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Final report

Literature research for the review of ecotoxicological Critical Limits for heavy metals as a basis for the calculation of Critical Loads in the Geneva Air Convention

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On behalf of the German Environment Agency

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Abstract: Literature research for the review of ecotoxicological critical limits for heavy metals as a basis for the calculation of critical loads in the Geneva Air Convention

Heavy metals, even being natural componets of the environment, are toxic to organisms surpassing specific thresholds. The organisms have adapted to the native occurence and bioavailablity of heavy metals depending on the actual situation (pH, organic content, …) by their choose of habitat. However, by anthropogenic activity the heavy metals are carried in the atmosphere over large distances and are accumulated after deposition in habitats like topsoil. If the bioavailable concentrations surpass specific threshold values, a harm on humans and environment cannot be excluded. Due to this, the release of heavy metals into the environment is regulated by law. To determine hazard-free heavy metal depositions, ecological threshold values – so called Critical Loads – are calculated for heavy metals. Critical Loads base on so called Critical Limits. These are concentrations in environmental matrices, which should not be exceeded in order to avoid harmful effects. In the scope of this project, a worldwide literature research should identify and list all relevant studies since 2005, dealing with chronic effects of increased heavy metal concentrations on terrestric organisms and ecosystem functions to find recent data to be used for a review of the Critical Limits calculation. It is not the goal of this project to discuss or evaluate the relevant data. All relevant data is submitted as a literature data base and an Excel data compilation including all relevant information necessary for calculation of Critical Limits to the German Environment Agency. Additionally, this summary report is prepared. The report lists the relevant references with effect concentrations, endpoints (e.g. reproduction), organism group and species, as well as soil parameters in tables and gives a short summary, separated for all heavy metals found in the literature reseach applied.

Kurzbeschreibung: Literaturrecherche für die Aktualisierung ökotoxikologischer Critical Limits für Schwermetalle als Grundlage für die Berechnung von Critical Loads in der Genfer Luftreinhaltekonvention

Schwermetalle, auch wenn sie natürliche Bestandteile unserer Umwelt sind, sind ab bestimmten Konzentrationen schädlich für Organismen. Organismen haben sich in ihrer Habitatwahl an das natürliche Vorkommen und die dortige Bioverfügbarkeit unter den gegebenen Umständen (pH, org-Gehalt, …) angepasst. Durch menschliche Aktivitäten werden Schwermetalle verstärkt über die Atmosphäre in Umlauf gebracht und über Deposition in Habitaten wie Oberboden angereichert. Überschreiten die verfügbaren Konzentrationen nun bestimmte Schwellenwerte, so können schädliche Wirkungen für Mensch und Umwelt nicht mehr ausgeschlossen werden. Aus diesem Grund ist die Freisetzung von Schwermetallen in die Umwelt rechtlich geregelt. Zur Festlegung ungefährlicher Schwermetalldepositionen werden ökologische Schwellenwerte, sogenannte „Critical Loads“ für Schwermetalle berechnet. Dabei handelt es sich um Konzentrationen in Umweltmatrizes, die nicht überschritten werden sollten um schädliche Effekte zu vermeiden. „Critical Loads“ basieren auf „Critical Limits“. Im Rahmen dieses Projektes sollen basierend auf einer weltweiten Literaturrecherche alle Studien seit einschließlich 2005, die sich mit chronischen Wirkungen erhöhter Schwermetallkonzentrationen auf terrestrische Lebewesen und Ökosystemfunktionen befassen, identifiziert und gelistet werden um aktuelle Werte zu finden, die für die „Critical Limits“-Berechnung genutzt werden können. Es ist nicht das Ziel dieses Projektes die relevanten Daten zu diskutieren oder auszuwerten. Eine Literaturdatenbank mit allen Referenzen und ein Excel-Dokument mit allen notwendigen Daten zur Berechnung von „Critical Limits“ wurde an das Deutsche Umweltbundesamt übergeben. Zusätzlich wurde dieser zusammenfassende Bericht erstellt. Der Bericht listet die relevanten Referenzen mit Effektkonzentrationen, Endpunkten (z.B. Reproduktion), Organismengruppe und Art, sowie Bodenparameter in Tabellen und gibt eine kurze Zusammenfassung, aufgeteilt nach allen Schwermetallen, die in der Recherche gefunden wurden.

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List of abbreviations

CLRTAP	Convention on long-range transboundary air pollution
Conc.	Concentration
Corg	Organic carbon
EC	Effect concentration
ECx	"Effect concentration" is the concentration of the heavy metal, which results in a X per cent reduction in the measured parameter relative to the control
ISO	International Organization for Standardization
LOEC	"Lowest Observed Effect Concentration" is the lowest concentration tested at which the measured parameter shows significant inhibition relative to the control
Meas. conc.	Measured concentration
NOEC	"No Observed Effect Concentration" is the highest concentration tested at which the measured parameter shows no significant inhibition relative to the control
Nom. conc.	Nominal concentration
OC	Organic content
OECD	Organisation for Economic Co-operation and Development
OTU	Operational taxonomic unit
SIN	Substrate induced nitrification
UBA	(Deutsches) Umweltbundesamt ((German) Environment Agency)
WHCmax	Maximum water holding capacity

1 Introduction

1.1 Background of the project

Heavy metals, even being natural componets of the environment, are toxic to organisms surpassing specific thresholds. The organisms have adapted to the native occurence and bioavailablity of heavy metals depending on the actual situation (pH, organic content, …) by their choose of habitat. Since centuries, heavy metals are circulated increasingly by anthropogenic activity. To some extend, they are carried in the atmosphere over large distances and are accumulated after deposition in habitats like topsoil. There, the bioavailability of the metals can increase due to (un)favaraourable environmental conditions (*pH dependent free metal ion concentration*). If the bioavailable concentrations surpass specific threshold values, a harm on humans and environment cannot be excluded. Due to this, the release of heavy metals into the environment by the humans as well as the permitted concentrations in diverse environmental matrices are regulated by law nationally and internationally. The Protocol on Heavy Metals of the Convention on Long-range Transboundary Air Pollution (Geneva Air Convention, CLRTAP. of 1998, amended in 2012), aims at reducing emissions of heavy metals in general, but regulates only lead (Pb), cadmium (Cd) and mercury (Hg) as priority metals.

For evaluating the effects of heavy metal deposition on human health and the environment, inter alia the "Critical Load approach" is used. In Europe this is done using the method as described in the Manual for Modelling and Mapping Critical Loads & Levels, Kapitel V.5. Critical Loads for Pb, Cd, Hg were deduced by an international expert panel and the Coordination Centre for Effects, the data centre of the International Cooperative Programme on Modelling and Mapping Critical Loads & Levels and Air Pollution Effects, Risks and Trends (ICP Modelling and Mapping), which works under the Working Group on Effects (WGE) of the CLRTAP until 2006 (e.g. De Vries et al. 2005, 2007a, b; Slootweg et al. 2007). For further heavy metals, values were also published later on (Hettelingh et al. 2007; and specificly for Germany by Schröder et al. 2018). In order to calculate the Critical Loads so called Critical Limits are needed. Going below these critical concentrations or threshold values in envoronmental matrices, organismens and ecosystematic functions are not expected to be affected.

Critical Limits applied for the calculation of the Critical Loads should be state of the art and therefore providing the highest possible prospective protection. For this, a regular proof and update where appropriate is essential. Due to this, current studies regarding chronic effects of heavy metals on soil organismens were to be identified and considered in this literature research study.

1.2 Content of this report

In the scope of this project, a worldwide literature research should identify and list all relevant studies since 2005, dealing with chronic effects of increased heavy metal concentrations on terrestric organisms and ecosystem functions. Additionally, these studies were reviewed for their applicability in deriving Critical Limits. The relevant data (NOEC, soil parameter) are listed. It is not the goal of this project to discuss or evaluate the relevant data. For submission of the results to the ICP Modelling and Mapping all relevant data is delivered to UBA as a literature data base and an Excel data compilation including all relevant information necessary for calculation of Critical Limits. Additionally, this summary report in english language is prepared. The report lists the relevant references with effect concentrations, endpoints (e.g. reproduction), organism group and species, as well as soil pH, organic content and clay content in tables and gives a short summary, separated for all heavy metals found in the the literature reseach applied. Besides the priority heavy metals lead (Pb), cadmium (Cd) and mercury (Hg), this compilation covers the heavy metals silver (Ag), arsenium (As), chrome (Cr), copper (Cu), lanthanum (La), molybdenum (Mb), nickel (Ni), vanadium (V), manganese (Mn), uranium (U), wolfram/tungsten (Wo) and zinc (Zn), as long as they were found by the search parameter „heavy metal“.

2 Methods

2.1 Main literature database

Applying an internet based worldwide literature research, studies on heavy metal effect studies in laboratory, artificial ecosystems and field published in 2005 or later should were searched.

The scientific online data bases considered are listed in the textbox below:

Scientific online data bases considered

- PubMed (http://www.ncbi.nlm.nih.gov/pubmed)
- Scopus (https://www.elsevier.com/solutions/scopus)
- Science Direct (http://www.sciencedirect.com)
- Web of Science (http://apps.webofknowledge.com)

At the start oft he project, additionally Google (https://google.com) or Google Scholar (https://scholar.google.de) and the European Chemicals Agency (ECHA) public domain substance data base were planned to be considered if necessary. However, the number of references found and the limited time available for the project, restricted the search on the four online data bases listed abhove. Additionally, the ECHA public domain substance data base allows only access on summaries. Therefore, not all necessary data needed for Critical Limits calculation are mandatorily available in this project.

The search included studies on single or combined effects of the - according to the heavy metal protocol oft he CLRTAP - top priority heavy metals lead (Pb), cadmium (Cd) and mercury (Hg). However, also finds on other metals (e.g. Cu, Zn, Ni, As, Cr, V, Mn, Sb, Ti, Tl, Co, Mb, Pt-group elements) are considered, if found by the search parameter „heavy metal“. The effects considered are chronic effects on terrestric organisms (especially plants and invertebrates), microbial processes and – as far as indicated – related affected ecological functions. References indicating effect concentrations or thresholds (e.g. in mg/kg soil or µg/L pore water) are of special interest, since generally only these can be considered for Critical Limits calculation.

The textblock below lists the initial search profile applied.

Initial search profile

- Group 1: “Heavy metal” OR “Pb” OR “Cd” OR “Hg” OR “lead” OR “cadmium” OR “mercury”
- Group 2: “Terrestrial organisms” OR “soil organisms” OR “soil microorganisms” OR “soil invertebrates” OR “earthworm” or “lumbricid” OR “plant” OR “collembola” OR “sprinmgtail” OR “soil function” OR “soil respiration” OR “nitrification” OR “litter decay” OR “decomposition”
- Group 3: “NOEC” OR “LOEC” OR “EC10” OR “chronic effects” OR “chronic ecotoxicological effects“

The search profile consisted of three groups. From each group at least one term must be found in the reference. E.g. „heavy metal" AND „soil organisms" AND „NOEC" must be found in a paper to be a find in according to the profile.

Within the literature found applying the search profile, it was also looked for studies or theoretical essays regarding data, methods or models on mobility and/or bioavailability of metals. However, there were no finds within this project.

The search in the four online data bases resulted in 568 (PubMed), 1184 (Scopus), 642 (Web of Science) and 1899 (ScienceDirect) finds. Excluding multiple finds of the same references in more than one data base, resulted in 3131 finds at all.

A first rough review revealed that the term „plant" in combination with „heavy metal" was leading in a lot of unsuitable finds. In most cases, „plant" was found in the meaning of factory, not in a biological meaning. Additionally, the terms „LOEC", „chronic effects" or "chronic ecotoxicological effects" without „NOEC" or „EC10" in combination did not reveal the effect thresholds necessary for Critical Limits calculation. Therefore, the final search profile was adapted. The textblock below lists the final search profile applied.

Final search profile

- Group 1: "Heavy metal" OR "Pb" OR "Cd" OR "Hg" OR "lead" OR "cadmium" OR "mercury"
- Group 2: "Terrestrial organisms" OR "soil organisms" OR "soil microorganisms" OR "soil invertebrates" OR "earthworm" or "lumbricid" OR "plant species" OR "terrestrial plant" OR "terrestric plant" OR "collembola" OR "sprinmgtail" OR "soil function" OR "soil respiration" OR "nitrification" OR "litter decay" OR "decomposition"
- Group 3: "NOEC" OR "EC10"

The search in the four online data bases resulted in 1293 finds at all. The lists were reduced to references for which full text acces was available without additional payment. The list was reduced to 686 references. The titles and/or abstracts of these 686 references were checked if they are really matching the theme ecotoxicology/heavy metals/terrestric environment. This review reduced the list of potentially appropriate references on 188. Even when the terms applied for searching mandatorily include a heavy metal, often publications dealing with organic substances were selcted. Also still a lot of studies dealing with aquatic species were selected. The 188 references were combined in an Endnote literature data base and submitted to the German UBA. The UBA intends to submit the data base to the ICP Modelling and Mapping of CLRTAP. The 188 references were searched through for the relevant parameters necessary for the calculation of Critical Limits. Only references with a complete data set of relevant parameters were included into the Excel data compilation. The final list contains 37 suitable references.

2.2 Criteria for references used in chapter 3

According to the call of bids, there was a list of selection and exclusion criteria for relevant references. The textbox below shows these criteria.

Selection and exclusion criteria for relevant references according to the call for bids

- Effect on structural (e.g. biomass, reproduction) or functional properties (e.g. litter decomposition, microbial activity).
- Effect threshold values are based directly on heavy metal concentrations related to soil (in mg/kg) or pore water (in µg/L) form effect studies in the laboratory, artificial ecosystems or field.
- Only NOEC values are accepted.
- Studies conducted according to or following standard guidelines (ISO, OECD, ASTM). Methods and test design are comprehensively documented.
- References must indicate soil pH, texture or clay (kaolin) content, organic matter/organic carbon content (in case of EC values based on mg/kg soil) or dissolved organic matter/carbon (in case of EC values based on µg/L pore water).
- Effect values in non-realistic magnitudes are not accepted (threshold of 90-percentile of german background values).
- Studies using water saturated or real contaminated field soil (e.g. mining sites or dumps) are not accepted.

Usually, for "Critical Limits" calculation, the NOEC as effect concentration based on mg/kg soil or µg/L pore water, and the soil/pore water parameters pH, organic matter/organic content (OC) or dissolved organic carbon and clay content must be available. However, a lot of experimental studies does not measure and indicate exactly these parameters. However, often comparable parameters are indicated like EC_{10} values, organic carbon content (Corg) or kaolin content in tests with artificial soil. Organcic matter e.g. can be roughly calculated from the Corg content. Therefore, it was already suggested in the quotation and discussed and decided in the kick-off meeting off the project, to keep the criteria for relevant references relatively wide to enable the use of data, even when not fitting the actual requirements of the call for bids. The textbox below shows the final selection and exclusion criteria for relevant references.

Selection and exclusion criteria for relevant references after adaptation

- Effect on structural (e.g. biomass, reproduction) or functional properties (e.g. litter decomposition, microbial activity).
- Effect threshold values are based directly on heavy metal concentrations related to soil (in mg/kg) or pore water (in µg/L) form effect studies in the laboratory, artificial ecosystems or field.
- EC_{10} values are accepted besides NOEC values.
- Studies conducted according to or following standard guidelines (ISO, OECD, ASTM) can be considered to be always of high quality. For other test designs, a comprehensive documentation of the method is required for acceptance.
- References must indicate soil pH, texture or clay (kaolin) content, organic matter/organic carbon content (in case of EC values based on mg/kg soil) or dissolved organic matter/carbon (in case of EC values based on µg/L pore water).

- Effect values in non-realistic magnitudes are accepted
- Studies using real contaminated field soil (e.g. mining sites or dumps) are not accepted.
- References do not mandatorily have to deal with Hg, Pb, Cd. All heavy metals are accepted.
- In laboratory studies according to standard guidelines, often artificial soil is used. Therefore, also data form studies with artificial soil is accepted.

Besides the EC values and the mandatory soil parameters, additional data of relevance is implemented in the Excel data compilation if available (see textblock below).

Information incorporated in the Excel data compilation

- Index number in the Endnote data base
- Authors of the reference
- Year of publishing
- Element (e.g. Pb)
- Form (e.g. sum formula of salt, pure metal, nano material)
- NOEC, LOEC, EC_{10} (mg/kg) in individual rows
- NOEC, LOEC, EC_{10} (mg/L) in individual rows
- Organism group (terrestrial plants, invertebrates, micro-organisms)
- Animal or plant species
- Endpoint (e.g. root length, reproduction, enzyme activity)
- Soil pH and solvent applied (H_2o, $CaCl_2$, KCl, soil solution) in individual rows
- Matrix (natural soil, artificial soil)
- Texture (%sand, %silt, %clay)
- Soil type (e.g. sandy loam, Entisol)
- (EC) Evaluation based on (e.g. nominal concentration, measured digested soil concentration)
- Solvent for metal extraction/digestion (e.g. $HClO_4$:HNO_3:HF (1:3:2) digestive at 175 °C)
- Organic content or organic carbon content (%) in individual rows
- Maximum water holding capacity (in g/kg)
- Dissolved organic matter or carbon (%) in individual rows
- Clay or kaolin content (%) in individual rows
- Guideline followed
- Remarks

For this summary report, the information listed in the tables of chapter 3 was reduced on the most relevant data. The textblock below shows the information incorporated in the summary report tables.

Information incorporated in the Excel data compilation

- Authors and year of the reference (title and paper indicated in chapter 4 “list of references”
- Form (e.g. sum formula of salt, pure metal, nano material)
- NOEC, LOEC, EC_{10} (mg/kg) or (mg/L)
- Endpoint (e.g. root length, reproduction, enzyme activity)
- Animal or plant species (table sorted by organism gropus)
- Organic content or organic carbon content (%)
- Clay or kaolin content (%)
- Soil pH and solvent applied (H_2o, $CaCl_2$, KCl, soil solution)
- Guidelines followed

3 Results of the literature research

3.1 Overview

Table 1: Literature generally suitable for calculation of Critical Limits

Heavy metal	Number of publications	Number of endpoints	Number of EC based on nominal concentration	Number of EC based on measured concentration	Number of soils
Lead (Pb)	2	2	-	16	16
Cadmium (Cd)	9	6	17	30	23
Mercury (Hg)	4	4	1	27	6
Silver (Ag)	2	3	-	12	4
Arsenic (As)	2	2	13	7	20
Chromium (Cr)	1	1	1	-	1
Copper (Cu)	9	4	14	70	67
Lanthanum (La)	1	5	-	10	1
Molybdenum (Mo)	1	3	-	30	10
Nickel (Ni)	3	2	4	-	3
Uranium (U)	1	5	5	-	1
Wolfram (Wo)	1	2	-	3	2
Zinc (Zn)	4	4	18	3	19

Endpoints are e.g. reproduction of earthworms, reproduction of springtails, biomass of plants, physiological activity of microorganisms, etc. EC = Effect concentration (NOEC, EC10, etc.). In some studies metal concentrations were measured with several methods (digestives, extracts, soil solution) and effect concentrations were calculated and indicated based on these concentrations. The EC values presented in the following chapters are based on total amounts when several methods were applied. All EC values and the respective methods were submitted to UBA by an Excel data compilation. Data not included in this report.

3.2 Terrestrial species investigated and their chronic response to Lead (Pb), Cadmium (Cd) and Mercury (Hg)

3.2.1 Lead (Pb)

Regarding chronic effects on terrestric organismens and soil functions originated by lead (Pb), two references were found. In these studies, 16 natural soils were applied and two different endpoints covering terrestric plants and soil micro-organisms were investigated (see Table 2).

There was one study investigating the standard endpoint shoot mass for one plant species in six soils. The study was conducted according to the standard guideline OECD 208.

The effect of lead on soil micro-organisms was investigated in one study with ten soils. Endpoint was enzyme activity. The study was conducted according to the SIN (substrate induced nitrification) assay.

Lead was applied as $PbCl_2$ or $Pb(NO_3)_2$. Effect concentrations were calculated based on measured extracted or measured digested soil concentration.

Ph of soils applied was in the range of 4.7 ($CaCl_2$) – 8.9 (H_2O). Organic content (OC) or organic carbon (Corg) content was in the range of 0.60% (OC) – 31.0% (Corg). The clay content was in the range of 3% - 66.1%.

Table 2: Literature generally suitable for calculation of Critical Limits for lead (Pb)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Cheyns et al. 2012	$PbCl_2$	NOEC 4400 mg/kg	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.4% Corg	16% clay	7.4 ($CaCl_2$)
Cheyns et al. 2012	$PbCl_2$	NOEC 750 mg/kg	Shoot mass	[1] *L. esculentum* (Solanaceae)	3.1% Corg	30% clay	6.5 ($CaCl_2$)
Cheyns et al. 2012	$PbCl_2$	NOEC < 250 mg/kg	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.0% Corg	12% clay	6.7 ($CaCl_2$)
Cheyns et al. 2012	$PbCl_2$	NOEC 440 mg/kg	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.5% Corg	3% clay	5.7 ($CaCl_2$)
Cheyns et al. 2012	$PbCl_2$	NOEC 260 mg/kg	Shoot mass	[1] *L. esculentum* (Solanaceae)	2.1% Corg	13% clay	5.2 ($CaCl_2$)
Cheyns et al. 2012	$PbCl_2$	NOEC 1100 mg/kg	Shoot mass	[1] *L. esculentum* (Solanaceae)	31.0% Corg	59% clay	4.7 ($CaCl_2$)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 160 mg/kg	SIN	[2] Soil micro-organisms	2.46% OC	38.9% clay	6.81 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 218 mg/kg	SIN	[2] Soil micro-organisms	0.99% OC	27.3% clay	7.12 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 166 mg/kg	SIN	[2] Soil micro-organisms	0.62% OC	27.5% clay	8.83 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 129 mg/kg	SIN	[2] Soil micro-organisms	1.57% OC	16.3% clay	8.86 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 169 mg/kg	SIN	[2] Soil micro-organisms	0.69% OC	17.6% clay	8.9 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 195 mg/kg	SIN	[2] Soil micro-organisms	1.02% OC	19.6% clay	8.86 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 207 mg/kg	SIN	[2] Soil micro-organisms	1.51% OC	66.1% clay	4.93 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 197 mg/kg	SIN	[2] Soil micro-organisms	1.42% OC	41.2% clay	6.71 (H_2O)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 86 mg/kg	SIN	[2] Soil micro-organisms	0.60% OC	10.1% clay	8.84 (H_2O)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Zheng at al. 2017	$Pb(NO_3)_2$	EC10 97 mg/kg	SIN	[2] Soil micro-organisms	1.43% OC	56.4% clay	4.95 (H_2O)

Guidelines: [1] OECD 208, [2] SIN assay. EC = Effect concentration (NOEC, EC10, etc.); SIN = Substrate induced nitrification; n.s. = natural soil; a.s. = artificial soil; OC = organic content; Corg = organic carbon; Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.2.2 Cadmium (Cd)

Regarding chronic effects on terrestric organismens and soil functions originated by cadmium (Cd), nine references were found. In these studies, 23 soils (21 natural soils and two artificial soils) were applied and six different endpoints covering terrestric plants, invertebrates and soil micro-organisms were investigated (see Table 3).

There were three studies covering three plant species and 15 soils, investigating the standard endpoint shoot mass but also one study covering three plant species and one soil investigating the non-standard endpoint plant enzyme activity. Nearly all studies were conducted according to or at least following standard guidelines (OECD or ISO). One plant study (da Rosa Corrêa et al. 2006) was conducted according to a standard guideline, but the endpoint enzyme activity (Glutathione reductase, Superoxide dismutase, Peroxidase, Catalase) was non-standard. However, the method for quantification of enzyme activity is well documented.

There were five studies with Oligochaeta (Lumbricidae and Enchytraeidae) in five soils and three studies with Collembola in six soils representing invertebrates. All studies investigated effects on reproduction. Nearly all studies were conducted according to or at least following standard guidelines (OECD or ISO).

The effect of cadmium on soil micro-organisms was investigated in one study with one soil. Endpoint was enzyme activity. One study regarding enzyme activity of soil micro-organisms (Caetano et al. 2016) was not following a standard guideline, but was well documented.

Cadmium was applied as $CdSO_4$, $CdCl_2$ or $Cd(NO_3)_2$. Effect concentrations were calculated based on nominal concentrations or measured digested soil concentration. Some studies additionally evaluated the effects based on measured extractable soil concentration or measured soil solution concentration. These values were submitted by an Excel data compilation. The data is not included in this report.

Ph of soils applied was in the range of 3.0 ($CaCl_2$) – 7.88 (orinial soil solution). Organic content (OC) or organic carbon (Corg) content was in the range of 0.67% (Corg) – 16.5% (OC). The clay content was in the range of 3.3% - 39.2%.

Table 3: Literature generally suitable for calculation of Critical Limits for cadmium (Cd)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Caetano et al. 2016	$CdSO_4$	NOEC <35 mg/kg	Shoot mass	[1] *L. sativa* (Compositae)	6.5% OC	3.3% clay	4.3 (KCl)
Du et al. 2014	CdCl2	EC10 11.5 mg/kg	Shoot mass	[1] *Z. mays* (Poaceae)	1.13% OC	11.6% clay	7.3 (uk)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 0.66 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	3.16% Corg	21.7% clay	4.9 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 6.8 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	2.23% Corg	9.2% clay	6.37 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 8.7 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	2.82% Corg	25.6% clay	5.55 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 6.3 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	0.67% Corg	13.0% clay	6.6 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 9.34 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	4.73% Corg	18.9% clay	5.81 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 9.5 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	2.23% Corg	27.7% clay	4.76 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 5.59 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	3.95% Corg	21.1% clay	5.34 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 11.0 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	1.92% Corg	20.1% clay	4.66 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 1.62 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	0.67% Corg	19.1% clay	7.44 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 3.0 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	1.54% Corg	39.2% clay	5.07 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 9.9 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	4.36% Corg	13.0% clay	5.67 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 7.66 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	2.14% Corg	11.2% clay	5.95 (s.s.)
Zhang et al. 2020	$Cd(NO_3)_2$	EC10 5.02 mg/kg	Shoot mass	[2] *L. japonicum* (Oleaceae)	7.28% Corg	11.3% clay	7.88 (s.s.)
da Rosa Corrêa et al. 2006	CdCl2	NOEC 0.19 mg/kg	Enzyme activity	[2] *A. sativa* (Poaceae)	3.0% OC 1.5% Corg	26% clay	6.6 (uk)
da Rosa Corrêa et al. 2006	CdCl2	NOEC 0.01 mg/kg	Enzyme activity	[2] *B. campestris* (Brassicaceae)	3.0% OC 1.5% Corg	26% clay	6.6 (uk)
da Rosa Corrêa et al. 2006	CdCl2	NOEC 0.01 mg/kg	Enzyme activity	[2] *L. sativa* (Compositae)	3.0% OC 1.5% Corg	26% clay	6.6 (uk)
Caetano et al. 2016	$CdSO_4$	NOEC 35.0 mg/kg	Repro	[3] *E. andrei* (Lumbricidae)	6.5% OC	3.3% clay	4.3 (KCl)
Du et al. 2014	CdCl2	EC10 58.1 mg/kg	Repro	[3] *E. fetida* (Lumbricidae)	1.13% OC	11.6% clay	7.3 (uk)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Caetano et al. 2016	$CdSO_4$	NOEC 7.0 mg/kg	Repro	[4] *E. crypticus* (Enchytraeidae)	6.5% OC	3.3% clay	4.3 (KCl)
Castro-Ferreira et al. 2012	CdCl2	EC10 15 mg/kg	Repro	[4] *E. crypticus* (Enchytraeidae)	4.0% OC	6.0% clay	5.5 ($CaCl_2$)
Novais et al. 2011	CdCl2	NOEC 1.0 mg/kg	Repro	[4] *E. albidus* (Enchytraeidae)	4.40% OC	6% clay	5.5 ($CaCl_2$)
Santos et al. 2022	CdCl2	EC10 26 mg/kg	Repro	[5*] *E. crypticus* (Enchytraeidae)	1.71% Corg	8.0% clay	5.6 ($CaCl_2$)
Bur et al. 2010	$Cd(NO_3)_2$	EC5 43.5 mg/kg	Repro	[6*] *F. candida* (Collembola)	1.4% Corg	16% clay	7.4 ($CaCl_2$)
Bur et al. 2010	$Cd(NO_3)_2$	EC5 56 mg/kg	Repro	[6*] *F. candida* (Collembola)	2.0% OC	37.2% clay	6.9 ($CaCl_2$)
Bur et al. 2010	$Cd(NO_3)_2$	EC5 15 mg/kg	Repro	[6*] *F. candida* (Collembola)	16.5% OC	19.4% clay	3.0 ($CaCl_2$)
Caetano et al. 2016	$CdSO_4$	NOEC 35.0 mg/kg	Repro	[6] *F. candida* (Collembola)	6.5% OC	3.3% clay	4.3 (KCl)
Son at al. 2009	CdCl2	NOEC < 12.3 mg/kg	Repro	[6] *P. kimi* (Collembola)	1.46% OC	20% kaolin #	4.87 (uk)
Son at al. 2009	CdCl2	NOEC < 12.3 mg/kg	Repro	[6] *P. kimi* (Collembola)	8.54% OC	20% kaolin #	6.63 (uk)
Caetano et al. 2016	$CdSO_4$	NOEC 13.4 mg/kg	Repro	Soil micro-organisms	6.5% OC	3.3% clay	4.3 (KCl)

Guidelines: [1] ISO 11269-2, [2] OECD 208 (*with modifications), [3] ISO 11268-2, [4] ISO 16387/OECD 220, [5] OECD 317 (*with modifications), [6] ISO 11267 (*with modifications). EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon; # artificial soil; uk = unknown; s.s. = soil solution. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.2.3 Mercury (Hg)

Regarding chronic effects on terrestric organismens and soil functions originated by mercury (Hg), two references were found. In these studies, four soils (three natural soils and one artificial soil) were applied and three different endpoints covering terrestric plants, invertebrates and soil micro-organisms were investigated (see Table 4).

There was one study (Mahbub et al. 2017) investigating the standard endpoint root length for three plant species in three natural soils. The study was not following a standard guideline, but was well documented.

There was one study (Gimbert et al. 2016) according to the ISO 15952 guideline, investigating the reproduction of the common garden snail in one artificial soil.

The effect of mercury on soil micro-organisms was investigated in one study (Mahbub et al. 2017) with three natural soils. Endpoint was enzyme activity. The study was not following a standard guideline, but was well documented.

Mercury was applied as $HgCl_2$. Effect concentrations were calculated based on nominal or measured digested soil concentration. One study additionally evaluated the effects based on measured extractable soil concentration. These values were submitted by an Excel data compilation. The data is not included in this report.

Ph of soils applied was in the range of 4.2 (H_2O) – 8.5 (H_2O). Organic content (OC) or organic carbon (Corg) content was in the range of 2.07% (Corg) – 10.0% (OC). The clay content was in the range of 2% - 14%.

Table 4: Literature generally suitable for calculation of Critical Limits for mercury (Hg)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Mahbub et al. 2017	$HgCl_2$	EC10 145 mg/kg	Root length	[1] *I. membranaceum* (Poaceae)	2.07% Corg	13% clay	7.6 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 0.95 mg/kg	Root length	[1] *I. membranaceum* (Poaceae)	2.2% Corg	14% clay	8.5 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 190 mg/kg	Root length	[1] *I. membranaceum* (Poaceae)	4.0% Corg	2% clay	4.2 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 103 mg/kg	Root length	[1] *D. sericeum* (Poaceae)	2.07% Corg	13% clay	7.6 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 115 mg/kg	Root length	[1] *D. sericeum* (Poaceae)	2.2% Corg	14% clay	8.5 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 >10^9 mg/kg	Root length	[1] *D. sericeum* (Poaceae)	4.0% Corg	2% clay	4.2 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 111 mg/kg	Root length	[1] *S. africanus* (Poaceae)	2.07% Corg	13% clay	7.6 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 98 mg/kg	Root length	[1] *S. africanus* (Poaceae)	2.2% Corg	14% clay	8.5 (H_2O)
Mahbub et al. 2017	$HgCl_2$	EC10 >5.3 mg/kg	Root length	[1] *S. africanus* (Poaceae)	4.0% Corg	2% clay	4.2 (H_2O)
Gimbert et al. 2016	$HgCl_2$	NOEC 100 mg/kg	Repro	[2] *C. aspersus aspersus* (Stylommato phora)	10% OC	20% kaolin #	6.7 (uk)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Mahbub et al. 2016	$HgCl_2$	EC10 0.11 mg/kg	Enzyme activity	Soil micro-organisms	2.07% Corg	13% clay	7.6 (H_2O)
Mahbub et al. 2016	$HgCl_2$	EC10 0.0006 mg/kg	Enzyme activity	Soil micro-organisms	2.2% Corg	14% clay	8.5 (H_2O)
Mahbub et al. 2016	$HgCl_2$	EC10 >300 mg/kg	Enzyme activity	Soil micro-organisms	4.0% Corg	2% clay	4.2 (H_2O)

Guidelines: [1] Well documented test method, [2] ISO 15952. EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon; # artificial soil; uk = unknown. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3 Terrestrial species investigated and their chronic response to further Heavy Metals

3.3.1 Silver (Ag)

Regarding chronic effects on terrestric organismens and soil functions originated by silver (Ag), two references were found. In these studies, four natural soils were applied and two different endpoints regarding soil microbial function were investigated (see Table 5).

The studies followed the OECD 216 and ISO 15685 guidelines. Endpoint was nitrification. One study additionally evaluated the molecular biological non-standard endpoint operational taxonomic units (OTUs).

Silver was applied as $AgNO_3$ or Ag and Ag_2S nano materials. Effect concentrations were calculated based on measured total or digested soil concentration.

Ph of soils applied was in the range of 5.1 ($CaCl_2$) – 7.3 ($CaCl_2$). Organic carbon (Corg) content was in the range of 1.30% – 6.9%. The clay content was in the range of 8% - 16%.

Table 5: Literature generally suitable for calculation of Critical Limits for silver (Ag)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Bollyn et al. 2018	$AgNO_3$	EC10 4.8 mg/kg	Nitrifi	[1] Soil micro-organisms	1.30% Corg	10% clay	7.3 ($CaCl_2$)
Bollyn et al. 2018	$AgNO_3$	EC10 3.8 mg/kg	Nitrifi	[1] Soil micro-organisms	1.61% Corg	8% clay	5.4 ($CaCl_2$)
Bollyn et al. 2018	$AgNO_3$	EC10 8.1 mg/kg	Nitrifi	[1] Soil micro-organisms	3.80% Corg	16% clay	6.0 ($CaCl_2$)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Bollyn et al. 2018	AgNM	EC10 9.0 mg/kg	Nitrifi	[1] Soil micro-organisms	1.30% Corg	10% clay	7.3 ($CaCl_2$)
Bollyn et al. 2018	AgNM	EC10 3.8 mg/kg	Nitrifi	[1] Soil micro-organisms	1.61% Corg	8% clay	5.4 ($CaCl_2$)
Bollyn et al. 2018	AgNM	EC10 29 mg/kg	Nitrifi	[1] Soil micro-organisms	3.80% Corg	16% clay	6.0 ($CaCl_2$)
Doolette et al. 2016	$AgNO_3$	EC10 8 mg/kg	Nitrifi	[2] Soil micro-organisms	6.9% Corg	14% clay	5.1 ($CaCl_2$)
Doolette et al. 2016	AgNM	EC10 7 mg/kg	Nitrifi	[2] Soil micro-organisms	6.9% Corg	14% clay	5.1 ($CaCl_2$)
Doolette et al. 2016	Ag_2S-NM	EC10 9 mg/kg	Nitrifi	[2] Soil micro-organisms	6.9% Corg	14% clay	5.1 ($CaCl_2$)
Doolette et al. 2016	$AgNO_3$	HC10 0.83 mg/kg	OTUs	[3] Soil micro-organisms	6.9% Corg	14% clay	5.1 ($CaCl_2$)
Doolette et al. 2016	AgNM	HC10 0.44 mg/kg	OTUs	[3] Soil micro-organisms	6.9% Corg	14% clay	5.1 ($CaCl_2$)
Doolette et al. 2016	Ag_2S-NM	HC10 1.2 mg/kg	OTUs	[3] Soil micro-organisms	6.9% Corg	14% clay	5.1 ($CaCl_2$)

Guidelines: [1] ISO 15685, [2] OECD 216, [3] Well documented test method. NM = Nanomaterial; EC = Effect concentration (NOEC, EC10, etc.); Nitrifi = nitrification; OTUs = operational taxonomic units as copy number of amoA gene; OC = organic content; Corg = organic carbon; Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.2 Arsenic (As)

Regarding chronic effects on terrestric organismens and soil functions originated by arsenic (As), two references were found. In these studies, 20 natural soils were applied and two different endpoints covering terrestric plants and soil micro-organisms were investigated (see Table 6).

There was one study investigating the standard endpoint root length for one plant species in 13 natural soils. The study was conducted according to the ISO 11269-1 guideline.

The effect of arsenic on soil micro-organisms was investigated in one study with seven natural soils. Endpoint was luminiscence. The study was conducted according to the Microtox® test, ASTM 2004.

Arsenic was applied as Na_2HAsO_4 or Na_2HAs. Effect concentrations were calculated based on nominal or measured digested soil concentration.

Ph of soils applied was in the range of 3.4 ($CaCl_2$) – 8.79 (H_2O). Organic carbon (Corg) content was in the range of 0.38% – 23.32%. The clay content was in the range of 7% - 54.76%.

Table 6: Literature generally suitable for calculation of Critical Limits for arsenic (As)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Song et al. 2006	Na_2HAs	NOEC 15 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.78% Corg	51% clay	5.4 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 11.8 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.53% Corg	20% clay	7.6 ($CaCl_2$)
Song et al. 2006	Na_2HAs	NOEC 30 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.38% Corg	25% clay	7.5 ($CaCl_2$)
Song et al. 2006	Na_2HAs	NOEC 15 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.63% Corg	7% clay	4.8 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 58.3 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.27% Corg	26% clay	7.5 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 11.4 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.76% Corg	9% clay	5.2 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 18.8 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	5.20% Corg	13% clay	3.4 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 4.2 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	12.9% Corg	13% clay	4.2 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 26.9 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.26% Corg	27% clay	7.4 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 50.7 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.41% Corg	38% clay	4.8 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 49.3 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	2.61% Corg	46% clay	7.4 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 13.9 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.98% Corg	15% clay	6.8 ($CaCl_2$)
Song et al. 2006	Na_2HAs	EC10 27.7 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	23.3% Corg	24% clay	4.7 ($CaCl_2$)
Romero-Freire et al. 2014	Na_2HAsO_4	EC10 58.21 mg/kg	Lumi	[2] *Vibrio fischeri* (Vibrionaceae)	5.43% Corg	23.6% clay	7.96 (H_2O)
Romero-Freire et al. 2014	Na_2HAsO_4	EC10 38.82 mg/kg	Lumi	[2] *Vibrio fischeri* (Vibrionaceae)	0.42% Corg	11.8% clay	8.67 (H_2O)
Romero-Freire et al. 2014	Na_2HAsO_4	EC10 5.51 mg/kg	Lumi	[2] *Vibrio fischeri* (Vibrionaceae)	0.38% Corg	7.70% clay	8.79 (H_2O)
Romero-Freire et al. 2014	Na_2HAsO_4	EC10 101.9 mg/kg	Lumi	[2] *Vibrio fischeri* (Vibrionaceae)	0.61% Corg	19.1% clay	6.74 (H_2O)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Romero-Freire et al. 2014	Na_2HAsO_4	EC10 44.38 mg/kg	Lumi	[2] *Vibrio fischeri* (Vibrionaceae)	8.22% Corg	23.8% clay	7.20 (H_2O)
Romero-Freire et al. 2014	Na_2HAsO_4	EC10 57.54 mg/kg	Lumi	[2] *Vibrio fischeri* (Vibrionaceae)	0.49% Corg	8.31% clay	5.87 (H_2O)
Romero-Freire et al. 2014	Na_2HAsO_4	EC10 332.1 mg/kg	Lumi	[2] *Vibrio fischeri* (Vibrionaceae)	0.66% Corg	54.8% clay	7.03 (H_2O)

Guidelines: [1] ISO 11269-1, [2] Microtox® test (ASTM 2004), comparable to ISO 11348-3. NM = Nanomaterial; EC = Effect concentration (NOEC, EC10, etc.); Lumi = luminiscence; OC = organic content; Corg = organic carbon. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.3 Chrome (Cr)

Regarding chronic effects on terrestric organismens and soil functions originated by chrome (Cr), one reference was found. In this study, one artificial soil was applied and the standard endpoint shoot mass of terrestric plants was investigated (see Table 7). The study was conducted according to the OECD 208 guideline.

Chrome was applied as $K_2Cr_2O_7$. Effect concentrations were calculated based on nominal concentration.

Ph of soils applied was 6.0 (unknown solvent). Organic content (OC) was 10%. The kaolin content was 20%.

Table 7: Literature generally suitable for calculation of Critical Limits for chrome (Cr)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Hu et al. 2016	$K_2Cr_2O_7$	NOEC 50 mg/kg	Shoot mass	[1] *B. rapa* (Brassicaceae)	10% Corg	20% kaolin #	6.0 (uk)

Guidelines: [1] OECD 208. EC = Effect concentration (NOEC, EC10, etc.); OC = organic content; Corg = organic carbon; # artificial soil; uk = unknown. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.4 Copper (Cu)

Regarding chronic effects on terrestric organismens and soil functions originated by copper (Cu), nine references were found. In these studies, 68 natural soils were applied and four different endpoints covering terrestric plants and invertebrates were investigated (see Table 8 and Table 9).

There were six studies covering eight plant species and 51 soils, investigating the standard endpoints shoot mass or root length. Nearly all studies were conducted according to or at least following standard guidelines (OECD or ISO). Two plant studies (Guo et al. 2010 and Liu et al. 2021) were well documented field studies.

There were two studies with Oligochaeta (Lumbricidae and Enchytraeidae) in 16 soils representing invertebrates. All studies investigated effects on reproduction. All studies were conducted according to or at least following standard guidelines (OECD or ISO).

Copper was applied as $CuSO_4$, $CuCl_2$ or $Cu(NO_3)_2$. Effect concentrations were calculated based on nominal concentrations, measured digested or extracted soil concentration. Some studies additionally evaluated the effects based on pore water concentration. These values were submitted by an Excel data compilation. The data is not included in this report. One study regarding effects on terrestric plants calculated effect concentrations only on the basis of measured soil solution concentration (Table 9).

Ph of soils applied was in the range of 3.4 ($CaCl_2$) – 8.9 (H_2O). Organic content (OC) or organic carbon (Corg) content was in the range of 0.37% (Corg) – 23.32% (Corg). The clay content was in the range of 5% - 68%.

Table 8: Literature generally suitable for calculation of Critical Limits for copper (Cu) (I)

Effect concentrations based on soil concentrations (mg/kg)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Engelhardt et al. 2020	$Cu(NO_3)_2$	NOEC 75 mg/kg	Shoot mass	[1] *O. sativa* (Poaceae)	2.11% OC	31% clay	4.9 (H_2O)
Engelhardt et al. 2020	$Cu(NO_3)_2$	EC10 103 mg/kg	Shoot mass	[1] *G. max* (Fabaceae)	3.99% OC	68% clay	4.4 (H_2O)
Guo et al. 2010	$CuCl_2$	EC10 542 mg/kg	Shoot mass	[2] *Z. mays* (Fabaceae)	0.69% Corg	18% clay	8.9 (H_2O)
Guo et al. 2010	$CuCl_2$	EC10 33 mg/kg	Shoot mass	[2] *Z. mays* (Fabaceae)	0.87% Corg	46% clay	5.3 (H_2O)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	2.99% Corg	20% clay	6.32 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	5.50% Corg	7.5% clay	7.31 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	1.48% Corg	20% clay	4.45 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	8.37% Corg	16% clay	5.68 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	3.86% Corg	10% clay	7.15 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	1.76% Corg	6.7% clay	7.73 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	3.49% Corg	36% clay	4.64 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	4.97% Corg	10% clay	4.92 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	3.54% Corg	18% clay	7.66 ($CaCl_2$)
Kader et al. 2016	$Cu(NO_3)_2$	EC10 626 mg/kg	Shoot mass	[2] *C. sativa* (Cucurbitaceae)	1.14% Corg	63% clay	6.19 ($CaCl_2$)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Li et al. 2010	$CuCl_2$	EC10 64 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	1.51% Corg	66% clay	4.93 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 31 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	0.87% Corg	46% clay	5.31 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 175 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	1.47% Corg	25% clay	7.27 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 110 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	1.42% Corg	41% clay	6.70 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 130 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	2.46% Corg	39% clay	6.80 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 133 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	0.99% Corg	27% clay	7.12 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 82 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	0.62% Corg	16% clay	8.83 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 76 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	1.57% Corg	28% clay	8.86 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 151 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	1.02% Corg	20% clay	8.86 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 86 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	0.69% Corg	18% clay	8.90 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 80 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	0.60% Corg	10% clay	8.84 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 84 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	1.01% Corg	21% clay	8.19 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 444 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	4.28% Corg	20% clay	7.48 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 221 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	2.66% Corg	37% clay	7.66 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 137 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	0.87% Corg	25% clay	8.72 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 393 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	2.17% Corg	45% clay	7.82 (H_2O)
Li et al. 2010	$CuCl_2$	EC10 325 mg/kg	Root length	[3] *H. vulgare* (Poaceae)	3.03% Corg	40% clay	6.56 (H_2O)
Liu et al. 2021	$Cu(NO_3)_2$	EC10 240 mg/kg	Shoot mass	[2] *S. oleracea (Amaranthaceae)*	0.95% OC	23% clay	8.35 (H_2O)
Recatala et al. 2012	$CuCl_2$	EC10 8 mg/kg	Shoot mass	[4] *L. sativa (Compositae)*	1.60% OC	25% clay	7.8 (H_2O)
Recatala et al. 2012	$CuCl_2$	EC10 46 mg/kg	Shoot mass	[4] *L. sativa (Compositae)*	3.50% OC	35% clay	8.5 (H_2O)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Recatala et al. 2012	$CuCl_2$	EC10 159 mg/kg	Shoot mass	[4] *L. sativa (Compositae)*	9.70% OC	41% clay	8.2 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 80.7 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.45% Corg	24.3% clay	8.2 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 80.7 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.45% Corg	24.3% clay	8.28 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 105.4 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	1.23% Corg	18.6% clay	8.20 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 269.8 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	3.06% Corg	39.4% clay	7.96 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 215.3 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	1.11% Corg	50.4% clay	6.45 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 60.5 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.78% Corg	15.2% clay	8.48 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 31.8 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.93% Corg	20.4% clay	7.88 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 54.7 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.56% Corg	21.8% clay	7.03 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 75.3 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.89% Corg	25.2% clay	6.56 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 10.0 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.90% Corg	16.0% clay	8.50 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 218.8 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	1.75% Corg	38.4% clay	8.08 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 105.7 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.86% Corg	35.0% clay	8.05 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 54.0 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.37% Corg	53.8% clay	5.76 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 19.1 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	1.17% Corg	49.8% clay	4.66 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 94.0 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	1.37% Corg	50.9% clay	3.95 (H_2O)
Duan et al. 2016	$CuSO_4$	EC10 28.9 mg/kg	Repro	[5] *E. fetida* (Lumbricidae)	0.14% Corg	12.2% clay	6.88 (H_2O)
Santos et al. 2012	$CuCl_2$	EC10 253 mg/kg	Repro	[6*] *E. crypticus* (Enchytraeidae)	1.71% Corg	8.0% clay	5.6 ($CaCl_2$)

Guidelines: [1] ISO 11269-2, [2] Well documented field study, [3] ISO 11269-1, [4] OECD 208, [5] ISO 11268-2, [6] OECD 317 (* modified). EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

Table 9: Literature generally suitable for calculation of Critical Limits for copper (Cu) (II)

Effect concentrations based on soil solution concentrations (mg/L)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Zhao et al. 2006	$CuCl_2$	EC10 0.13 mg/L	Root length	[1] *H. vulgare* (Poaceae)	1.48% Corg	21% clay	7.5 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.034 mg/L	Root length	[1] *H. vulgare* (Poaceae)	1.51% Corg	50% clay	7.5 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.023 mg/L	Root length	[1] *H. vulgare* (Poaceae)	0.38% Corg	25% clay	3.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.026 mg/L	Root length	[1] *H. vulgare* (Poaceae)	1.86% Corg	5% clay	3.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.043 mg/L	Root length	[1] *H. vulgare* (Poaceae)	1.63% Corg	7% clay	4.8 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.11 mg/L	Root length	[1] *H. vulgare* (Poaceae)	2.35% Corg	9% clay	5.1 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.094 mg/L	Root length	[1] *H. vulgare* (Poaceae)	1.27% Corg	26% clay	7.5 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.48 mg/L	Root length	[1] *H. vulgare* (Poaceae)	0.76% Corg	9% clay	5.2 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.48 mg/L	Root length	[1] *H. vulgare* (Poaceae)	5.20% Corg	13% clay	3.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.34 mg/L	Root length	[1] *H. vulgare* (Poaceae)	12.94% Corg	13% clay	4.2 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.066 mg/L	Root length	[1] *H. vulgare* (Poaceae)	1.26% Corg	27% clay	7.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.002 mg/L	Root length	[1] *H. vulgare* (Poaceae)	0.41% Corg	38% clay	4.8 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.058 mg/L	Root length	[1] *H. vulgare* (Poaceae)	2.61% Corg	46% clay	7.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.2 mg/L	Root length	[1] *H. vulgare* (Poaceae)	0.98% Corg	15% clay	6.8 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.002 mg/L	Root length	[1] *H. vulgare* (Poaceae)	1.47% Corg	38% clay	7.3 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.24 mg/L	Root length	[1] *H. vulgare* (Poaceae)	4.40% Corg	21% clay	6.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.24 mg/L	Root length	[1] *H. vulgare* (Poaceae)	23.32% Corg	24% clay	4.7 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.08 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	0.87% Corg	51% clay	5.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.14 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.48% Corg	21% clay	7.5 ($CaCl_2$)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Zhao et al. 2006	$CuCl_2$	EC10 0.03 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	0.38% Corg	25% clay	3.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.17 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.86% Corg	5% clay	3.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.06 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.63% Corg	7% clay	4.8 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 1.20 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	2.35% Corg	9% clay	5.1 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.11 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.27% Corg	26% clay	7.5 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.52 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	0.76% Corg	9% clay	5.2 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.19 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	5.20% Corg	13% clay	3.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.43 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	12.94% Corg	13% clay	4.2 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.16 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	1.26% Corg	27% clay	7.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.001 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	0.41% Corg	38% clay	4.8 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.07 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	2.61% Corg	46% clay	7.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.22 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	4.40% Corg	21% clay	6.4 ($CaCl_2$)
Zhao et al. 2006	$CuCl_2$	EC10 0.28 mg/L	Shoot mass	[1] *L. esculentum* (Solanaceae)	23.32% Corg	24% clay	4.7 ($CaCl_2$)

Guidelines: [1] ISO 11269-2. EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.5 Lanthanum (La)

Regarding chronic effects on terrestric organismens and soil functions originated by lanthanum (La), one reference was found. In this study, one natural soil was applied and the standard endpoint reproduction of four invertebrate spiecies was investigated (see Table 10). The study was conducted according to ISO and OECD guidelines. An additional non-standard test with a non-standard invertebrate (isopods) was conducted in this study, looking for weight gain as the endpoint. The test was not following a standard guideline, but was well documented.

Lanthanum was applied as $La(NO_3)_3$. Effect concentrations were calculated based on measured digeted soil concentration. The studys additionally evaluated the effects based on measured extractable soil concentration. These values were submitted by an Excel data compilation. The data is not included in this report.

Ph of soils applied was 5.5 ($CaCl_2$). Organic carbon content (Corg) was 2.3%. The clay content was 11%.

Table 10: Literature generally suitable for calculation of Critical Limits for lanthanum (La)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Li et al. 2018	$La(NO_3)_3$	EC10 350 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	2.3% Corg	11% clay	5.5 ($CaCl_2$)
Li et al. 2018	$La(NO_3)_3$	EC10 870 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	2.3% Corg	11% clay	5.5 ($CaCl_2$)
Li et al. 2018	$La(NO_3)_3$	EC10 1120 mg/kg	Repro	[3] *F. candida* (Collembola)	2.3% Corg	11% clay	5.5 ($CaCl_2$)
Li et al. 2018	$La(NO_3)_3$	EC10 1000 mg/kg	Repro	[4] *O. nitens* (Oribatida)	2.3% Corg	11% clay	5.5 ($CaCl_2$)
Li et al. 2018	$La(NO_3)_3$	EC10 69 mg/kg	Weight gain	[4] *P. scaber (Isopoda)*	2.3% Corg	11% clay	5.5 ($CaCl_2$)

Guidelines: [1] OECD 222, [2] OECD 220, [3] OECD 232, [4] Well documented method. EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.6 Molybdenum (Mo)

Regarding chronic effects on terrestric organismens and soil functions originated by molybdenum (Mo), one reference was found. In this study, ten natural soils were applied and the standard endpoint reproduction of three invertebrates (Oligochaeta: Lumbricidae and Enchytraeidae; Collembola) was investigated (see Table 11). The study was conducted according to OECD and ISO guidelines.

Molybdenum was applied as Na_2MoO_4. Effect concentrations were calculated based on measured digested soil concentration.

Ph of soils applied was in the range of 4.4 ($CaCl_2$) – 7.8 ($CaCl_2$). Organic carbon content (Corg) was in the range of 0.6% – 30.7%. The clay content was in the range of 2% - 33%.

Table 11: Literature generally suitable for calculation of Critical Limits for molybdenum (Mo)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
van Gestel et al. 2011	Na_2MoO_4	NOEC 993 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	30.7% Corg	20% clay	4.4 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 79.7 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	2.0% Corg	3% clay	5.0 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 78.1 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	2.8% Corg	2% clay	5.2 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 89.1 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	1.8% Corg	2% clay	5.2 ($CaCl_2$)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
van Gestel et al. 2011	Na_2MoO_4	NOEC 799 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	3.6% Corg	27% clay	6.3 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 8.88 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	0.9% Corg	12% clay	6.7 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 917 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	0.6% Corg	33% clay	6.8 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 83 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	2.8% Corg	10% clay	7.3 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 78.9 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	2.7% Corg	18% clay	7.6 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 24.8 mg/kg	Repro	[1] *E. andrei* (Lumbricidae)	3.6% Corg	11% clay	7.8 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2721 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	30.7% Corg	20% clay	4.4 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 239 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	2.0% Corg	3% clay	5.0 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 78.1 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	2.8% Corg	2% clay	5.2 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 277 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	1.8% Corg	2% clay	5.2 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 255 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	3.6% Corg	27% clay	6.3 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 87.6 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	0.9% Corg	12% clay	6.7 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >763 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	0.6% Corg	33% clay	6.8 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 742 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	2.8% Corg	10% clay	7.3 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2817 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	2.7% Corg	18% clay	7.6 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >867 mg/kg	Repro	[2] *E. crypticus* (Enchytraeidae)	3.6% Corg	11% clay	7.8 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2721 mg/kg	Repro	[3] *F. candida* (Collembola)	30.7% Corg	20% clay	4.4 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 25.8 mg/kg	Repro	[3] *F. candida* (Collembola)	2.0% Corg	3% clay	5.0 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 836 mg/kg	Repro	[3] *F. candida* (Collembola)	2.8% Corg	2% clay	5.2 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	NOEC 277 mg/kg	Repro	[3] *F. candida* (Collembola)	1.8% Corg	2% clay	5.2 ($CaCl_2$)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
van Gestel et al. 2011	Na_2MoO_4	EC10 >3396 mg/kg	Repro	[3] *F. candida* (Collembola)	3.6% Corg	27% clay	6.3 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2896 mg/kg	Repro	[3] *F. candida* (Collembola)	0.9% Corg	12% clay	6.7 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2744 mg/kg	Repro	[3] *F. candida* (Collembola)	0.6% Corg	33% clay	6.8 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2844 mg/kg	Repro	[3] *F. candida* (Collembola)	2.8% Corg	10% clay	7.3 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2817 mg/kg	Repro	[3] *F. candida* (Collembola)	2.7% Corg	18% clay	7.6 ($CaCl_2$)
van Gestel et al. 2011	Na_2MoO_4	EC10 >2821 mg/kg	Repro	[3] *F. candida* (Collembola)	3.6% Corg	11% clay	7.8 ($CaCl_2$)

Guidelines: [1] OECD 222, [2] OECD 220, [3] ISO 11267. EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.7 Nickel (Ni)

Regarding chronic effects on terrestric organismens and soil functions originated by nickel (Ni), three references were found. In these studies, four soils (three natural soils and one artificial soil) were applied and two different endpoints covering terrestric plants and invertebrates were investigated (see Table 12).

There were two studies covering two plant species and three soils, investigating the standard endpoint shoot mass. One study was conducted according to the OECD 208 guideline. One study was not following a standard guideline. However, it was a well documented field study (Guo et al. 2010).

There was one study with Oligochaeta (Enchytraeidae) in one soil representing invertebrates. The study investigated effects on reproduction. The study was conducted according to the OECD 220 guideline).

Nickel was applied as $NiCl_2$ and NiO nano materials. Effect concentrations were calculated based on nominal concentration.

Ph of soils applied was in the range of 5.3 (H_2O) – 8.9 (H_2O). Organic carbon content (Corg) was in the range of 0.69% – 5.0%. The clay content was in the range of 6% - 46%.

Table 12: Literature generally suitable for calculation of Critical Limits for nickel (Ni)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Guo et al. 2010	$NiCl_2$	EC10 372 mg/kg	Shoot mass	[1] *Z. mays* (Poaceae)	0.69% Corg	18% clay	8.9 (H_2O)
Guo et al. 2010	$NiCl_2$	EC10 26 mg/kg	Shoot mass	[1] *Z. mays* (Poaceae)	0.87% Corg	46% clay	5.3 (H_2O)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Soares et al. 2016	NiO NM	EC10 13.8 mg/kg	Shoot mass	[2] *H. vulgare* (Poaceae)	5.0% Corg	20% kaolin #	5.5 (uk)
Gomes et al. 2014	$NiCl_2$	EC10 12 mg/kg	Repro	[3] *E. albidus* (Enchytraeidae)	2.3% Corg	6% clay	5.5 ($CaCl_2$)

Guidelines: [1] Well documented field study, [2] OECD 208, [3] OECD 220. NM = Nanomaterial; EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon; # artificial soil; uk = unknown. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.8 Uranium (U)

Regarding chronic effects on terrestric organismens and soil functions originated by uranium (U), one reference was found. In this study, one natural soil was applied and four different endpoints covering terrestric plants, invertebrates and soil micro-organisms were investigated (see Table 13).

The test with terrestrial plants covered one plant species, investigating the standard endpoint shoot mass. The test was conducted according to the ISO 11269-2 guideline.

There were two tests with Oligochaeta (Lumbricidae and Enchytraeidae) and one test with Collembola representing invertebrates. The tests investigated effects on reproduction and were conducted according to the ISO 11268-2, ISO 16387and ISO 11267 guidelines. The effect of uranium on soil micro-organisms was investigated in a non-standard test. Endpoint was enzyme activity.

Uranium was applied as $UO_2(NO_3)_2$. Effect concentrations were calculated based on nominal concentration. Ph of soils applied was 4.3 (KCl). Organic content (OC) was 6.5%. The clay content was 3.3%.

Table 13: Literature generally suitable for calculation of Critical Limits for uranium (U)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Caetano et al. 2014	$UO_2(NO_3)_2$	NOEC <167 mg/kg	Shoot mass	[1] *L. sativa* (Compositae)	6.5% OC	3.3% clay	4.3 (KCl)
Caetano et al. 2014	$UO_2(NO_3)_2$	NOEC 500 mg/kg	Repro	[2] *E. andrei* (Lumbricidae)	6.5% OC	3.3% clay	4.3 (KCl)
Caetano et al. 2014	$UO_2(NO_3)_2$	NOEC 421 mg/kg	Repro	[3] *E. crypticus* (Enchytraeidae)	6.5% OC	3.3% clay	4.3 (KCl)
Caetano et al. 2014	$UO_2(NO_3)_2$	NOEC 676 mg/kg	Repro	[4] *F. candida* (Collembola)	6.5% OC	3.3% clay	4.3 (KCl)
Caetano et al. 2014	$UO_2(NO_3)_2$	EC20 34.9 mg/kg	Enzyme activity	Soil micro-organisms	6.5% OC	3.3% clay	4.3 (KCl)

Guidelines: [1] ISO 11269-2, [2] ISO 11268-2, [3] ISO 16387, [4] ISO 11267. NM = Nanomaterial; EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.9 Wolfram (Wo)

Regarding chronic effects on terrestric organismens and soil functions originated by wolfram/tungsten (Wo), one reference was found. In this study, two soils (one natural soil and one artificial soil) were applied and and two different endpoints covering terrestric plants and invertebrates were investigated (see Table 14).

The test with terrestrial plants covered two plant species, investigating the standard endpoint shoot mass. The test was conducted according to the OECD 208 guideline.

There was one test with Oligochaeta (Lumbricidae) representing invertebrates. The test investigated effects on reproduction and was conducted according to the OECD 222 guideline.

Wolfram/tungsten was applied as Na_2Wo_4. Effect concentrations were calculated based on Measured concentration in spiking solutions (total soil concentration).

Ph of soils applied was in the range of 6.5 (unknown solvent) – 7.8 (unknown solvent). Organic content (OC) was in the range of 0.6% – 11%. The clay or kaolin content was in the range of 0.6% (clay) - 20% (kaolin).

Table 14: Literature generally suitable for calculation of Critical Limits for wolfram (Wo)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Bamford et al. 2011	Na_2Wo_4	NOEC 21.7 mg/kg	Shoot mass	[1] *L. sativa* (Compositae)	0.6% OC	8% clay	7.8 (uk)
Bamford et al. 2011	Na_2Wo_4	NOEC 65 mg/kg	Shoot mass	[1] *R. sativus* (Cruciferae)	0.6% OC	8% clay	7.8 (uk)
Bamford et al. 2011	Na_2Wo_4	NOEC ≥586 mg/kg	Repro	[2] *E. fetida* (Lumbricidae)	11% OC	20% kaolin #	6.5 (uk)

Guidelines: [1] OECD 208, [2] OECD 222; EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon; # artificial soil; uk = unknown. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

3.3.10 Zinc (Zn)

Regarding chronic effects on terrestric organismens and soil functions originated by zinic (Zn), four references were found. In these studies, 19 natural soils were applied and and three different endpoints covering terrestric plants and invertebrates were investigated (see Table 15).

There was one study with terrestrial plants covering one species and 16 soils, investigating the standard endpoint root length. The test was conducted according to the ISO 11269-1 guideline.

There were two studies with Oligochaeta (Lumbricidae and Enchytraeidae) and one study with Collembola representing invertebrates. The studies investigated effects on reproduction and were conducted following OECD and ISO guidelines.

Zinc was applied as $ZnCl_2$, ZnO and ZnO nano material. Effect concentrations were calculated based on nominal concentration or measured digested soil concentration.

Ph of soils applied was in the range of 4.93 (H_2O) – 8.90 (H_2O). Organic content (OC) or organic carbon content (Corg) was in the range of 0.60% (Corg) – 4.28% (Corg). The clay content was in the range of 6% - 66%.

Table 15: Literature generally suitable for calculation of Critical Limits for zinc (Zn)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Quin et al. 2021	$ZnCl_2$	EC10 18.5 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.51% Corg	66% clay	4.93 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 69 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.87% Corg	46% clay	5.31 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 391.6 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	3.03% Corg	40% clay	6.56 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 962.9 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.42% Corg	39% clay	6.70 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 42.9 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	2.46% Corg	41% clay	6.80 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 481.1 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.99% Corg	27% clay	7.12 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 406.6 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.47% Corg	25% clay	7.27 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 235.7 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	4.28% Corg	20% clay	7.48 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 267.2 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	2.66% Corg	37% clay	7.66 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 503.2 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	2.17% Corg	45% clay	7.82 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 70.7 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.00% Corg	10% clay	8.19 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 1619 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.62% Corg	16% clay	8.83 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 335.9 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.60% Corg	21% clay	8.84 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 51.5 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.57% Corg	28% clay	8.86 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 1515 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	1.02% Corg	20% clay	8.86 (H_2O)
Quin et al. 2021	$ZnCl_2$	EC10 146.3 mg/kg	Root length	[1] *H. vulgare* (Poaceae)	0.69% Corg	18% clay	8.90 (H_2O)
Fernández et al. 2021	ZnO-NM	NOEC 20 mg/kg	Repro	[2] *E. andrei* (Lumbricidae)	1.69% OC	18% clay	5.4 (H_2O)
Novais et al. 2011	$ZnCl_2$	NOEC 18.0 mg/kg	Repro	[3] *E. albidus* (Enchytraeidae)	4.40% OC	6% clay	5.5 ($CaCl_2$)
Kool et al. 2011	ZnO-NM	EC10 1678 mg/kg	Repro	[4] *F. candida* (Collembola)	2.09% Corg	11% clay	5.5 ($CaCl_2$)

Reference	Form	EC	Endpoint	Species	OC / Corg	Clay / Kaolin	pH (solvent)
Kool et al. 2011	ZnO	EC10 1383 mg/kg	Repro	[4] *F. candida* (Collembola)	2.09% Corg	11% clay	5.5 ($CaCl_2$)
Kool et al. 2011	$ZnCl_2$	EC10 108 mg/kg	Repro	[4] *F. candida* (Collembola)	2.09% Corg	11% clay	5.5 ($CaCl_2$)

Guidelines: [1] ISO 11269-1, [2] Following ISO 11268-2, [3] OECD 220, [4] ISO 11267; NM = Nanomaterial; EC = Effect concentration (NOEC, EC10, etc.); Repro = reproduction; OC = organic content; Corg = organic carbon. Further information like organism group, soil texture and/or type and WHCmax (if available), metal concentration based on nominal or measured values and extraction method and metal analysis if applied, were submitted to UBA by an Excel data compilation. Data not included in this report.

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