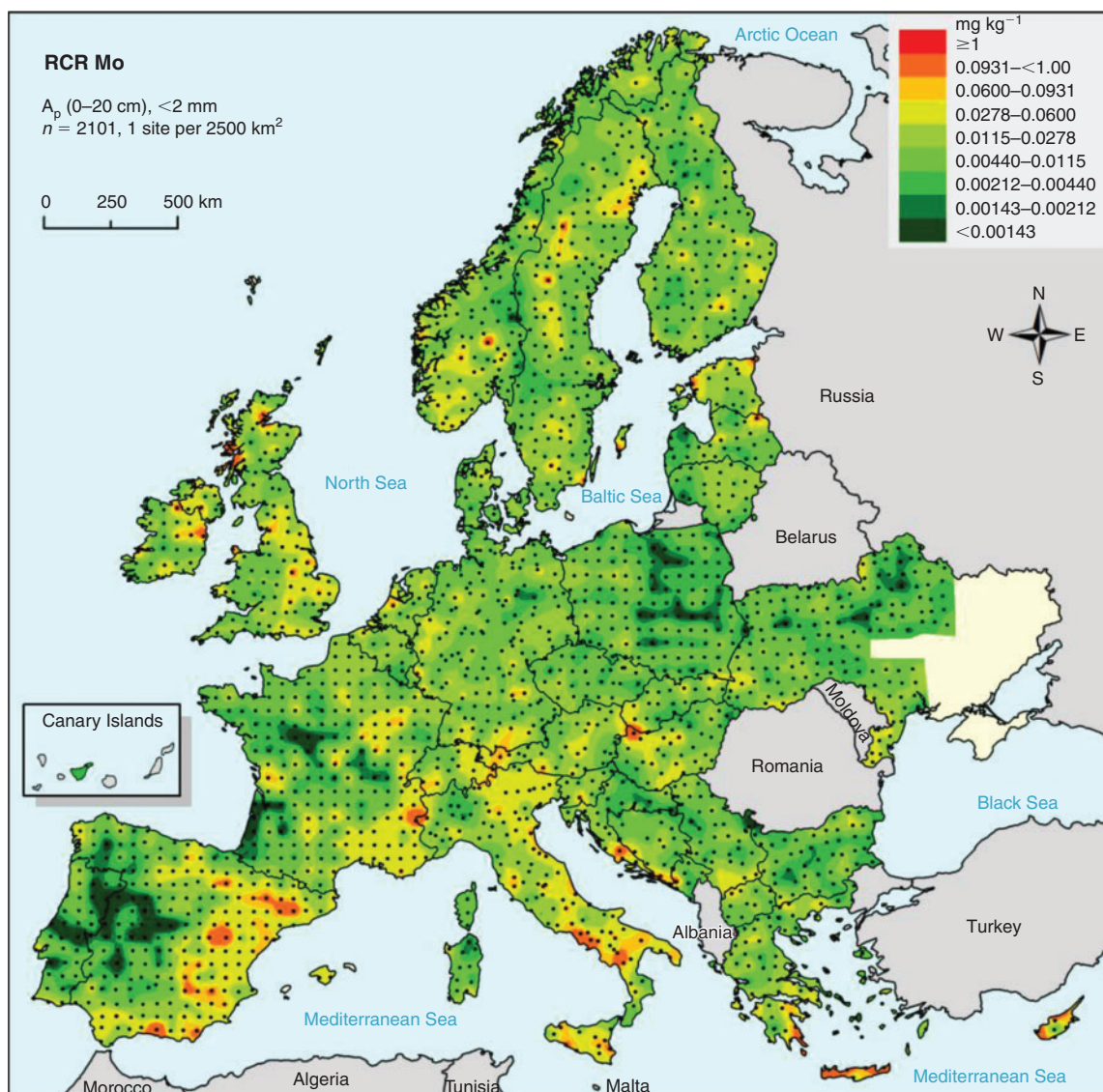
toxicity data, and this value is therefore considered a protective threshold for effects of MoO<sub>4</sub><sup>2-</sup> under field conditions.

Based on the above evaluation, and in particular the availability of normalisation models and field validation, it can be concluded that the available toxicity data and models allow the derivation of an HC<sub>5-50</sub> that is protective against direct MoO<sub>4</sub><sup>2-</sup> toxicity in the soil. This provides a robust and ecologically relevant PNEC to be retained for use in risk characterisation.

#### *Risk assessment of arable and grazing land soils in Europe*

The results from the GEMAS survey demonstrate the large range in ambient background concentrations of Mo and soil properties affecting MoO<sub>4</sub><sup>2-</sup> toxicity in soil across Europe (Table 5, Fig. 3). PNEC values for all 2108 arable land soils sampled in the GEMAS project vary by three orders of magnitude (0.6–458 mg Mo kg<sup>-1</sup> DW), with a median value of 34.1 mg Mo kg<sup>-1</sup> DW (Table 5, Fig. 4). The large spatial variation in both exposure and PNECs of Mo in soil stresses the





**Fig. 5.** Risk characterisation ratios (RCR), calculated as the ratio of measured *aqua regia*-extractable concentration over the corresponding predicted no-effect concentration, for molybdenum in agricultural land soils in Europe. Dots represent sampling points. Areas between sample points were interpolated using the Kriging technique. Colour classes are based on 5th, 10th, 25th, 50th, 75th, 90th and 95th percentiles. Data from the GEMAS project.<sup>[41]</sup> ( $A_p$ , arable land.)

need to take into account this variation in ambient background concentrations and soil properties when assessing effects and risks of metals in soil. Similar results were obtained for grassland soils in Europe (data not shown). The 10th percentile of all individual PNEC values, being  $10.7 \text{ mg Mo kg}^{-1} \text{ DW}$ , can be suggested as a reasonable worst-case PNEC value for a generic risk assessment in the absence of information on pH and clay content of the soil.

Risk characterisation ratios (RCRs), calculated as the ratio of measured *aqua regia*-extractable molybdenum concentration over the corresponding PNEC, range from 0.0002 to a maximum of 1.1 with 50th and 90th percentiles of 0.01 and 0.06 respectively (Table 5, Fig. 5). Because the 90th percentile of the RCR values is well below 1, i.e. the exposure concentration is well below the PNEC, it can be concluded that prevailing Mo concentrations in arable soils do not pose a risk for  $\text{MoO}_4^{2-}$  toxicity at the regional scale in Europe. The largest RCR values are generally observed for countries in southern Europe (France,

Greece, Italy and Spain), mainly owing the combination of high background concentrations of Mo and high pH and hence low PNEC values. Only one site, with the highest *aqua regia*-extractable Mo concentration observed and a rather low PNEC (<20th percentile for Europe), has an RCR value larger than 1, which is an indication for a potential risk of  $\text{MoO}_4^{2-}$  toxicity to soil organisms. A reasonable worst-case value for RCR in a regional risk assessment for Europe can be calculated as the median of the 90th percentiles of the RCR for all regions (= countries) covered. This approach results in a reasonable worst-case RCR of 0.05 for arable land and this value can be used as a generic estimate for risk due to regional background concentrations in a risk assessment of sites with additional local point emissions (e.g. from smelters).

As a reference, a generic PNEC derived as the  $\text{HC}_{5-50}$  of a species sensitivity distribution with the raw  $\text{EC}_{10}$  data without corrections for aging or normalisation to reference soil properties is  $5.1 \text{ mg Mo kg}^{-1} \text{ soil}$ . Applied to the European data set for

arable soils, this yields an RCR ranging from 0.03 to 0.24 (10th–90th percentile) and predicts risk at ambient (mostly natural) concentration in 16 sites instead of in one. Such risk at background concentrations is difficult to defend because these background concentrations are also the reference in the toxicity tests. This illustrates that bioavailability correction does remove prediction of risk at natural background concentrations while still ensuring adequate protection of soil organisms against Mo toxicity.

## Conclusions

Over the last few years, a comprehensive data set describing  $\text{MoO}_4^{2-}$  toxicity to soil organisms (plants, invertebrates and microorganisms) has been generated. Molybdate toxicity data for the same endpoint, but tested in various soils, varied from 13-fold to more than 500-fold and this illustrates the importance of soil properties for  $\text{MoO}_4^{2-}$  bioavailability and toxicity in soils. Furthermore, the bioavailability and toxicity of  $\text{MoO}_4^{2-}$  in soil decrease with longer equilibration time after incorporation. A correction factor of 2.0 was selected to account for the difference in  $\text{MoO}_4^{2-}$  toxicity between typical laboratory conditions (no leaching, no aging) and field conditions (leaching of excess ions with percolating rainwater and long-term equilibration). Molybdate toxicity for the various endpoints studied was best correlated with soil pH and clay content and regression models were developed to normalise toxicity data for variation of these properties among soils. Toxicity thresholds were determined as the  $\text{HC}_{5-50}$  (i.e. median 95% protection level) as derived from the SSD of ecotoxicity data after correction for leaching and aging processes as well as normalised to clay content and pH. An uncertainty analysis of this  $\text{HC}_{5-50}$  showed that it was protective against direct toxicity of Mo and Mo compounds in the terrestrial environment and that it provides a robust and ecologically relevant PNEC to be determined for risk characterisation purposes. PNEC values in European arable land soils varied commonly between 10.7 and 168 mg Mo  $\text{kg}^{-1}$  DW (10th and 90th percentile respectively) and no risk of Mo toxicity to soil organisms was expected at the European regional scale. The information presented in the present paper can be used for national and international regulatory purposes for the setting of soil quality criteria for Mo and Mo compounds.

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