

Topical Scientific Workshop on Soil Risk Assessment Helsinki, 7 – 8 October, 2015

Posters





Topic 1: Problem definition and conceptual model for soil risk assessment

Poster 1

A Comparison of Functional and Structural Soil Testing for Risk Assessment of Plant Protection Products

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Keywords: Protection goal, functional test, structural test, risk assessment

For registration of plant protection products in the EU, effects on non-target soil organisms have to be investigated. Next to earthworms soil non-target meso- and macrofauna have been proposed and required to be tested. Test systems comprised functional parameters/tests (i.e. organic matter breakdown) and structural/species-specific tests (e.g. with Collembola and soil mite species). More recently structural testing was given a preference while testing of individual surrogate species is missing a direct link to the proposed protection goals derived from the Ecosystem Services (EsS) concept. Instead, functional testing, like the measurement of organic matter breakdown can be directly linked to relevant ecosystems services, like nutrient cycling.

In the development process of a new active substance of a plant protection product (PPP), an insecticide, functional tests and structural tests were performed, which allows a comprehensive assessment of effects on functional and structural soil parameters. In the functional field study - a test on the breakdown of organic matter following OECD GD 56 (2006) and EPFES WS recommendations (2002) – slight transient effects due to the insecticide were found on mass loss of organic matter at 1 month after treatment, while at 3 and 6 months after treatment no significant impact on organic matter decomposition was found. The potential effects of the test substance on structural parameter (soil mites, and collembolans for which a potential risk was identified based on worst-case laboratory testing) were addressed via additional soil samplings and organisms extraction on the same plots of the organic matter breakdown study. Samplings performed at 3 and 5 months after treatment indicated no significant effects on the structural parameters (soil mites and collembolans). In another field study including higher tested insecticide soil concentrations - with focus on the structure of Collembola populations showed some initial transient effects, but also no longer term impact.

On the basis of these results it can be concluded that the functional field test on organic matter breakdown is sensitive and covers the effects on soil mites and Collembola.

Sensitivity and initial effects in field tests on organic matter breakdown were also reported in a previous industry-wide ECPA review of such data (Dinter et al 2008). It is concluded that functional test system on organic matter breakdown is sensitive and covering potential effects on soil non-target meso- und macrofauna and considered – due to the direct link to protection goals derived from the EsS concept (plus the robustness of the test conduct itself) a superior testing tool for soil risk assessment of plant protection products.

Poster 2

Biodiversity and structural diversity in the agricultural landscape – An overall concept relevant for soil risk assessment?

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Keywords: Agricultural landscape, biodiversity, Leitbild, pesticide risk assessment

Biodiversity is the basic resource maintaining and supporting ecosystem services and functions. Biodiversity is realized at different levels in the complexity of a given landscape. Therefore it is necessary to analyse and classify the distribution of different organism groups, in order to elucidate the interrelationship between diversity and structural elements in the landscape.

It is widely accepted, however controversially discussed, that species diversity and habitats quality dramatically decrease with increasing intensity of agricultural land-use.

An approach is presented, that considers landscape structure by classifying landscape elements according to their biodiversity of various arthropod groups. We propose to apply an 'ecological value', that reflects the habitat quality and depends on the spatial distribution of typical elements of terrestrial agricultural landscapes. 50 types of ecologically relevant landscape elements (LE-types), exemplified by two regions in Germany, were defined, which were automatically derived from remote sensing data or land utilisation data. The methodology is considered well established but needs calibration by data from different landscapes all over Europe.

In this way, the impact of risk mitigation measures in the context of pesticide registration to increase the ecological value of a landscape can be simulated and evaluated.

In order to address the potential for biodiversity for agricultural landscapes, it is necessary to define a corresponding landscape typical "Leitbild" (overall concept).

Iterative adaptive monitoring to link the gaps in current risk assessment

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Keywords: Biodiversity, monitoring, risk assessment, reference values, soil organisms

The target of risk assessment (RA) is to protect biodiversity as basic resource to maintain soil health i.e. maintain ecological services and functions. The risks for soil organisms arising from an application of PPP depend – besides the concentration of the chemical in the soil matrix - on the spatial and temporal distribution of the animals in the soil profile (i.e. their exposure) as well as their specific sensitivity to the chemical. This multidimensional relationship between exposure and effects as well as the specific effect patterns of pesticide mixtures are not yet sufficiently considered in soil RA. In a research project in Terrestrial Model Ecosystems (TME) funded by the Federal Environmental Agency (Germany) effects on the soil community were assessed. The results indicated that the current approach underestimates the effects on soil communities. This is due to the fact that most of the soil species occur in close relation to the soil surface and due to their behaviour i.e. the vertical movement of species. This research in TME under real life conditions, i.e. natural species composition considering the natural competition between species, helps us to encrypt the main drivers in natural system that are not known so far.

There are a lot of appropriate approaches and possibilities to measure the impact of PPP but one of the main gaps is to link emerging results of the different scales, laboratory, semi-field, field with the reality i.e. what happens in the landscape. Furthermore, to assess the risk of PPP on the soil community a threshold for an unacceptable change in the community must be defined beforehand. References (good ecological values) for the different habitat types in the arable landscape must be developed, which can only be derived from field data that are not yet sufficiently evaluated and available.

We propose how to connect prospective RA with retrospective RA for an iterative adaptable monitoring. It is necessary to have both to control and adapt the effect level on laboratory scale with the one in the communities at the field. Furthermore, we will present how to connect the different scales laboratory, semi-field and field. This is necessary to derive meaningful indicator species and test communities and it promote basic knowledge, necessary to develop models of the soil system leading to a better understanding. Models will help to see more of the whole picture of complex effect patterns in arable landscapes. Using these tools the limits of pesticide use could be defined on a landscape level and this will give the opportunity to sustainably manage the soil ecosystem.

Soil risk assessment for glyphosate and AMPA

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Keywords: Glyphosate; AMPA; earthworm; *Folsomia*; *Hypoaspis*; nitrogen transformation

The herbicidal active substance glyphosate is a broad-spectrum systemic herbicide used widely in agriculture, horticulture, private gardens and in public infrastructure, where it is applied to areas such as road sides, railway tracks and parks to control the growth of weeds. The exposure risk from glyphosate and the primary soil metabolite aminomethylphosphonic acid (AMPA) on representative species of soil structuring earthworms (Eisenia fetida), re-cycling springtails (Folsomia candida), beneficial predatory soil mites (Hypoaspis aculeifer) and effects on nitrogen-transformation processes by nutrient cycling soil micro-organisms was assessed under controlled laboratory conditions based on internationally recognized testing guidelines (OECD).

For earthworms, the reproductive no observed effect concentration (NOEC) was > 473 mg acid equivalent (a.e.)/kg dry soil for glyphosate, and 198.1 mg/kg dry soil for AMPA. For predatory mites, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 320 mg/kg dry soil for AMPA. For springtails, the reproductive NOEC was 587 mg a.e./kg dry soil for glyphosate and 315 mg/kg dry soil for AMPA. Soil nitrogen transformation processes were unaffected by glyphosate and AMPA at 33.1 mg a.e./kg soil and 160 mg/kg soil, respectively.

Comparison of the achieved soil organism endpoints with worst-case soil concentrations (Max PECsoil) expected for glyphosate (6.62 mg a.e./kg dry wt soil) and AMPA (6.18 mg a.e./kg dry wt soil) for a bare soil application of a glyphosate containing formulation, indicate that the risk to soil invertebrates and microorganisms from glyphosate and AMPA is acceptable with exposure safety margins of between 5 and 88.

Making soil protection goals based on the ecosystem services concept operational in ecotoxicological risk assessments

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Keywords: protection goals, structural tests, functional tests, landscape

Abstract:

What to protect, where to protect and when to protect are fundamental questions that need to be addressed before developing risk assessment schemes. The millennium ecosystem assessment initiative and the therein specified ecosystem services (EsS) concept can give guidance for risk assessment schemes. Here we use the EsS concept to propose a novel and science based soil risk assessment for PPPs.

As a first step we define registration principles followed by identification of soil relevant EsS. Thereafter, we formulate relevant protection goals which are transferred into test systems. Finally, a tiered risk assessment based on acceptability criteria will be defined.

The registration principles we identified were that risk assessments should be workable, scientifically sound, socially viable and politically acceptable. A well-structured risk assessment should follow a tiered approach, reduce uncertainties and data gaps concerning organisms potentially at risk. A risk assessment scheme should use validated test systems only, needs to have conservative trigger values (see Poster Mike Coulson *et al.*), and clear acceptability criteria.

Soil relevant EsS (e.g. food production & nutrient cycling) can be affected by use of PPPs. While it is relatively easy to define general protection goals, it can be quite challenging to define specific protection goals. In the soil area the current focus is on structural protection goals (testing of surrogate species) and lacks a clear link to protection goals derived from EsS. On the other hand functional protection goals can be directly connected to EsS. Thus suitable test systems based on functional parameters will reduce uncertainties with regard to EsS. Although several functional tests are already available a critical evaluation is needed to select representative and sensitive test systems. Additionally, these novel functional tests need to be linked to information obtained in structural tests for a comprehensive risk assessment. The EsS and related protection goals might be weighted differently on a spatial scale in agricultural landscapes: for in-crop areas functional aspects are in focus (e.g. food production & nutrient cycling) whereas in off-crop areas structural aspects are additionally of interest.

EsS and the protection goals derived from them are suitable to design a new soil risk assessment scheme. Several EsS are relevant to soil and can be affected by PPPs. Ongoing research will help to define a workable risk assessment scheme for soil that combines structural and functional endpoints. (see Poster Gregor Ernst *et al.*). We are of the opinion that the integration of functional tests can substantially improve the soil risk assessment. Potential effects on EsS should be addressed by validated and suitable

functional test systems as these are directly linked to protection goals and thus fulfil registration principles, which is in contrast to structural test systems with single species.

Poster 6

Innovative Biofumigation Technologies for Soil risk Assessment

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Keywords: Biofumigation, Soil Management, Soil risk Assessment, Organic Agriculture, Methyl Bromide alternatives

We adopted new innovative technologies to develop sustainable soil-borne disease management tactics suitable for use in organic or Global GAP vegetable and fruit production systems. We aimed to develop tactics that build healthy soils and promote microbial ecosystems that challenge these potentially devastating, broad spectrum pathogens. We adapted three innovative approaches of synergistic biofumigation (Syn-Biofum) to control soil-borne diseases, root-knot nematode and aggressive weeds. Our approaches are synergistic physical-biofumigation, synergistic biocontrol-biofumigation, and synergistic chemical-biofumigation. These technologies can efficiently suppress soil-borne diseases, plant parasitic nematodes, and aggressive weeds instead of Methyl Bromide (MB). These new technologies are eco-friendly, safe, cheap and can work under organic agricultural and global gap systems worldwide. We are working by these technologies here in Egypt and we developed 2 Eco-friendly biofumigated compost companies that produce more than 360,000 ton/year. This is a unique technology and we are working across Egypt in Organic and global gap agricultural systems.

Making use of publicly available studies within the REACH Regulation: An overview of submitted terrestrial toxicity data

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Keywords: publicly available studies, REACH information requirements, terrestrial toxicity

Testing on animals to fulfil REACH information requirements should only be undertaken as a last resort – when there is no other scientifically reliable way to examine the impact of chemicals.

Registrants use a number of alternative methods to generate information on the hazards of chemicals, such as predicting substance properties by read-across, combining information together from different sources (weight of evidence), or by using different modelling approaches. Additionally, registrants can fulfil REACH requirements by submitting information already available from different sources (scientific journals, handbooks, published reports).

This poster provides an overview of this type of information based on the registration dossiers submitted to ECHA from 1 June 2008 to 1 August 2015, covering the first two registration deadlines.

The results of the conducted analysis for terrestrial toxicity endpoints indicate the following:

- For studies submitted both within the read-across approach and as experimental data, publicly available studies account for more than 60 % of endpoint study records in the REACH database for all three terrestrial trophic levels.
- In the majority of cases, registrants report studies from publicly available sources as key studies, meaning that they consider them as being able to fulfil a REACH information requirement on their own.
- The majority of publicly available studies are being classified as reliable studies by the registrants.
- The majority of publicly available studies submitted within the REACH framework consists of publications from scientific journals.
- In most cases, the same publication was submitted multiple times for different substances, dossiers, or endpoint study records.

According to the REACH Regulation, ECHA shall check the compliance of at least 5 % of registration dossiers received by the Agency for each tonnage band, and may request further studies if considered necessary. Therefore, when interpreting the findings of the current analysis it should be noted that in principle the current results may be affected by the outcome of the dossier evaluation works.

How can soil risk assessment data be used in ecosystem services analysis for agrochemicals regulation?

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Keywords: Soil, risk assessment, ecosystem services

Many European policies and regulations, including those relating to agrochemicals, attempt to incorporate ecosystem services (ES) concepts when defining protection goals. In the case of agrochemicals (pesticides), protection goals are being defined in relation to the ES "represented" by the testing plans constituting regulatory dossiers. The related risk assessment process includes a thorough evaluation of the risks to the environment designed to provide a high level of protection of all these services. The process however discusses risks in disconnection to their conditions and likelihood of occurrence, nor to the context and consequences of potential decisions made based on the risk assessment. As a consequence policy decisions that rely on these risk assessments are unlikely to have considered the risks and benefits of the product in a comprehensive way, nor of alternative pest control strategies including land use changes, which can in turn lead to unintended consequences for soil, water, wildlife and local communities. Approaches involving holistic ES analysis would in this context allow to inform policy makers on all the aspects at stake in decision making and help implementing a sustainable use of pesticides (and other crop protection methods).

In order for decision makers to be able to consider such approaches for chemical regulation, the data provided by a risk assessment needs to relate to functional ecosystem services-based protection goals. For this aim, the outcome of a risk assessment should first be discussed in terms of expected consequences on the ES in the crops concerned. Then changes to services such as the maintenance of soil fertility, waste regulation, soil erosion prevention and a habitat for soil organisms can be compared with potential changes to other ecosystem services provided by a habitat in order to decide whether the use of a pesticide provides a net environmental benefit or whether some services need to be compensated or losses accepted (a trade-off).

Through a series of case studies, the authors have developed and evolved a framework that embeds the ecosystem services approach into a tool that puts potential environmental risks and benefits into context by comparing these with the cultures and traditions of farming and economic prosperity. The poster will focus on how soil risk assessment data has been used in ES analyses, including assumptions and data extrapolations that have been needed in order to interpret traditional risk data in terms of ecosystem services. Case studies have also included assessments of non-chemical products for comparison.

Topic 2: Environmental exposure assessment and in-soil fate processes

Poster 9

Environmental risk assessment of agriculture soils towards food safety and food security requirements

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Keywords: Agriculture, soil, food security, food safety

The organic carbon (OC) content of agriculture soils is acknowledged as a key factor to guarantee the food security. Biosolids from civil wastewater treatment plants (WWTPs) have been proposed as a direct OC source, and may enter also in the composition of other top soil improvers (TSI) such as digestates and mixed composts. The proposed use of biosolids-derived TSI with a OC content up to the 30% on dry matter basis can reach 35 ton/ha/year. Therefore, the driving force consists on the regular use of WWTPsanyway, such use may determine a pressure from bioaccumulative derived TSI; persistent organic pollutants, that concentrate in biosolids due to their high KOC. Such pressure may exitate in a compromised top soil quality of impact on food producing animal welfare and health (i.e. via endocrine disruption of thyroid gland and of the reproductive system), on the compliance with regulatory limits of bio-accumulative contaminants in food, and on human health (toxicological guidance values for chronic intakes trespassed when local food is consumed). The direct intake of soils, of related earthworms, and of soil-contaminated grass and forages from extensively reared flocks and herds represent the main pathway, with an impact both on food security (reduction of the quality/quantity of animal products) and on feed&food safety. The response to such biosolids-driven pressure could rely on a soil risk assessment focused on the georeferenced agronomic use of OC sources, on the related food production systems, and on the food consumption habits of target populations. The POPs burden associated to OC inputs in soil, its animals intake, and the POPs carry-over to food items are the data necessary to elaborate a response based on the definition of agriculture soil environmental quality standards (EQS). Such EQS are the starting point to draft the endof-waste criteria for the safe use of bio-wastes in agriculture. An example of such perfluorochemicals, brominated flame assessment for retardands, and polychlorobiphenyls associated to biosolids, along with the related uncertainties, is given for dairy production the Mediterranean Region. The potential extension of such an assessment to those pharmaceuticals that show a strong binding with the OC of top soils, along with the opportunities represented by soil remediation from the use lowcontaminated biowastes are discussed.

Towards a unified approach for the determination of the bioaccessibility of organic pollutants

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Keywords: Risk assessment, bioacessibility, unified format, organic pollutants, validation studies

Bioaccessibility studies have been widely used as a research tool to determine the potential human exposure to ingested contaminants. More recently they have been practically applied for soil borne toxic elements. This paper reviews the application of bioaccessibility tests across a range of organic pollutants and contaminated matrices. Important factors are reported to be: the physiological relevance of the test, the components in the gut media, the size fraction chosen for the test and whether it contains a sorptive sink. The bioaccessibility is also a function of the composition of the matrix (e.g. organic carbon content of soils) and the physico-chemical characteristics of the pollutant under test. Despite the widespread use of these tests, there are a large number of formats used and very few validation studies with animal models. We propose a unified format for a bioaccessibility test for organic pollutants. The robustness of this test should first be confirmed through inter laboratory comparison, then tested in-vivo.

Poster 11

French Regulatory Feedback on new EFSA guidance for predicting concentration of active substances of plant protection products in soil: Impact on the risk assessment

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Keywords: Active substance, soil; PEC, PERSAM

The draft Guidance Document on "Predicting environmental concentrations of active substances of plant protection products and their transformation products in soil" was released by EFSA in July 2014 for public consultation⁽¹⁾. This document provides procedures for calculating concentrations that are potentially needed for terrestrial risk assessment. The current draft guidance is devoted to the spray applications on annual crops under conventional and reduced tillage (excluding tillage systems with ridges and furrow). The guidance document proposes a tiered approach (from Tier 1 to Tier 5) including both analytical and numerical models. To achieve PECsoil calculations the PERSAM tool was also made available in addition to the FOCUS tools already available at the European level.

This new guidance on PECsoil calculations aims at producing a more robust risk assessment for soil organisms consistent with the assessment performed for groundwater and surface water compartments.

During the commenting period, issues regarding the results from the PERSAM tool as proposed with the draft guidance and the impact of the tiered approach as proposed in this guidance document on the risk assessment were raised.

Anses proposes some feedbacks on the use of this draft guidance on the foreseen consequences on terrestrial risk assessment at zonal level. It includes worked examples to allow discussion on the foreseen impact when using this new guidance.

¹ European Food Safety Authority, Draft EFSA Guidance Document for predicting environmental concentrations of active substances of plant protection products and transformation products of these active substances in soil. 20YY. EFSA Journal 2014; Draft document

Poster 12

Comparing PRZM, PEARL and MACRO using field data from a case study of pesticide leaching in Norway

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Keywords: Leaching, metribuzin, cold climate, modelling, PRZM, PEARL, MACRO.

A data set from a Norwegian site monitored by Bioforsk was used to compare the three leaching models PRZM, PEARL and MACRO. Data from two experimental periodes on soil temperature, water contents, bromide and metributzin in a silt loam soil (0-80cm), was used. The three models simulated the bromide concentration quit well for both experimentals periods, although MACRO and PRZM overestimated the bromide data by an factor of 1.7. The particular cold climate conditions of Norway were poorly simulated (temperature and water content). PRZM simulation of pesticide transfer differed largely from MACRO and PEARL.

Degradation, persistence and exposure of pesticides in soil under different environmental scenarios - simulation and measurements

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Keywords: Degradation, persistence, soil, exposure assessment, scenarios, simulation, measurements, temperature, moisture

For chemicals released into the terrestrial environment soil is a filter, buffer and transformation system with physical, chemical and biotic constituents. It is influenced by and in interaction with variable environmental boundary conditions (i.e. weather). Key elements for fate and behavior of chemicals in soil are the properties of the soil matrix which vary in space (vertical and horizontal dimension) and in time (daily, seasonal; temperature and moisture), the intrinsic physico-chemical characteristics of the chemicals and the transformation and transport processes. Processes relevant for exposure and fate are degradation (biotic, photolytic, hydrolytic etc.), and disappearance pathways (e.g. volatilization, leaching). The basic concepts for description and modelling of the temporal variation of pesticide degradation in response to fluctuating soil temperature and moisture have been outlined already in the early 1970s e.g. by Walker (1974). They have been tested and verified against laboratory and field conditions and have been used since then for research as well as for regulatory purposes. Modelling allows the scaling and extrapolation of information gathered under specific circumstances to a multitude of conditions. For the extrapolation step from the incubation conditions to the environmental conditions following aspects have to be considered:

a) the degradation has to be determined under conditions that are environmentally relevant; i.e. the incubation system should represent a "snapshot" of the possible environmental conditions and should, considering and minimizing as far as possible the limitations of the test system such as maintenance of the biological activity b) a statistically sound kinetic analysis of the data is essential before extrapolation of the incubation conditions to other environmental situations.

b) the transfer from the incubation to the exposure conditions is done with an accepted and validated model concept.

c) incubation and exposure conditions are within the validity range of the model concept.'

d) The exposure conditions have to be captured in scenarios, which capture the pedoclimatic and agricultural conditions for which the risk assessment is warranted.

Examples are presented for simulation and measurement of pesticide (as example chemicals) degradation and persistence in the soil environment after single or multiple applications in a single year as well as in multiyear accumulation studies with annual applications. Degradation rates are estimated from laboratory as well as field studies and used for extrapolation.

Multi-year soil accumulation studies (in total \geq 50 study sites) show for different environmental scenarios and even for the more slowly degrading substances no further increase of the maximum concentration after a few years (2-5) with maximum accmulated concentrations being only ~< 2x higher than after first application and. The

residue patterns in soil at the different scenarios could in general be described well with simulation models and normalized degradation rates extrapolated to the local soil temperature and moisture conditions considering local weather data.

References:

Walker, A. (1974) A simulation model for prediction of herbicide persistence.- Journal of Environmental Quality, 3, 396-401

Poster 14

Unsaturated column for evaluation of pesticide behavior in soil

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Keywords: Unsaturated column, pesticide degradation, time dependent sorption

In evaluating the potential for environmental exposure to pesticides, regulatory agencies rely on parameters derived from laboratory soil degradation and equilibrium sorption studies. These studies are conducted as batch experiments, using small amounts of soil. The static conditions in the batch study do not allow dynamic exchange of air, moisture or nutrient supply. Further, degradation and sorption are evaluated separately. Field dissipation studies, on the other hand, are conducted in a more realistic environment, but under uncontrolled conditions and include processes besides sorption and degradation. These two types of studies often produce differing results which can be attributed to the basic design of the experiments: controlled but static in the laboratory or uncontrolled but dynamic in the field. An option to bridge the two is an unsaturated soil column which allows evaluation of combined effects of sorption and degradation under dynamic flow conditions in the presence of a continuous gas phase, as is normally the case in natural surface soils.

We designed an unsaturated soil column which has several unique features. The column contains two series of ports on opposite sides along the length of the column. By applying a controlled air pressure to the side ports, the pressure head and thus the water content within the soil can be maintained constant over time. Furthermore, by applying a downward unit hydraulic gradient, the water content within the column is spatially uniform. The design allows uniform aerobic conditions to be maintained in the column. It allows periodic sampling of both the gas phase and the solution phase, allowing calculation of mass balances. In addition, because the pressure head is maintained using a positive gas-phase pressure, there are no chambers or components under negative pressure and both addition and collection of solution proceed at atmospheric pressure.

The column design was tested with a non-reactive tracer for a period of three months at an unsaturated flow rate of approximately 5 cm per day. Results showed that the unsaturated hydraulic conductivity was reasonably steady and can be easily maintained. The breakthrough curve of the tracer was fairly symmetrical, as predicted by theory, with a remarkably good mass balance. The approach has a great potential to be used to bridge gaps between the laboratory batch studies and field experiments.

Chemical bioavailability of vanadium species in soil – effect of soil pH and organic matter

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Keywords: Bioavailability vanadium IV V soil pH organic matter sequential extraction speciation species

Vanadium (V) is a redox sensitive transition metal. In soils, two oxidation states are prevalent: +IV and +V. The species formation, as well as the biological activity, is dictated by the oxidation state. The toxicity and mobility of pentavalent species is considered higher than that of tetravalent species. The aim of this study was to discover the effect of soil pH and organic matter on the chemical bioavailability of V(+IV) and V(+V). Two soils (fine sand), differing mainly in their soil organic matter (SOM, 3.2 %and 0.5 %), were incubated at three pH levels (pH \sim 4-7) separately with V(+V) (NaVO3) and V(+IV)(VOSO4). Easily soluble V,V adsorbed by ligand exchange, organic V and strongly bound V were extracted by using a specific sequential extraction method (1:10 dw/V, 0.25 M KCl; 0.1 M KH2/K2HPO4; 0.1 M NaOH; 0.25 M H2SO4) and analyzed by ICP-OES (290.8/292.4 nm). Potential chemical bioavailability of V (easily soluble/ ligand exchangeable species) was lower in soil rich in SOM. Declined availability was attributable to SOM being able to act as adsorbent of V species. Furthermore, SOM lowered chemical bioavailability of V by reducing V(+V) to less mobile V(+IV). Chemical reactions of V in soils were regulated by pH. Higher pHs enhanced the predominance of V(+V) species and consequently soluble V and ligand exchangeable V fractions increased at elevating pHs. Pattern was similar in both soils. Overall, the accessibility of V in soils was determined by soil properties.

Poster 16

GEMAS: An overview of the distribution of metals in agricultural soil at the continental (European) scale

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Keywords: Metals, monitoring, geochemical background, soil

The geochemical background distribution of trace elements and their bioavailability in soil must be known at a variety of different scales and for different soil types for a sound risk assessment. The GEMAS (Geochemical Mapping of Agricultural and Grazing Land Soil) project had the aim to map the geochemical background for over 60 chemical elements in addition to the parameters determining their availability in soil across Western Europe. The European REACH Regulation specifies that industry must prove that it can produce and use its substances safely and risks, due to the exposure to a

substance during production and use at the local, regional and European scale, all need to be reliably assessed. The GEMAS project was carried out by the EuroGeoSurveys (EGS) Geochemistry Expert Group in cooperation with Eurometaux and the aim of this project was to produce harmonised data with respect to the spatial scale (sampling density), analytical methodology and landuse (comparable level of diffuse emissions). Across Europe more than 2000 samples each (in total 4132 samples), of agricultural land (Ap horizon, regularly ploughed) and grazing land (topsoil, 0-10 cm) were collected during 2008 following a common field protocol. Sampling took place in 33 European countries and covered 5,6 million km2. All samples were prepared in just one laboratory and analysed for each extraction/set of parameters in just one laboratory following strict external quality control procedures. The GEMAS data provide a strong basis for taking into account the spatial variability of both exposure (metal concentrations) and effect concentrations (considering bioavailability through variation in soil properties) in a risk assessment for metals in soils. More than 60 chemical elements were analysed in a variety of extractions. Total concentrations for 40+ elements were determined by X-ray fluorescence, 53 elements were analysed in an aqua regia extraction and 47 elements were determined in a weak mobile metal ion extraction. The data sets are freely available to the general public. The poster provides an easy overview of the analytical breadth of the project.

Poster 17

European standardised scenarios for exposure of soil organisms to pesticides

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Keywords: Soil organisms, scenarios, pesticides, exposure

European pesticide authorisation procedures require an effect assessment for soil organisms to be carried out. Predicting the environmental concentrations of pesticides by means of exposure models is an essential part of such an effect assessment. The European Food Safety Authority (EFSA) published a guidance document for predicting environmental concentrations of pesticides and their metabolites in soil^{(1).} Guidance is provided for all types of concentrations that are potentially needed for assessing ecotoxicological effects, i.e. the concentration in total soil and the concentration in pore water, both averaged over various depths and time windows.

The recommended exposure assessment procedure consists of five tiers. Standardised exposure assessment scenarios play an important role in the lower tiers of the assessment (a scenario is a combination of climate, weather and crop data to be used in exposure models). The goal of the exposure assessment is the 90th percentile of the exposure concentration in the area of agricultural use of a pesticide in each of three regulatory European zones (North, Central, South). Separate scenarios were developed for the concentration in total soil (mg/kg) and for the concentration in the liquid phase (mg/L) so that the total number of scenarios developed was six. A statistical approach was adopted to find scenarios that are consistent with this exposure goal^(2,3). Scenario development began with the simulation of the concentration in the entire area of use by means of a simple analytical model. In subsequent steps, procedures

were applied to account for parameter uncertainty and scenario uncertainty (i.e. the likelihood that a scenario that is derived for one pesticide is not conservative enough for another pesticide). In the final step, the scenarios were defined by their average temperature, soil organic-matter content and their soil textural class.

Organic matter of the scenarios decreased in the order North-Central-South. Because organic matter has a different effect on the concentration in total soil than it has on the concentration in the liquid phase, the concentration of pesticides in total soil decreased in the order North-Central-South whereas the concentration in the liquid phase decreased in the opposite order.

To facilitate efficient use of the guidance document and the scenarios in regulatory practice, user-friendly software tools were developed. Practical examples on how to use the guidance and the software tools are provided in the case studies as part of the session on exposure and fate.

Disclaimer

This abstract presents a summary according to the authors view and does not necessarily represent the position of EFSA.

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Hazardous organic compounds in biogas plant digestates — Soil burden and risk to food safety

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Keywords: Biogas plant, Hazardous organic compound, Organic fertilizer

Biogas technology is a sustainable method for managing biodegradable material and for energy production. Use of biological fertilisers such as biogas plants digestates (BGD) is favourable for recycling nutrients because phosphorus is a depleting nutrient and nitrogen fertilizers are manufactured in an energy-intensive process. If the raw material for a biogas plant contains organic compounds, they may end up in the digestate, and finally in agricultural soil. Some of these compounds may have the potential to accumulate in the food chain.

We analysed concentrations of ten hazardous organic compounds or compound groups in BGDs from ten biogas production lines in Finland and assessed their environmental burden and potential risk to food safety. The compounds included in the study were: polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCB(7)), polyaromatic hydrocarbons (PAH(16)), bis-(2-ethylhexyl) phthalate (DEHP), perfluorinated alkyl compounds (PFCs), linear alkylbenzene sulfonates (LASs), nonylphenols and nonylphenol ethoxylates (NP+NPEOs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA).

For most of the compounds, a median burden of the pollutants after a single addition of digestate (15 tonnes f.w./ha) was of the same order of magnitude as air deposition of the same compound or compound group in Finland or other Nordic countries. However, the burden for PBDEs was 400 to 1000 times higher than the PBDE air deposition in Finland. Highly persistent compounds, such as PBDE- and PFC-compounds may accumulate in the agricultural soil after repeated use of organic fertilizers containing these compounds.

With PBDEs, PFCs and HBCD, the impact of the use of BGDs should be a focus of further research. For other compounds included in this study, agricultural use of BGDs is unlikely to cause risk to food safety in Finland. Many compounds may be rapidly degraded in the environment (e.g. bisphenol A, DEHP, NP+NPEO). However, compounds "not persistent but persistently present", need attention since they are widely in use and there is a continuous flow of these compounds into the environment.

Topic 3: Effect assessment

Poster 19

Ecosystem services approach to pesticide risk assessment and management of non-target terrestrial plants: recommendations from two SETAC Europe workshops

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Keywords: risk assessment, effect assessment, terrestrial, soil, plant testing, plant protection product, field

In the field of soil risk assessment scientific principles and approaches for assessing the ecological risks of chemical substances, including pesticides and biocides, released to/or reaching soil, are under discussion. Here, we report the recommendations from two recently organised stakeholder workshops focusing on non-target terrestrial plant risk assessment. In 2014 the first workshop was held under the auspices of the Society of Environmental Toxicology and Chemistry (SETAC) Europe, with sponsorship from the European Crop Protection Association and participating stakeholder representatives. The workshop included invited experts from academia, regulatory bodies and business. The aim of this workshop was to consolidate current knowledge and expertise to aid the further development of testing and assessment procedures for non-target terrestrial plants and resulted in a set of agreed recommendations and a workshop report is being finalised. Meanwhile, EFSA published its opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. With participation from EFSA, a second SETAC stakeholder workshop was organized to build upon these documents in September 2015. Both workshops focused on the risk assessment and risk management of Plant Protection Products (PPPs) (Regulation 1107/2009), but the principles discussed and agreed upon might be extrapolated to and relevant for other risk assessment frameworks. The aims of the workshops were:

• developing a framework for a higher-tier approach to assessing the risk of PPPs to NTTPs;

• providing expert opinion and advice as input for the ongoing revision of the terrestrial ecotoxicology guidance document and NTTP risk assessment procedures.

The recommendations agreed to by the first workshop relate to the three main themes, i.e. specific protection goals, risk assessment and mitigation. The participants of the workshop adopted the European Food Safety Authority (EFSA) approach of using an ecosystem services framework for identifying specific protection goals. First, delivery and protection of ecosystem services were discussed for in-crop, in-field and off-crop, and off-field areas. Second, lower and higher tier risk assessment methods, including modelling approaches, were evaluated and the benefits from these options were addressed. Third, options for risk mitigation of spray drift and run-off were discussed and evaluated. The workshop participants agreed that the type and relative importance of ecosystem services provided by NTTPs differ between areas both in-field and off-field. A number of concerns were raised during the workshop and literature reviews were performed and data collected in order to reduce uncertainty. These actions focussed on the protectiveness of standard test species for wild species; the protectiveness of regulatory endpoints for reproductive endpoints; the methods and endpoints for multispecies or field-studies; and drift reducing technology and buffer zones to reduce the exposure of non-target plants. The second workshop built upon the results of these literature reviews, the recommendations of the first workshop and the EFSA opinion on risk assessment of PPPs for NTTPs. The main charge questions identified for the second workshop were: how to address reproductive endpoints; how to mitigate risks; how to conduct higher tier tests. Recommendations from both workshops will be presented.

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Approaches and tools to correct ageing effects and bioavailability in ecological risk assessment of lead in soil

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Keywords: Lead, Ageing, Bioavailability, Soil, Risk Assessment, Effect, PNEC

Metal bioavailability and toxicity in soil can be strongly affected by soil physico-chemical properties and ageing. In a multi-year study, the lead industry has recently addressed these issues with a view to providing state-of-the-science data for terrestrial lead risk assessment during its product registration for REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) in Europe. The study included measuring toxicity of lead (Pb) for 6 endpoints (2 plants, 2 invertebrates and 2 microbial endpoints) in a set of 7 soils covering a representative range of soil properties (pH, organic matter content, texture and eCEC). The effect of ageing was quantified by studying toxicity of Pb after 3 different contamination conditions: i) freshly spiked with PbCl2, ii) freshly spiked, leached and pH corrected in order to remove salinity and pH stress, and iii) freshly spiked and aged for 5 years. Variation in soil properties significantly explained variation in Pb toxicity for some endpoints (e.g. nitrification), but not for other endpoints (e.g. microbial respiration, Folsomia candida reproduction). Leaching and long-term aging of soils attenuated Pb toxicity significantly with a median factor of 4.7, confirming higher toxicity in freshly spiked soils compared to soils equilibrated under field conditions. Several bioavailability models, developed relating lead toxicity to soil properties, allowed normalization of soil toxicity data to a relevant range (10th-90th percentile) of soil properties across European soils yielding safe levels of lead between 171 and 442 mg Pb/kg. A computer interface for user-friendly uses of the above models together with species sensitivity distribution (SSD) analyses and other risk characterization options is currently available (http://www.arche-consulting.be/en/ourtools/soil-pnec-calculator/) and will be illustrated in the context of implementing bioavailability for predicting ecological risks of Pb in soil for a given site.

Re-calibration of the earthworm tier 1 risk assessment of plant protection products

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Keywords: Earthworms, Risk assessment, Plant protection products, Conservatism, Trigger value

To address potential risks of plant protection products to earthworms, a risk assessment is required. This risk assessment comprises two steps 1) deriving no-observed effect levels (NOEL) from laboratory reproduction tests and applying a trigger value, to cover uncertainties, and if this indicates a potential risk, 2) conducting field studies. In this review paper the tier 1 earthworm risk assessment for plant protection products is calibrated by comparing the NOEL in laboratory earthworm reproduction tests with effect levels on earthworm populations under realistic field conditions. A dataset of 54 pairs of studies conducted in the laboratory and in the field with the same plant protection product was compiled, allowing a direct comparison of relevant endpoints. The results indicate that a tier 1 assessment factor (AF) of 5 combined with a regulatory relevant soil layer of 0-5 cm provides a conservative tier 1 risk assessment. A risk was identified by the tier 1 risk assessment in the majority of the cases at application rates which were of low risk for natural earthworm populations under field conditions. Increasing the conservatism in the tier 1 risk assessment by reducing the depth of the regulatory relevant soil layer or by increasing the tier 1 AF would increase the number of false positives and trigger a large number of additional field studies. This would however not increase the margin of safety for earthworm populations. The analysis revealed that the risk assessment is conservative if an AF of 5 and a regulatory relevant soil layer of 0-5 cm are used.

Soil functional test systems for an in-crop soil risk assessment of plant protection products

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Keywords: Soil function, organic matter degradation, Soil micro-arthropods, ecosystem services, plant protection products, risk assessment

Soil functional test systems provide valuable and ecologically relevant information for the risk assessment of plant protection products. Functional tests directly measure ecosystem functions and services which are provided by soils and soil organisms (e.g. organic matter degradation and mineralization). Focusing on structural endpoints in the risk assessment for plant protection products lacks a clear link to the protection goals derived from ecosystem services (see poster of Bergtold et al.). Directly measuring soil functions and services can help to better assess the impact of a stressor on the fertility of soils. Furthermore, functional test systems can help to evaluate the ecological relevance of a density change of a soil organism population affected by a certain stressor. In order to improve the current toolbox for the soil risk assessment, a project on soil functional test systems was initiated by the European Crop Protection Association in 2014.

In a first step a literature search identified several promising functional test systems which could provide valuable and ecologically relevant information. Additionally these test systems are classified as having a high degree of reproducibility and standardization potential. In a second step a field study was set up in 2015 which measures the impact of two insecticides (Methamidophos and Lindane) on organic matter degradation based on methods described in Eisenbeis et al., 1999 (minicontainer test), von Törne, 1990 (bait lamina test), and the litter bag test (OECD guidance No. 56, 2006). Soil microarthropod abundances (i.e. Collembola and Acari) are monitored in parallel to determine the link between effects on the structure of soil micro-arthropods and their soil functional implications (i.e. organic matter degradation). In this study the suitability of the different functional test systems related to organic matter degradation is evaluated with regard to their potential use in soil risk assessment of plant protection products. Preliminary results of this project are shown and proposals on the use of functional endpoints in the risk assessment are provided. With this poster we would like to receive feedback from stakeholders and thus fine-tune this promising project for a 2016 field study aiming to better link protection goals with test systems.

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Environmental risks of biochar in soils: ecotoxicological effects on plants and microarthropos

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Keywords: Biochar, Environmental Risk, Ecotoxicity, Soil microarthropods, *Folsomia candida*

Biochar is the solid residue produced by the pyrolysis bio-wastes (biomasses, sludges, agricultural wastes) for energetic purposes. Its application to soil is considered a promising tool to improve soil fertility and to mitigate climate change. Several benefits are been described using biochar in agriculture or in remediation. However high salinity, high alkalinity and the presence of contaminants (heavy metals and polycuclic aromatic hydrocarbons deriving from the originating feedstocks or enhanced by the production methods) might induce detrimental effects on soil habitat and soil biota. These potential risks can't be ignored and it is necessary ensure the safety of biochar before to add it to soils.

We present some ecotoxicological results of four biochars, deriving from different biomass feedstocks from a gasification plant ($^{\textcircled{o}}$ AGT s.r.l., Cremona, Italy), on plants and soil microarthropods.

The biochars, deriving from conifer -CO-, Grape Marc -GM-, Poplar -PO- and Wheat Straw -WS-, were pulverized and mixed to standard soil at increasing w/w concentrations (0.5- 1- 2- 5- 10- 20- 50 %). Phytotoxicity was assessed using 5 L. (cucumber), 5 L. (watercress) and *Sorghum saccharatum* (L.) Moench (sorghum) performing the UNICHIM (2003) procedures. The seeds were placed in Petri dishes containing 15 g of biochar mixes and incubated at 25 ± 2 °C for 72h. Then thee germination rate and the root length were determined. Ecotoxicity was evaluated by using the collembolan *Folsomia candida* in a inhibition of reproduction test (ISO 11267:1999) and in an avoidance test (ISO 17512-2:2011). For the first test, synchronized juveniles (10-12 days old) were placed in Petri dishes with biochar mixes, kept at 20 ± 2 °C for 28 days, then adults and juveniles were counted after flotation. For the second one, 10 synchronized juveniles were disposed on Petri dishes in the conjunction between biochar mixes and standard soil. The test lasted 48h, then the substrates were separated with a plastic bar and counted after flotation.

The results showed that both plants and animals were affected by biochar, especially at higher concentrations. The origin of biomasses seemed to impact in different way the germination and the elongation of seeds and the survival and reproduction of collembolans, outlining differences between wood derived biochar (CO and PO) and herbaceous biochar (GM and WS).

Among the biochars, the GM revealed the worse effects on root length of seeds and on survival and reproduction of springtails. This point unveils the necessity of test biochars before the application on agricultural fields in order to avoid detrimental effects.

Using higher-tier data in the nickel terrestrial effects assessment: reducing residual uncertainty?

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Keywords: Nickel, Field-effects, Bioavailability

Nickel rarely occurs geochemically as a single element, often being accompanied by other trace elements such as copper, cobalt and manganese. Therefore, because of this co-occurrence soils receiving emissions from nickel mining, smelting, refining and processing activities often have elevated concentrations of multiple elements in addition to nickel.

The terrestrial risk assessments of nickel under both the ESR Programme and REACH identified the absence of higher tier data for nickel effects in the field as a source of potential uncertainty in the effects assessment. However, since these assessments were made new field-based terrestrial ecotoxcity data have become available at nickel ore processing sites in Canada that are impacted by a mixture of current and historic emissions. Importantly, The form of Ni used in laboratory toxicity tests was limited to soluble Ni (e.g., NiCl₂), whereas Ni deposited to soils surrounding the Canadian nickel processing sites was in the form of particulate matter (e.g., NO_x). The ramifications of these differences in terms of soil risk assessment will also be discussed.

This poster will provide an assessment of these data in regard to the influence of aging on nickel field effects and the application of previously developed bioavailability relationships to these higher tier data and a comparison of laboratory and field generated endpoints.

Methods and challenges of using such high tier data will be discussed in context of potential reductions in residual uncertainties of the nickel terrestrial effects assessment.

Tracking soil contaminants using in vitro toxicity assays

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Keywords: Soil, contamination, in-vitro, toxicity

Soil contamination, primarily anthropogenic in nature, poses a significant and ongoing risk to both the environment and human health. However, the effects of contamination remain poorly understood. Standard assessment of soil contamination often relies upon the quantitative measurement of known contaminants in the soil. However, although this approach permits the degree of contamination to be determined (where contaminant guideline values exist), it neither reflects the effects of contamination on human health; considers the possible additive toxic effects of contaminants in combination nor identifies the presence of previously unidentified toxic chemicals. We hypothesised that soil samples could be collected and extracts prepared and tested in cell based assays in order to screen for toxic effects using a variety of viability and gene reporter assays.

To test the hypothesis, multiple soil samples were taken from around the boundary of a functioning waste site and for comparison from 3 distant control sites (2 suburban and 1 rural) from which aqueous, alcohol and organic extracts were generated by methanol or chloroform extraction. The effect of these extracts on a range of toxic endpoints was then determined in a range of cell types.

Initial screening showed that several extracts, particularly phosphate (aqueous) extracts 1 and 2, inhibited the proliferation of liver progenitor cells and within 72 hours, induced apoptosis. Screening for mitochondrial toxicity indicated that although several extracts mildly inhibited mitochondrial respiration, phosphate extracts 1 and 2 were potent inhibitors, potentially acting through inhibition of complex V. This inhibition of oxidative phosphorylation was associated with a complementary increase in glycolysis. Measurement of gene expression indicated that all organic and alcohol extracts significantly activated the aryl hydrocarbon receptor and most of the alcohol extracts activated the oestrogen receptor. In contrast, control soil sample extracts were less potent in essentially all measured parameters.

These data demonstrate that extracts, suitable in-vitro experimentation, can be generated from soil and tested in cell-based toxicity screens. In this work, this approach demonstrated that soil from around a waste site contains chemicals which had toxic effects in-vitro and could therefore pose a risk to human health. Use of this approach at contaminated locations would enhance our understanding of the potential effects of contamination upon disease.

Assessment of the impact of an abandoned dump site on the health of adjacent soil ecosystems by in vivo and in vitro testing with *Eisenia fetida*

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Keywords: dumpsite, Eisenia, coelomocytes, in vivo, in vitro, biological effects

1. Introduction

Earthworms are considered as sentinel organisms for soil health assessment, and among them, Eisenia fetida has been selected for the development and application of standard tests due to its sensitivity and easy maintenance. E. fetida has been successfully employed for real soil assessment through biomarker measurements in vitro (e.g., Neutral Red Uptake -NRU- by coelomocytes) in and in toto (methallotionein induction) after *in vivo* exposure⁽¹⁾. Recently, an *in vitro* test with *E. fetida* coelomocytes was developed, including in vitro exposure to metals and elutriates of standard and real soils contaminated with pollutants alone or in mixtures^(2,3). Such *in vitro* exposure approach would enable soil health assessment within the framework of the 3R (Reduction, Refinement, Replacement) foundations⁽⁴⁾. Presently, biomarkers were determined *in vitro* in coelomocytes after both in vivo exposure (real soils) of earthworms and in vitro exposure (real soil elutriates) of coelomocyte explants extruded from stock healthy earthworms. The in vitro responses after in vitro and in vivo exposures were compared, aimed to replace the in vivo exposure approach in the future and contribute thus to reduce the use of animals in soil toxicity testing. For comparisons, two biomarkers (NRU and riboflavin content) were used for ecosystem health screening in soils surrounding an abandoned dumpsite.

2. Materials and methods

A dumpsite located in Hernani (Basque Country), which received industrial and urban wastes between 1972 and 1989, was used as experimental basin. Two field samplings were carried out in February and June of 2011. Sampling sites (following the run-off from the dumpsite, from KAT in the border to CARR further downhill) were selected according to a gradient after *in situ* measurement of metals by XRF. A reference site (REF) was sampled in the vicinity of the dumping area. 4 soil replicates were taken from each place, sieved, air dried and hydrated (60% WHC). The points of the runoff KAT and CAR had shown more alkaline pH (KAT:6,2-7,8; CAR:5,6) in comparison with the natural pH showed in the REF site (4,5-4,8). In order to predict the behavior of the site in the future, a set of soils was treated with CaCl₂ and HNO₃ to modify the capacity of the tested soils for metal retention and thus enhance metal bioaccesibility. This might happen when environmental factors or soil physicochemical conditions change due to soil tendency to recover its natural pH. Metal contents in soil were measured *in situ* by XRF, and soil pH, total organic matter, total hydrocarbon content, nitrogen, and ammonium were determined in the laboratory.

In vivo *exposure* - in vitro *biomarkers. In vivo* exposure was carried out following the Acute Toxicity Test N.207 (OECD, 1984) procedure. Earthworms were weighted after 0 and 14 d exposure. Tissue metal concentrations were quantified by ICP-MS in earthworm

soft tissues at Day 14. At Days 3 and 14 d, coelomocytes were harvested and seeded in a microplate $(2 \times 10^5 \text{ cells/well})$ and the riboflavin fluorescence signal was measured. Then, the same coelomocytes were incubated in NR dye (% 0.02) for 30 min. After washing, the dye was extracted and the absorbance was measured at 540 nm to determine NRU⁽³⁾.

In vitro *exposure and biomarkers*. Coelomocytes were extruded from earthworms maintained in the laboratory and seeded in L-15 medium supplemented with antibiotics. After 24 h, new culture medium containing elutriates from the collected soils were added. Elutriates of soils were obtained following the DIN 38 414 standard as specified in the Spanish legislation. 24 h after exposure, NRU assay was performed as above explained.

3. Results and discussion

In vivo *exposure* - in vitro *biomarkers.* No significant mortality was recorded. Weight loss was higher in earthworms exposed to REF soil that in those exposed to KAT and CARR soils. In contrast, tissue metal concentration was much higher in earthworms exposed to KAT and CARR soils than in those exposed to REF soil. NRU decreased after 3 d exposure to KAT and CARR soils but increased after 14 d. Overall, riboflavin content decreased after exposure to KAT and CARR soils. CaCl₂ treatment decreased riboflavin signal and HNO₃ treatment affected NRU capacity.

In vitro *exposure and biomarkers.* A significant decrease in NRU capacity was recorded but the riboflavin content remained unchanged on exposure to KAT and CARR soil elutriates. $CaCl_2$ and HNO_3 treatments did not cause any significant effect on NRU and riboflavin content.

4. Conclusions

In vitro testing of coelomocytes after both *in vitro* exposure of coelomocytes and *in vivo* exposure of earthworms provided similar information about the health of soils surrounding the dumping site. The selected cellular biomarkers discriminated polluted and unpolluted soils, even though pollution levels were low (low metal bioavailability). Therefore, when biomarker endpoints such as NRU capacity and riboflavin content are measured *in vitro*, the *in vitro* exposure approach is suitable to replace the *in vivo* exposure assays for soil health assessment.

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How well can standard soil tests provide the needed evidence for risk assessment of nanomaterials?

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Keywords: Environmental fate of nanomaterials, time dependency, bioavailability, bioaccumulation

Over the recent years it has become clear that ranking toxicities of nanomaterials through the testing of "pristine" as made particles in clean media may not provide much relevance in terms of the environmental risk their released forms potentially present. While it is clear that dealing with detailed phys-chem characterisation of the multiple forms in which the nanomaterials may be released from all stages (particle production; incorporation; use and disposal phases) of a nano enabled products life-cycle is impossible. Then it is equally clear that adequate and efficient risk assessment cannot be done by simply comparing PNECs from "short lab test with pristine NM forms" with the PECs from simple mass flow based models that do not take the transformations of nanomaterials both pre and post release to the environment into account. For one the fate processes and behaviour of the released materials depend on the new phys-chem properties developed in such transformations. Again tracking such transformations in details and doing so in environmentally relevant media and possibly concentrations is technically challenging and resource intensive beyond most available means. Therefore, we propose to move focus away from the physical/chemical properties of pristine ENMs and to aim to understand the functional and behaviour patterns of release relevant ENMs in exposure relevant environments. The need for this will be highlighted through presentation of a series of recent non-standard experiments that aim to get as relevant nanomaterial exposures as possible, each addressing a different element of fate, transformation or aging. Through these experiments we will show how presenting an exposure into a system as nano vs ionic metals lead to diverse and in some cases unexplainable response patterns.

Integrated testing strategy for effects on terrestrial organisms under the REACH Regulation

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Keywords: soil hazard assessment, integrated testing strategy

Chapter R.7C of ECHA's *Guidance on information requirements and chemical safety assessment* describes the general scheme for soil risk assessment including the integrated testing strategy with screening risk assessment approach for terrestrial organisms.

Since 2009, ECHA has cooperated with other stakeholders to process a number of dossier evaluations where issues regarding the screening risk assessment approach summarised in the Guidance were raised. The issues included discussing the acceptability of the test guidelines and relevant number of species for assessing long-term soil plants and invertebrates toxicity testing. In addition, the necessity of considering toxicity to soil microorganisms as well as the equilibrium partitioning method-based screening risk assessment was addressed.

Based on these discussions, the screening assessment approach for soil was further clarified. Furthermore, based on new regulatory knowledge the criteria to define substances with high potential for adsorption and substances very toxic to aquatic organisms were further clarified.

The poster summarises these developments/clarifications and provides a schematic presentation of the currently applied screening risk assessment approach for soil.

Analysis of experimental terrestrial toxicity studies submitted in the framework of the REACH Regulation

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Keywords: standard test guidelines, soil invertebrates, terrestrial plants, microbial tests, test species

This poster summarises the availability of experimental data on terrestrial ecotoxicity submitted in REACH registration dossiers within two registration deadlines (1 December 2010 and 1 June 2013). This information has been disseminated on ECHA's website. The similar analysis performed after the first registration deadline was published in 2013 by Versonnen et al.1 The current analysis covers the submitted data after the two registration deadlines and the experimental data produced as a consequence of the evaluation of testing proposals and the compliance checks. As in the previous analysis, both the most used test guidelines and test species were investigated.

As the outcome of the conducted analysis, it can be concluded that:

- for many cases, the information on the test guideline according to which the study was conducted, was not reported or was not reported correctly (for over 30 % of invertebrate studies; for about 60 % of plant tests and for over 70 % of microbi tests);
- for soil invertebrate tests, a clear prevalence has been detected for testing on the species recommended by the standard test guidelines. On the other hand, the reporting included a large variety of species from very different families, demonstrating the feasibility for conducting toxicity tests on a number of relevant groups e.g. for future species sensitivity distribution approaches;
- for terrestrial plants, the most extensively reported test guidelines were OECD 208, ISO 11269-1 and ISO 11269-1;
- the experimental tests were available for only about 35 % of the substances for which the terrestrial information was included in the dossier;
- adaptations and waiving justifications, based on various groundings, were the most commonly used ways to omit the experimental testing for soil terrestrial toxicity.
- when interpreting the findings of the current analysis, it should, however, be noted that in principle the current results may be affected by the outcome of the dossier evaluation works performed by ECHA.

By the end of August 2015, ECHA had issued 70 final decisions following the evaluation of the testing proposal requests for additional testing. In addition, as a result of the evaluation of the provided information, ECHA had issued 27 compliance check final decisions by the end of December 2014, which requested terrestrial toxicity studies to be performed according to standard test guidelines. The analysis of the outcome of ECHA decisions requesting terrestrial toxicity testing is presented on the current poster.

¹ Bram Versonnen, Jose V. Tarazona, Romanas Cesnaitis, Marta A. Sobanska, Tomasz Sobanski, Vincent Bonnomet, Wim De Coen. 2014. Analysis of the ecotoxicity data submitted within the framework of the REACH Regulation, Part 4. Experimental terrestrial toxicity assays. Sci. Total Environ. 475:123-131.