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IMPROVING SUBSTANCE INFORMATION IN USETOX®, PART 1: DISCUSSION ON DATA AND APPROACHES FOR ESTIMATING FRESHWATER ECOTOXICITY EFFECT FACTORS

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Abstract: The scientific consensus model USEtox® is recommended by the European Commission as the reference model to characterize life cycle chemical emissions in terms of their potential human toxicity and freshwater aquatic ecotoxicity impacts in the context of the International Reference Life Cycle Data System (ILCD) Handbook and the Environmental Footprint pilot phase looking at products (PEF) and organisations (OEF). Consequently, this model has been systematically used within the PEF/OEF pilot phase by 25 EU industry sectors, which manufacture a wide variety of consumer products. This testing phase has raised some questions regarding the derivation of and the data used for the chemical-specific freshwater ecotoxicity effect factor in USEtox®. For calculating the potential freshwater aquatic ecotoxicity impacts, USEtox® bases the effect factor on the chronic hazard concentration (HC50) value for a chemical calculated as the arithmetic mean of all logarithmized geometric means of species-specific chronic lethal (or effect) concentrations (L(E)C50). We investigated the dependency of the USEtox® effect factor on the selection of ecotoxicological data source and toxicological endpoints, and we found that both influence the ecotoxicity ranking of chemicals and may hence influence the conclusions of a PEF/OEF study. We furthermore compared the average measure (HC50) to other types of ecotoxicity effect indicators like the lowest species EC50 or NOEC, frequently used in regulatory risk assessment, and demonstrated how they may also influence the ecotoxicity ranking of chemicals. We acknowledge that these indicators represent different aspects of a chemical’s ecotoxicity potential and discuss their pros and cons for a comparative chemical assessment as performed in LCA and in particular within the PEF/OEF context. This article is protected by copyright. All rights reserved

Keywords: USEtox®; Fate modelling; Chemical regulation; Environmental toxicology; Product Environmental Footprint; Organisation Environmental Footprint; Life cycle assessment
INTRODUCTION

The main goal of life cycle assessment (LCA) is to quantify and compare the potential impacts on the environment including ecosystem quality, human health, and natural resources occurring along the life cycle of products and services (from extraction of raw materials to end of life treatment). Potential impacts are thereby associated with the consumption of natural resources and emissions of chemical substances into air, soil and aquatic environments. Originally, the LCA methodology developed in the late 1960’s focused mainly on the accounting of resources and energy flows (and related greenhouse gas (GHG) emissions into air). Steadily, new impact categories have been added to LCA including depletion of stratospheric ozone, acidification and eutrophication of terrestrial and aquatic ecosystems, abiotic resources depletion, ecotoxicity and human toxicity, impacts due to land and water use, etc. Each impact category indicator covers a different impact pathway and relies on models that describe these impact pathways by linking the resources used or chemical emissions into the environment as quantified in the life cycle inventory (LCI) phase to impact along a cause-effect chain as quantified in the life cycle impact assessment (LCIA) phase. For the characterization of each type of impact, different models are usually available [1–3].

Over the years, several models for characterizing freshwater ecotoxicity impacts have been developed that are based on different assumptions and algorithms and that can lead to results which differ by several orders of magnitude [4]. To overcome intrinsic differences between models and capitalise on available knowledge, the scientific consensus model USEtox® has been developed since 2003 under the auspices of the UNEP-SETAC Life Cycle Initiative [4–6]. USEtox® aims at characterizing the toxicity-related impacts of chemical emissions on freshwater ecosystems and on humans by combining multimedia environmental fate modelling to estimate chemical distributions in various environmental compartments with exposure and effect assessment. After a review of several models performed by the European Commission – Joint Research Centre (EC-JRC) [1,2], USEtox® has been retained as reference model for human toxicity and freshwater ecotoxicity impact characterization. Indeed, USEtox® is the reference model in the International Reference Life Cycle Data
System (ILCD) recommendations [2], and is consequently also applied in the context of the European Commission’s Product and Organization Environmental Footprint (PEF/OEF) pilot phase [7,8]. USEtox® is a screening-level model that aims to help identifying out of hundreds of chemicals emitted along product life cycles those emissions with the greatest contribution to potential aquatic ecotoxicity and human toxicity profiles [9]. It will be further evaluated if the outcome of the USEtox® calculation helps with the identification of the chemical of concerns in the context of the PEF/OEF, and the identification of environmentally preferable products, as far as potential toxicity is concerned.

To assess the overall potential human toxicity and freshwater ecotoxicity impacts of a product, the mass of each chemical emitted along the product’s life cycle into particular environmental compartments is multiplied by its corresponding characterisation factors (CFs), representing the potency of chemicals toward causing human toxicity and/or freshwater ecotoxicity impacts. In a product life cycle, there can be thousands of different chemicals that are emitted to air, water and soil. USEtox® (version 1.01) already provides 2498 CFs for freshwater ecotoxicity. For each substance emitted to compartment $i$, ecotoxicity CFs are calculated from the combination of matrices containing fate factors (FF), exposure factors related to freshwater compartment $w$ (XF) and ecotoxicity effect factors (EF) with $\text{CF}_i = \text{FF}_{i,w} \times \text{XF}_w \times \text{EF}_w$. Since its first release in 2008, USEtox® has been widely used but only recently systematically applied and evaluated across industry sectors for the purpose of product comparison and communication in the PEF/OEF pilot phase (2013-2017). In 2015, the European Commission organized a workshop with the PEF/OEF pilots, which have been using USEtox® 1.01 in their screening studies. The main conclusions from this workshop were that using USEtox® in PEF/OEF might lead to results, which are difficult to understand and interpret. Moreover, USEtox® substance-related input data including physicochemical properties, chemical half-lives and freshwater ecotoxicity data should be aligned with the most recent data sources, e.g. the IUCLID database of the European Chemical Agency (ECHA) that is used for the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) in the European Union [10]. After this workshop, the EC –JRC has conducted a re-evaluation of USEtox® model
under the light of the newly performed PEF/OEF screening studies, aiming at increasing the acceptability of toxicity and ecotoxicity characterization factors. The results of this evaluation related to the calculation of ecotoxicity effect factors are summarized in the present paper and apply to both USEtox® versions 1.01 and 2.0 as the underlying approach and the related input data are identical except for some substances regarding the latter.

The rationale behind the USEtox methodology has been published [3,11–16], and is also the result of a series of scientific consensus workshops between LCA and Environmental Risk Assessment experts. Furthermore, USEtox® has the endorsement of the UNEP-SETAC Life Cycle Initiative [17]. Via this paper, we examine some of the consequences of the current approach as an input to the scientific developments around USEtox, and by presenting 2 case studies we illustrate the potential issues encountered while applying the recommended model.

Our analysis is divided into four main blocks.

Firstly, we present a critical discussion on the methodology and assumptions applied by USEtox® to derive freshwater ecotoxicity effect factors to be used in the EU context with the PEF/OEF activities. This topic has been (and still is) debated within the LCA and the risk assessment communities both for the ecological and human health related impacts. Due to the collaborative nature of this article (some authors are risk assessment experts, some are LCA experts and have actively participated at the development of USEtox, others have double expertise in LCA and ERA), no final recommendation is provided on how effect factors should be calculated, but rather five possible options to calculate effect factor in the context of the PEF/OEF are presented. The paper does not provide case studies where all the different options are tested and compared. This will be done in future work.

Our analysis is further developed by: 2), a comparison between USEtox® effect factors and how ecotoxicity is dealt with in European chemicals regulation; 3), an illustrative case study to highlight the

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implications of the original methodological choices in the PEF/OEF context; and 4), a comparison of USEtox® HC50 values with metrics used in regulatory chemical environmental risk assessment (ERA).

MATERIALS AND METHODS

As basis for critically discussing the approach that USEtox® follows for deriving the freshwater ecotoxicity effect factor, we reviewed the user manuals for version 1.01 [11], the official model publications [12], [13–15]; and two related book chapters [16,18]. Furthermore, two case studies were designed to analyse the influence of input data source on effect factor results and to compare official USEtox® effect factors with ecotoxicity effect indicators used in the context of the European chemicals regulation, respectively. USEtox® aims at quantifying potential ecotoxicity impacts from any studied product system and to help identifying the 10-20 most contributing chemicals out of the potentially thousands of chemicals emitted to air, water and soil from a product life cycle inventory. The analysis of a dozen of recent food, housing and mobility LCAs has shown that in most cases few metals and a few organic chemicals (often pesticides) are identified as the most contributing chemicals [19,20]. The USEtox model being specifically suited for organic chemicals, we have selected chemicals that are both ‘organic’ and for which the input data to run the model are easily retrievable and of high quality. By high quality we mean that all the data have been peer-reviewed and ‘validated’ by a competent authority (e.g. EFSA) for use in an environmental risk assessment. EFSA produces scientific opinions and advice for policy support on food and feed safety, nutrition, animal health and welfare, plant protection and plant health. In this context, EFSA performs risk assessments of pesticides and thorough assessments of all data needed for ERA including physicochemical substance properties, and data relevant for environmental fate and ecotoxicity. Related EFSA reports are available on the authority’s website (http://efsa.europa.eu/efsajournal). Draft assessment reports are prepared by the reporting Member States, which are then peer-reviewed by EFSA, resulting in the “Conclusions on Pesticides”. The observations we are presenting on pesticides should equally be applicable to a wider range of organic chemicals, although the risk assessment procedure between industrial chemicals and pesticides differs.
We therefore defined two sets of chemical substances – all being active ingredients of plant protection products (PPPs) currently used on the European market - because:

1) Pesticide active ingredients are designed to be toxic to certain target organisms,
2) Pesticides are widely applied in EU food production and hence represent important chemical flows toward ecosystems to be considered in an LCA or PEF/OEF study,
3) Peer-reviewed risk assessment reports from the European Food Safety Authority EFSA are publicly available for many pesticides.

However, the observations described in this paper also apply to organic substances in general. A first set of the 15 most recently approved pesticides was compiled from the ‘EU Pesticides database’ [21]. From this list, 6 pesticides were kept for further analysis as they: i) had an available EFSA “Conclusion on Pesticides”; ii) were included in the USEtox® organic substances database; iii) had a complete inventory of physicochemical and ecotoxicity data in the EFSA reports. For the 6 pesticides, EFSA ‘Conclusion on Pesticides’ data were extracted and compared with corresponding data in the USEtox® organic substances database. To strengthen the observations made on this set of 6 pesticides, a second set of 34 pesticides was compiled from the ‘EU Pesticide database’ using the following search criteria: i) approved for the EU market; ii) classified as “aquatic chronic 1” [22], i.e. non-rapidly degradable with chronic NOEC ≤ 0.1 mg/L or rapidly biodegradable, and with chronic NOEC ≤ 0.01 mg/L; and iii) fulfilling either persistent or bio accumulative criteria. Out of these 34 pesticides, 26 were present in USEtox® and hence allowed a comparison of ecotoxicity effect factors, expressed as hazardous concentration (HC50), based on applying different underlying substance input data sources, namely:

- The USEtox® organic substances databases 1.01 containing effect factors calculated with USEtox® (http://usetox.org/current-version);
- The EFSA reports compilation of a database on ecotoxicological properties of active substances and PPPs (http://efsa.europa.eu/supporting/pub/364e). This database, hereafter referred to as ‘EFSA DB’,
contains reports with data on aquatic and terrestrial ecotoxicity representing the agreed endpoints to be used for pesticides ERA in the EU;

- The pesticide properties database (PPDB) (http://sitem.herts.ac.uk/aeru/iupac/), which amongst data from various sources of different quality and reliability includes data originating from EFSA ‘Conclusions on Pesticides’ [23]; and
- The ‘Aquatic Impact Indicator Database’ (AiiDA) that provides pre-calculated HC50 values, which could potentially be used as input for deriving effect factors, but which would need to be extracted manually for each considered chemical (http://aiida.tools4env.com) [24].

RESULTS AND DISCUSSION

USEtox effect factor: Description of the approach and critical discussion

The freshwater ecotoxicity effect factor (EF) in USEtox® represents the potential toxicity of individual chemical substances to freshwater aquatic ecosystems. The equation to calculate substance-specific EF in USEtox® is EF = 0.5/HC50, where HC50 is the hazardous concentration at which 50% of the species tested are exposed above their chronic L(E)C50. In USEtox®, log HC50 is derived from first taking the geometric mean across \( i \in \{1, \ldots, m\} \) available chronic L(E)C50 data points per species and then taking the arithmetic mean of the logarithmic values for all \( j \in \{1, \ldots, n\} \) species-specific chronic L(E)C50 geometric mean values as in equation 1 (L stands for lethal effect; E for other type of effect, both affecting 50% of the tested organisms):

\[
\log_{10} HC50 = \frac{1}{n} \sum_{j=1}^{n} \log_{10} \left[ \prod_{i=1}^{m} L(E)C50_{i,j} \right]^{\frac{1}{m}} = \frac{1}{n} \sum_{j=1}^{n} \frac{1}{m} \sum_{i=1}^{m} \log_{10} L(E)C50_{i,j} \quad (1)
\]

In a concentration-effect graph with the concentration along the x-axis and the effect on the y-axis, the EF corresponds to the slope of a straight line connecting the point (HC50, 0.5) with the origin (0, 0) [16]. This approach corresponds to assuming linearity between the concentration and the response (percentage of affected species). The slope is, therefore, used as an indicator of a chemical’s ecotoxicity potency, i.e. the more ecotoxic...
the chemical, the steeper the slope and hence the higher EF. Assuming linearity between concentration and effect is a straightforward way to attribute an ecotoxicity score to the emitted mass of a chemical, which is the only information available in a life cycle inventory. This assumption thereby accommodates the fact that little is typically known about the shape of all the chemical- and species-specific concentration-effect curves at very low concentrations that are relevant for environmental exposure, and that in LCA we normally have no information about the background concentration of chemicals in the environmental compartments that receive the emission from the product system along its life cycle.

\( HC_{50} \) is chemical-specific and based on all available aquatic ecotoxicity data. Chronic L(E)C50 data are preferred over acute L(E)C50 data, but an extrapolation factor of 2 is suggested to convert acute data to chronic data for chemicals where insufficient or no chronic aquatic ecotoxicity data are available. This factor is based on an analysis of 92 compounds (18 organics, 22 inorganics, 54 pesticides) [25]. For chemicals not already included in USEtox® and for which the user has to calculate a HC50 value, guidance is provided on page 17 of the USEtox® 1.01 user manual [11]. For calculating EFs, USEtox® has so far relied on two ecotoxicological data sources providing the underlying acute and chronic L(E)C50 data. The first source contains acute L(E)C50 from the RIVM e-toxBase [26] and the second source contains mainly acute and chronic data compiled by [27] for the Assessment of the Mean Impact (AMI) method. The rationale for using in USEtox the arithmetic mean of all species-specific geometric means of the log of L(E)C50 values to derive the HC50 as well as the linear relationship between concentration and response is documented in [3,11–16] and relies on several key points.

In the following sections, we discuss the justifications provided by USEtox for choosing this approach to derive chemical effect factors. Paragraphs in italic refer to text copied from USEtox publications and manuals.

‘A HC50 based on L(E)C50 values represents a best estimate, while using a metric like the risk assessment related PNEC would introduce significant levels of conservatism due to the use of the NOEC and introducing assessment factors by regulatory agencies to set PNECs [9,12,14,16]’
The HC50 is not by definition a “best estimate” for comparing chemical ecotoxicity, but represents an average ecotoxicity-related pressure on the entire exposed ecosystem. It is the least sensitive value regarding inclusion of additional data above or below the HC50, i.e. the HC50 is the value on a species sensitivity distribution curve at which statistical variability is minimized. Variability is also minimized in regulatory approaches based on establishing the PNEC (and hence representing conservative rather than average estimates), which includes safety factors applied on the lowest valid ecotoxicity values (EC50 or NOEC), by taking into account the number of species and trophic levels tested, and the type of tests (acute or chronic) [28,29]. However, the NOEC or LOEC values do not carry any conservatism (there are the actual outcomes of an experimental test even if the underlying statistic to derive the NOEC/LOEC is questionable) and these values could be used to present another way to derive the effect factor in PEF/OEF.

“The HC50 is more robust as (a) it is derived from all available data and hence less sensitive to new data points than risk assessment metrics (i.e. PNECs) that only use the lowest available and validated ecotoxicity value [9,14], and (b) it is the point on the concentration-response curve associated with the less statistical uncertainty than other toxicity-based estimates like the HC5 or the predicted no effect concentration (PNEC), where this uncertainty can be estimated and used in calculations of the uncertainty accompanying the overall freshwater ecotoxicity characterization factor [12,14,15].”

The HC50 is indeed more robust and less affected by introduction of new ecotoxicity data than other effect indicators like the lowest chronic test result, PNEC or HC5. However, availability of new or additional data might potentially suggest that one or more ecosystem taxa are more sensitive to a particular substance. Such values impact the lower extreme of the species sensitivity relationship more than a central value like HC50. Such new information may be ecologically important, because in cases where it might indicate that not just the tested species but the whole trophic level is impacted, it is the full ecosystem structure and functioning that is impacted.
In risk assessment decisions, all data are also used. The fact that the lowest value is chosen to base future decisions upon does not mean the other data do not contribute to the decision and provide support for that value. The lowest value can, however, potentially change a lot as new data are developed and this usually increases ecological relevance and protection. Thereby, using the lowest available toxicity value will only change when a more sensitive species is tested. In regulatory assessment, in this context, it is not the actual lowest value of a set of toxicological data that is used, but the lowest validated data, meeting strict data quality criteria covering relevance, reliability and adequacy. Finally, as new data are generated, also HC50 values might change (to a lesser extent though, as the effect will be moderated by the bulk of data). [30,31].

‘LCA commonly uses averages or best estimates and assumes linear relationships between inventory flows (reported in mass) and environmental responses to estimate impacts of processes on human health, ecosystem quality, and resources [3], and additivity of ecotoxicity can readily be incorporated into LCA with a linear concentration-response model [9]’;

This assumption (linearity) ignores the possible existence of a threshold below which the chemical has no potential ecotoxicity effects on aquatic ecosystems and ascribes an ecotoxicity effect to any amount of chemical emitted to the environment proportional to the mass emitted. In reality, the concentration-response is usually not linear and threshold concentrations below which no effects are observed for individual species or species groups can be established for different chemicals [32–34]. The assumption is, however, needed for two reasons. First, the LCI reports emissions in mass related to the functional unit (i.e. function upon which product systems are ultimately compared) and brings no information about the total emission over time from each process to the receiving environments (e.g. small or big river, sea or soil), permitting to calculate an exposure expressed in concentration, which would be needed to judge whether a potential low threshold is exceeded or not. Second, the exceedance of thresholds also depends on the background concentration of the chemical in the exposed environments, and this information is usually not possible to attain for all processes involved in a considered product life cycle. The use of a linear concentration-response curve corresponds to the assumption of toxicity
additivity meaning that even if thresholds exist for individual chemicals, and they are not exceeded for any chemical in a concrete exposure situation, with the presence of a multitude of chemicals at the same time in the same compartment, toxicity additivity may still lead to an effect (cocktail or combined toxicity effect). Inherent in the linearity assumption is that any quantity of a chemical emitted will contribute to a potential ecotoxicity impact. This linearity assumption however clearly overestimates toxicity in the lower part of the ‘S’ curve and underestimates the toxicity in the upper part, but HC50 being based on the slope between 0 and 50% effect, this under- or over estimation is of low consequence.

Assuming additivity of ecotoxicity effects in LCA is a pragmatic solution to allow the calculation of one single score for a full product LCA in which hundreds of chemicals may be emitted. The reality is of course more complex and while chemicals present at the same time in the same exposure medium can exert combined effects in an additive way, also synergistic or antagonistic effects are possible [35–37]. Additive combined effects are mainly elicited by co-exposure to chemicals acting with similar toxic mode of action (TMoA), as chemicals with different TMoA are theoretically not believed to contribute to the combined effect, if they are present below their individual threshold concentration [36]. There are thus large numbers of chemicals which may not contribute to combined toxicity, although in environmental risk assessment combination effects could not be ruled out [38]. On the other hand, the life cycle emissions from a product system interact not just with other emissions from the same product system but also with other chemicals that are present in the environment and that originate from other man-made activities with no relation to the studied life cycle. It is therefore difficult to know the nature of all occurring chemical interactions, and ecotoxicity additivity has been assumed as a straightforward proxy solution [28,29].

Comparison of ecotoxicity effect approaches in LCA and in chemical risk assessment

The USEtox® approach to characterize potential freshwater ecotoxicity of chemicals – like other methods for ecotoxicity characterization in LCA – differs from approaches used in European chemical safety assessments and regulatory schemes (REACH, Classification and Labelling, Plant Protection Products)
General differences and similarities between LCA and risk assessment have been addressed elsewhere [41–45]. In short, regulatory environmental risk assessment for industrial chemicals is performed one chemical at a time, and requires the estimation of a predicted environmental concentration in a specific compartment (river water, sediment or soil) using actual usage of the substance (tonnage, emission scenario) and the estimation of a Predicted No Effect concentration – PNEC - (using standard ecotoxicological tests). If the ratio PEC/PNEC is below 1, the conclusion can be drawn that the chemical is of low or no concern. For pesticides, the procedure is slightly different. A standard set of ecotoxicity tests is provided according to the legal data requirements. Based on these, a so-called Regulatory Acceptable Concentration (RAC) is derived for different organism groups. The lowest RAC is then used for the risk assessment, putting it into context with the predicted exposure, usually modelled using FOCUS scenarios. If the ratio of predicted concentration / RAC is <1 there is low concern. If the ratio is close to or >1 more refined higher tier testing is an option. For pesticides, it has to be decided whether slight population effects followed by recovery are considered acceptable or not [46]. Pharmaceuticals and Biocides have also their specificities but all are assessed using the same principle of exposure estimate over toxicity indicator. For all categories of chemicals, the toxicity indicator is established in order to protect the most sensitive species / trophic level.

The present paper, in contrast, focuses on differences in characterizing chemical ecotoxicity in the context of PEF/OEF. Table 1 summarizes the main differences between the general LCA (e.g. USEtox®) approach and the general approach used in EU chemical regulation for characterizing chemical ecotoxicity (presenting succinctly the approach for industrial chemicals and pesticides).

While USEtox® relies on all available L(E)C50 data as explained above, ecotoxicological endpoints including EC10, NOEC and LOEC, which are used to report chronic toxicity test results, are presently not considered. In contrast, in risk assessment or labelling approaches, all data available for selected endpoints for a chemical are used to understand the impacts of short term and long-term exposures in support of any final conclusion. For long-term environmental exposures, there is a focus on the most sensitive species from at least

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three trophic levels representative of essential ecosystem functions to be protected. These trophic levels refer to producers (photosynthetic organisms like algae and plants), primary consumers (herbivores like *Daphnia* species), and secondary consumers (predators like carnivorous fish species). If one of these trophic levels disappears from the ecological food web, the ecosystem might collapse. Usually, the lowest ecotoxicity value from these trophic levels is used per chemical to represent its ecotoxicity and to protect the entire ecosystem. Alternatively, when ecotoxicity values are available for more than 10 exposed species, a cumulative species sensitivity distribution (SSD) can be used to derive the specific endpoint, often HC5 in ERA, where the median HC5 is the concentration that with 50% certainty is below other ecotoxicity values (e.g. EC50s) for 95% of the species tested [33,34]. In regulatory risk assessment and depending on the type of data available (acute, chronic, controlled mesocosm studies), safety factors are added. The lowest validated endpoint is also used to assess hazard criteria (persistence, bioaccumulation and toxicity, PBT) for priority setting and for deriving the classification and labelling of chemicals. The exact procedure used to classify chemicals is complex as all considered tests must be scrutinized to ensure their reliability, accuracy and adequacy. There are also important subtleties on how to deal with ecotoxicity data used for ERA and classification and labelling, which will not be further discussed in the context of the present paper. In summary, USEtox® uses an average of all species-specific, aggregated EC50 values, whereas chemical risk assessment, PBT assessment, priority setting, and classification and labelling use one of the lowest validated endpoints (i.e. EC50, NOEC, HC5, etc.).

Results of applying the USEtox® approach in the PEF/OEF context

Following the USEtox® recommended steps for deriving HC50 (see Equation 1), we calculated the HC50 and EF values for the 6 selected pesticides using ecotoxicological test results for algae, aquatic plants, invertebrates, and fish as reported in the EFSA database (DB). Table 2 summarizes the results of the calculation for the pesticide clomazone (CAS 81777-89-1), while details for the other five pesticides (namely fludioxonil (CAS 131341-86-1), halosulfuron methyl (CAS 100784-20-1), prosulfocarb (CAS 52888-80-9); teflubenzuron (CAS 83121-18-0), and fenbutatin oxide (CAS 13356-08-6)) as well as a link to the corresponding EFSA...
‘Conclusions on Pesticides’ are available in the Supplementary Material (Table S1). From Table 2 (and Table S1), we made the following observations:

1. Out of 21 ecotoxicological endpoints for 9 species available in the EFSA conclusion on clomazone, 11 tests are excluded from the analysis, because they were performed either on a formulation containing clomazone or on metabolites and, hence, these data do not represent the ecotoxicity of clomazone. Further, two tests on marine species are excluded as the effect factor refers to freshwater ecosystems.

2. Three chronic tests on two organisms (one fish species and one *Daphnia* species) are excluded, because values are expressed as NOEC, whereas USEtox® currently only uses L(E)C50 data. In acute ecotoxicity tests, L(E)C50 are the most commonly reported endpoints; however, for chronic ecotoxicity tests typically EC10, NOEC or LOEC values are reported. This results in disregarding potentially valuable chronic data for chemical ecotoxicity characterisation when only considering L(E)C50. In consequence, only five acute L(E)C50 values can be used for the final calculation of the HC50 in USEtox® for our clomazone case study.

3. Clear rules on selecting and interpreting ecotoxicological tests are lacking within the current USEtox® HC50 calculation procedure. For example, is a 7 days’ exposure duration for a macrophyte (e.g. *Lemna gibba*) an acute or a chronic exposure? Should biomass or growth rate data be used on macrophytes (analogous to algae or in contrast)? Should an EC50 value from an algae test be considered as acute, knowing that the cells divide every 20-30 min so going through a multicycle reproduction process during the 72h of test? Depending on the answer, within USEtox®, a factor of 2 will be used to extrapolate from acute to chronic. A response to these questions can be found in the literature but requires effort, relevant ecotoxicological expertise, and consensus by experts to ensure similar endpoints are handled consistently for all materials going forward [32,47–49]. A compiled overview of acute and chronic exposure durations for about 550 aquatic ecosystem species including their trophic level information is given in Table S2 of Müller et al. [50], but this list should be extended and included in
any upcoming USEtox® documentation along with additional guidance of how to properly process any related ecotoxicity test data.

4. The selection of correct input data for ecotoxicity EFs is also problematic for the average LCA or PEF/OEF practitioner. Within USEtox®, detailed guidance is currently lacking but foreseen to be included in the upcoming official documentation (http://usetox.org/documentation) on how to select the appropriate data from the literature to derive HC50 values. Without extensive guidance combined with ecotoxicological background knowledge, a practitioner may use all available data, including those that should potentially be rejected as not being reliable and/or toxicologically invalid (e.g., high mortality in the control, test item concentration not measured or not appropriately maintained). The reason for building criteria which may exclude results of a test are numerous and specific guidance is provided in the relevant EU chemicals regulation guidelines to avoid that chemical substances are assessed with invalid endpoints. In principle, each test should be assessed for its relevance, reliability and adequacy. For substances currently included in USEtox®, effect factors have been derived from a database on which a first level of scrutiny was applied; however, not all available endpoints might necessarily be fit-for-purpose. The current lack of specific guidance may lead to inconsistency in the selection of ecotoxicity data and thereby affect the calculation of effect factors.

5. Although for our case study all data were extracted from the EFSA DB, to which a high level of review and scrutiny has already been applied, the interpretation and selection of the correct endpoint for the derivation of the HC50 was nevertheless complex and time consuming. Applying this approach to thousands of chemicals, as usually reported in PEF/OEF studies, is not only a difficult task, but will likely lead to varying HC50 estimations depending on the level of expertise of the practitioner performing the work. Table 3 shows that the compiled HC50 for the six case study pesticides can vary by up to three orders of magnitude as a function of which underlying data source is applied (i.e. HC50 reported in USEtox®, pre-compiled HC50 from the AiiDA database, or HC50 based on EFSA data).
is also visible that each estimation method has used a different number of tests, species and trophic levels. Information on individual ecotoxicological tests are either not available (USEtox®) or not specified (AiiDA), making it currently impossible for users to verify which data points were taken into account in the calculation of the final HC50. Hence, this information should be made available in USEtox® for any chemical included in the future to provide maximum transparency and reproducibility of ecotoxicity EFs for PEF/OEF and LCA practitioners.

Comparison of USEtox® HC50 with values used for risk assessment

The initial investigation on 6 pesticides has been complemented with the additional selection of 26 pesticides (being also very toxic, persistent and or bio accumulative). USEtox® HC50 values are compared also for the additional set of pesticides with the lowest validated chronic toxicity value for algae, aquatic plants, Daphnia, and fish extracted from the Pesticide Properties Database (PPDB, Table 4 and Table S2). Ratios between the HC50 based on USEtox® and the lowest available value from PPDB ranged from 0.14 to > 50’000, thus differing up to 4 orders of magnitude. For 26 out of the total set of 32 case study pesticides, the ratio between the values from the two sources is greater than 10. Note that in most cases NOEC or LOEC values represent the lowest endpoints from a risk assessment perspective, while USEtox® HC50 are based mainly on (estimated) chronic values extrapolated from acute EC50 (with a factor of 2), leading to some expected inherent difference. This extrapolation factor of 2 appears to be low relative to similar factors published in the peer-reviewed literature [51,52] and it is questionable to apply it in the same way to thousands of chemicals disregarding their different properties and modes of action.

Moving from the USEtox® average approach to an approach based on the lowest chronic toxicity as a potential alternative for PEF/OEF might have a large influence on the effect factor and might potentially also affect any substance ranking in terms of ecotoxicity. The ecotoxicity ranking of the selected pesticides does not only change depending on the method used to derive the effect factor, but the absolute ratio between the values for some of those pesticides might also change.

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In an LCA context, chemicals are compared with each other as practitioners seek to confront impacts of different sets of chemicals associated with a product system life cycle and ideally identify those chemicals (or products) with lower impact on the environment. In other words, when comparing two agricultural products which include the use of pesticides for their production in terms of their overall ecotoxicity profiles, the use of average toxicity could lead to a small difference between the product systems, while using the lowest agreed toxicity value from risk assessment might show that one farm is using a much more toxic pesticide than another.

It should be recalled, that in the USEtox® characterisation factor applied in LCA, differences in the ecotoxicity of pesticides can be mitigated by differences in their fate and exposure factors leading to a potentially different contribution in the freshwater ecotoxicity impact category.

CONCLUSION & OUTLOOK

The main conclusion to be drawn from this investigation is that the USEtox model to estimate the chemical effect value has a clear impact on the conclusion to be drawn out of an PEF/OEF study. In the case of pesticides, the shift from basing the EF on average endpoint to lowest endpoint can lead to opposite conclusions on the question which product is the environmentally preferable option. It is expected that the same observations can be made for a wide range of industrial chemicals as previously demonstrated by Larsen and Hauschild (2007) [14]. We also demonstrated that the selection of the underlying data needs clear guidelines and that the use of all ecotoxicological end points (EC50 but also NOEC and LOEC) will be helpful to strengthen the comparison of chemical toxicity, as most chronic experimental data do not report EC50. As a consequence, USEtox presently does not make use of all toxicity information that may be seen as relevant when comparing the potential toxicity effects of chemicals.

Comparing chemical ecotoxicity to freshwater ecosystems ‘on a fair basis’ for use in LCA in general and for use in PEF/OEF in particular is a major challenge. The ecotoxicity of a chemical can vary between species, within the same species depending on life stage, exposure duration, endpoint assessed (mortality, reproduction, etc.), and test conditions (water hardness, pH, temperature, dissolved matters, etc.). Some
chemicals are difficult to dissolve in water, others volatilize or (bio)degrade quickly, etc. Many ecotoxicological tests failed because the test conditions were not maintained, but the results of these tests still end up in a database, because they may bring some information to those that are able to interpret them. The amount of information on ecotoxicity also varies between substances with several hundred experimental data for some and only few data points for others. In this context, comparing ecotoxicity of chemicals is not a straightforward task.

Because of this complexity, we recommend that the data selection procedure is being harmonized and clearly described and made available to users to avoid personal interpretation of the data that may lead to different estimation of effect values used in LCA and PEF/OEF.

More specifically, some substances cause effects in a narrow concentration range to different organisms (i.e. different organisms have similar ecotoxicological sensitivity), while others (especially pesticides and pharmaceuticals) may cause ecotoxicity effects to different species across a wide range of concentrations. Averaging ecotoxicity data puts generally less weight on particularly sensitive species than applying data for the most sensitive species only. In USEtox®, the toxicity of chemicals is assessed based on an arithmetic mean of the logarithm of all species-specific geometric mean L(EC)50 values. A geometric-based HC50 was chosen because it puts more weight on the lower values and hence on the more sensitive species while maintaining the statistical robustness that lies in being based on an average of effect data and offering an empirically-based quantitative link to ecosystem damage in the form of disappearance of species. However, the use of average condition ignores biological variability. It remains to be further investigated which of the two approaches (average versus most sensitive species) can be ecologically more relevant in an LCA or PEF/OEF context [53,54]. To derive an ecotoxicity effect factor to be used in LCA, different options would have to be considered in such an investigation. (1) The average HC50 takes into account all species data but tones down the influence of very sensitive species and ignores inter-species variability. (2) The use of HC5 considering the whole range of ecotoxicity data but putting more emphasis on the more sensitive species than a HC50. The use of SSD-based solutions like HC50 and HC5 has the advantage that the whole range of values across all tested species is
considered. Disadvantages of using HC5 are the higher uncertainty that accompanies it and the more cumbersome way of calculating it, compared to calculations needed for determining the HC50 or selecting the lowest toxicity value. (3) The use of the PNEC is another alternative and has the advantage to be readily available for chemicals that have been risk assessed, thanks to the REACH regulation, although the quantity of available PNEC is probably limited. (4) The use of the most sensitive species value takes into account stronger specific effects but also introduces a stronger dependence on the selection of species assessed. (5) The use of the weighted average of lowest toxicity for three trophic levels might be another alternative, but has not yet been tested in the context of LCA [55]. This approach builds largely on using consistently the same species, while weights for different species need to be further explored and validated. Table 5 summarizes the pros and cons of these 5 possible alternatives to derive an effect factor to be potentially used in a PEF/OEF context.

Since the effect factor is one of the factors that control the freshwater ecotoxicity characterisation factor [15], the possible methods used to derive this parameter deserve further analysis for their capability to identify substance of concerns. These alternatives would need to be tested on a larger set of substances and results would need to be compared to current ecotoxicity classification of chemicals (CLP/GHS) to evaluate if what is already classified as eco-toxic in EU and global chemical legislation is also considered toxic in a PEF/OEF context, and if not, what the reasons for this are.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at 10.1002/etc.xxxx.

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Data availability—All data and model are freely downloadable from public internet websites. Urls are provided directly in the paper.
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Table 1: Different approaches to derive an ‘effect’ indicator for chemicals ecotoxicity in EU chemical regulation (REACH and Plant Protection Products) and USEtox.

| USEtox®                                                                 | EU chemical regulation: REACH                                      | EU chemical regulation: PPP                                      |
|------------------------------------------------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------|
| Principle: Average toxicity from all toxicity tests                    | Principle: Lowest (valid and relevant) most sensitive trophic level. | Principle: Lowest (valid and relevant) most sensitive trophic level. |
| Gather existing experimental EC50 data for the chemical of interest. EC10, EC20, NOEC and LOEC are not used. | Collect all data and assign a Klimisch score (K1: reliable without restriction; K2: reliable with restriction; K3: unreliable...) according to guidelines. | Ecotoxicity data for a pre-defined set of aquatic organisms are provided by the applicant according to the legal data requirements and available guidance. This includes EC50, EC10 or NOEC values. |
| Specify for every EC50-value whether it is chronic or acute. If acute, extrapolate to chronic by dividing by 2. | Use only K1 and K2 and select the lowest for each trophic level: i.e. algae, invertebrate, fish, etc. | At tier 1, for each trophic level (i.e. algae/plants, invertebrates (e.g. crustaceans/insects), vertebrates (fish, amphibians)), the regulatory acceptable concentration (RAC) is calculated using the lowest endpoint (EC50 for acute, EC10/NOEC for chronic) and a safety factor (SF). In tier 1 a SF of 100 is applied to acute data and of 10 to chronic data. |
| Calculate the geometric mean EC50 (mg/l) of the data available for each individual species. | Take the lowest of the three trophic levels. In some circumstances, it is possible to calculate the geometric mean of multiple comparable toxicity values for the same species and the same endpoint. | For higher tier risk assessment, geometric means within a taxonomic group (arthropods, vertebrates, algae, etc..) are calculated if more data than from standard data requirements are available. Then the lowest geometric mean is used for the risk assessment with the same SF. |
| Take the log of the geometric means = logEC50 (mg/l) Calculate the arithmetic average of the log-values. | The effect factor EF is then calculated by dividing 0.5 by the inverse of the avlogEC50. | The effect factor EF is then calculated by dividing 0.5 by the inverse of the logEC50. |
| The effect factor EF is then calculated by dividing 0.5 by the inverse of the logEC50. | Apply safety factor (SF) to count for intra and inter species variability, and laboratory to field extrapolation: i.e. SF of 1000 if acute tests, 10 if chronic tests with different species representing three trophic levels. | In data rich situations, Species Sensitivity Distributions HC5 can be calculated and used to derive the RAC applying lower SFs. |

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| This final result gives the effect factor which characterize chemical toxicity | The result gives the PNEC: predicted no effect concentration used in ERA | The lowest derived RAC is used in the ERA which is protective for all organism groups. |
Table 2: The USEtox® approach applied on the eco-toxicological end-points of the pesticide Clomazone (CAS 81777-89-1) available in the EFSA ‘Conclusions on Pesticides’ report. The equivalent table (Table S2) for the remaining 5 pesticides can be found in supplementary materials.

| Test substance | Group | Species/Phytoplankton (genus, strain) | test (h) | Time-scale | Acute or Chronic | Endpoint | Material tested | Toxicity (mg/l) | Present in other Pesticide DB | Reason for not selecting for EC50 calculation | Extrapol. Factor | Toxicity Extrapolation (mg/l) | Geometric mean of individual species (mg/l) | Log of geometric means (mg/l) | Average of log values + LogEC10 (mg/l) | EC50 (mg/l) |
|----------------|-------|-------------------------------|---------|-----------|-----------------|----------|----------------|----------------|---------------------------|---------------------------------------------|----------------|-------------------------------|------------------------------------------|----------------|-----------------------------|--------------------------|------------------|
| Clomazone | Fish | Oncorhynchus mykiss | 16 hours (static) | Acute | LC50 | Active substance | 15.5 | In PPDB, EFSA lowest | | | 2 | 7.750 | 7.750 | 0.889 | || |
| | | | 24 hours (static) | Acute | LC50 | Formulation (exp. As a.s.) | 187.9 | | | | | | | | | 2 | 7.750 | 7.750 | 0.889 | |
| | | | 48 hours (static) | Acute | NOEC | Metabolite 1 (exp. As a.s.) | 20 | | | | | | | | | | | | |
| | | | 72 hours (static) | Acute | NOEC | Metabolite 2 (exp. As a.s.) | 20 | | | | | | | | | | | | |
| | | | 2 days (flow-through) | Chronic | NOEC (sub-lethal) | Active substance | 2.3 | In PPDB, EFSA lowest | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| | | Daphnia magna | 24 hours (flow-through) | Acute | EC50 (reduction in shell deposition) | 5.3 | Marine species | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| | | Maydopsis bahia | 96 hours (flow-through) | Acute | EC50 (immobility) | 0.57 | In PPDB, marine | Marine species | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| | | Navicula pelliculosa | 120 hours (static) | Acute | EC50 (bionass reduction) | 0.196 | In PPDB, EFSA lowest | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| | | Pseudokirchneriella subcapitata (= selenotrophi capri) | 72 hours (static) | Acute | EC50 | Active substance | 2 | | | | | | | | | | | |
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PPDB: Pesticide properties database (17)
EFSA lowest: agreed end-point to be used in environmental risk assessment http://www.efsa.europa.eu/en/supporting/pub/364e

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Table 3: Comparison of the HC50 values from the USEtox, the AiiDA and our own calculation (this paper) for six pesticides. Number of ecotoxicological tests, species and phylum used for the calculation of the effect factor are reported when available.

| Name              | Database | HC50 (mg/l) chronic | Total tests | Total nb of tests on active substance | Number of tests that can be used according to USEtox | Number of tests extrapolated (Acute to chronic) | Total nb species tested | Number trophic levels | Total phylum |
|-------------------|----------|---------------------|-------------|--------------------------------------|-----------------------------------------------------|-------------------------------------------------|------------------------|----------------------|---------------|
| Clomazone         | USEtox   | 2.5                 | ns          | ns                                   | ns                                                  | ns                                               | ns                     | ns                   | ns            |
|                   | EFSA DB  | 2.6                 | 21          | 10                                   | 5                                                   | 5                                                | 9                      | 5                    | ns            |
|                   | AiiDA    | 4.7                 | 41          | ns                                   | ns                                                  | 29                                               | 20                     | ns                   | 9             |
| Clomazone         | USEtox   | 0.16                | ns          | ns                                   | ns                                                  | ns                                               | ns                     | ns                   | ns            |
|                   | EFSA DB  | 0.15                | 25          | 8                                    | 3                                                   | 3                                                | 10                     | 5                    | ns            |
|                   | AiiDA    | 0.08                | 37          | ns                                   | ns                                                  | 30                                               | 16                     | ns                   | 6             |
| Clomazone         | USEtox   | 0.17                | ns          | ns                                   | ns                                                  | ns                                               | ns                     | ns                   | ns            |
|                   | EFSA DB  | 0.0008              | 29          | 14                                   | 3                                                   | 3                                                | 14                     | 5                    | ns            |
|                   | AiiDA    | 0.42                | 29          | ns                                   | ns                                                  | 24                                               | 14                     | ns                   | 7             |
| Prossulfocarb     | USEtox   | 0.27                | ns          | ns                                   | ns                                                  | ns                                               | ns                     | 11                   | 3             |
|                   | EFSA DB  | 0.32                | 11          | 8                                    | 5                                                   | 5                                                | 6                      | 5                    | ns            |
|                   | AiiDA    | 0.55                | 11          | 8                                    | 5                                                   | 5                                                | 6                      | 5                    | ns            |
| Teflubenzuron     | USEtox   | 1E-03               | ns          | ns                                   | ns                                                  | 3                                                | 4                      | ns                   | 4             |
|                   | EFSA DB  | 7E-03               | 15          | 7                                    | 1                                                   | 1                                                | 6                      | 4                    | ns            |
|                   | AiiDA    | 4E-02               | 7           | ns                                   | ns                                                  | 6                                                | 4                      | ns                   | 1             |
| Fenbutatin        | USEtox   | 9E-03               | ns          | ns                                   | ns                                                  | ns                                               | ns                     | 11                   | 3             |
|                   | EFSA DB  | 2E-03               | 17          | 11                                   | 3                                                   | 3                                                | 8                      | 4                    | ns            |
|                   | AiiDA    | 2E-04               | 56          | ns                                   | ns                                                  | 45                                               | 27                     | ns                   | 7             |

ns: not specified
Table 4: Comparison of the USEtox® average toxicity (HC50 mg/L) and the lowest agreed toxicity value for aquatic toxicity for algae, fish, invertebrate and aquatic plants retained by EFSA for performing aquatic environmental risk assessment. Data were extracted from the Pesticide Properties DB (PPDB).

| Name             | CAS      | USEtox_avEC50 (mg/L) | Chronic validated Endpoints (mg/L) | Ratio: HC50 USEtox/Lowest chronic (rounded number) |
|------------------|----------|----------------------|-----------------------------------|-----------------------------------------------|
|                  |          |                      | NOEC Fish¹ | NOEC Daphnia¹ | LC50 Algae¹ | Plant EC50 |                                  |
| Etofenprox       | 80844-07-1 | 2.70                 | 0.0032 | 0.000054 | > 0.15 | na | 50074                            |
| Pirimicarb       | 23103-98-2 | 16.28                | <18     | 0.0009 | 140    | na | 18093                            |
| Halosulfuron methyl | 100784-20-1 | 0.17                 | 34      | > 6.9  | 0.0053 | 0.0005 | 845                              |
| Teflubenzuron    | 83121-18-0 | 0.04                 | 0.0186 | 0.000062 | 0.02 | na | 720                              |
| Imazamox         | 114311-32-9 | 5.37                | > 122   | 137    | 0.011 | 0.014 | 488                              |
| Bifenthrin       | 82657-04-3 | 0.00036              | 0.000012 | 0.0000095 | 8   | na | 381                              |
| Chlorotoluron    | 15545-48-9 | 7.24                 | 0.4     | 16.7   | 0.024 | 0.041 | 302                              |
| Tri-allate       | 2303-17-5  | 0.60                 | 0.038   | 0.013  | 0.0022 | 2.6 | 274                              |
| Flufenacet       | 142459-58-3 | 0.26                | 0.2     | 3.26   | 0.00204 | 0.005 | 130                              |
| Metribuzin       | 21087-64-9 | 2.07                 | 5.6     | 0.32   | 0.02  | 0.011 | 103                              |
| Cyprodinil       | 121552-61-2 | 0.67                | 0.083   | 0.0088 | 2.6   | 7.74 | 76                               |
| Triasulfuron     | 82097-50-5 | 2.40                 | 36.6    | 10     | 0.035 | 0.000071 | 69                           |
| Metsulfuron-methyl | 74223-64-6 | 1.26                | 68      | 150    | NOEC 0.02 | 0.00039 | 63                           |
| Cyproconazole    | 94361-06-5 | 5.76                 | 0.65    | 0.29   | 0.099 | 0.062 | 58                               |
| Fludioxonil      | 131341-86-1 | 0.26                | 0.04    | 0.005  | 0.024 | 0.95 | 52                               |
| Lenacil          | 2164-08-1  | 0.33                 | 2.3     | 0.48   | 0.0077 | 0.022 | 47                               |
| Oxadiazon        | 19666-30-9 | 0.04                 | 0.00088 | 0.03   | 0.004 | 0.060 | 43                               |
| Tebuconazole     | 107534-96-3 | 0.38                | 0.012   | 0.01   | 1.96  | 0.147 | 32                               |
| Prosulfuron      | 94125-34-5 | 0.25                 | 5.8     | 148    | 0.0089 | 0.00129 | 28                          |
| Clomazone        | 81777-89-1 | 2.49                 | 2.3     | 2.2    | 0.136 | 37 | 18                              |

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| Compound                  | CAS Number | EC50 1 | EC50 2 | EC50 3 | EC50 4 | EC50 5 | EC50 6 |
|---------------------------|------------|--------|--------|--------|--------|--------|--------|
| Propiconazole             | 60207-90-1 | 1.15   | 0.068  | 0.31   | 0.093  | 4.12   | 17     |
| Esfenvalerate             | 66230-04-4 | 0.0008 | 0.00025| 0.00052| 0.0065 | na     | 15     |
| Isoproturon               | 34123-59-6 | 0.17   | 1      | 0.12   | 0.013  | 0.034  | 13     |
| Prosulfocarb              | 52888-80-9 | 0.55   | 0.31   | 0.045  | 0.049  | 0.72   | 12     |
| Pendimethalin             | 40487-42-1 | 0.05   | 0.006  | 0.0145 | 0.004  | 0.025  | 11     |
| Diquat (dibromide)        | 2764-72-9  | 0.12   | 0.12   | 0.125  | 0.011  | na     | 11     |
| Aclonifen                 | 74070-46-5 | 0.04   | 0.005  | 0.016  | 0.47   | 0.009  | 8      |
| Fenbutatin oxide          | 13356-08-6 | 0.01   | 0.00127| 0.016  | >0.0036| na     | 7      |
| Ziram                     | 137-30-4   | 0.06   | 0.189  | 0.01   | 0.066  | na     | 6      |
| Prochloraz                | 67747-09-5 | 0.11   | 0.049  | EC50 4.3| >0.0055| 0.174  | 2      |
| Flumetralin               | 62924-70-3 | 0.02   | EC50 0.023| EC50 >0.16| 0.85  | 0.18   | 1      |
| Lambda-Cyhalothrin        | 91465-08-6 | 0.0003 | 0.00025| 0.3    | >0.3   | na     | 0.1    |

na: not available; 1: unless otherwise specified
Table 5: Comparison of several options for deriving the Effect factor to be used in assessing the toxic impact of chemical in LCA via the USEtox model.

| Pro’s                                                                 | Con’s                                                                 | Feasibility                                                                 |
|-----------------------------------------------------------------------|-----------------------------------------------------------------------|----------------------------------------------------------------------------|
| HCS0¹                                                                | - Statistically more robust                                          | - Difficult: all toxicity data need to be collected, interpreted correctly, and finally processed to calculate the HC50. |
|                                                                       | - Less influenced by extreme values                                   | - Requires considerable ecotoxicology expertise.                            |
|                                                                       | - Recommended indicator from USEtox consensus workshop               |                                                                           |
|                                                                       | - Lends itself to damage modelling                                    |                                                                           |
|                                                                       | - Chemical toxicity ranking different from other schemes (CLP/GHS) used internationally. |                                                                           |
|                                                                       | - Some chemicals classified very toxic according to worldwide regulatory schemes may be considered less toxic. |                                                                           |
|                                                                       | - Due to absence of reported EC50 for chronic toxicity (usually expressed as NOEC or LOEC), the majority of chronic toxicity data are not used to derived the HC50. |                                                                           |
|                                                                       | - Higher variability                                                  |                                                                           |
|                                                                       | - the result depends on the number of underlying data points and the model chosen to calculate the HCS. |                                                                           |
|                                                                       | - PNEC can be derived from NOEC which is a statistically weak toxicity end point (values influenced by the test design). |                                                                           |
|                                                                       | - Data rich chemicals are penalized (the more a chemical is tested, more likely a lower value will be found). |                                                                           |
|                                                                       | - Easy to extract or to calculate from existing databases (e.g REACH) or pesticides properties DB. |                                                                           |
|                                                                       | - Only limited expertise required (value often already calculated by experts) |                                                                           |
| HCS                                                                  | - Statistical measure that takes into account all available data      |                                                                           |
|                                                                       | - Better accounts for more sensitive species than HCS0                |                                                                           |
|                                                                       | - Lends itself to damage modelling                                    |                                                                           |
|                                                                       | - Statistical measure that takes into account all available data      |                                                                           |
|                                                                       | - Better accounts for more sensitive species than HCS0                |                                                                           |
|                                                                       | - Lends itself to damage modelling                                    |                                                                           |
|                                                                       | - Higher variability                                                  |                                                                           |
|                                                                       | - The result depends on the number of underlying data points and the model chosen to calculate the HCS. |                                                                           |
| PNEC²                                                                | - Used in most worldwide regulatory schemes to assess chemical safety. |                                                                           |
|                                                                       | - Available for > thousands of chemicals in REACH and other chemical databases. |                                                                           |
|                                                                       | - Extrapolation factors are used to compensate lack of ecotoxicological data. |                                                                           |
|                                                                       | - PNEC can be derived from NOEC which is a statistically weak toxicity end point (values influenced by the test design). |                                                                           |
|                                                                       | - Data rich chemicals are penalized (the more a chemical is tested, more likely a lower value will be found). |                                                                           |
|                                                                       | - Easy to extract or to calculate from existing databases (e.g REACH) or pesticides properties DB. |                                                                           |
|                                                                       | - Only limited expertise required (value often already calculated by experts) |                                                                           |
| Lowest validated end point (lowest EC50 or NOEC, or EC10) across at least 3 trophic levels³ | - Represent the toxicity of concern of a chemical (to which trophic level the chemical is truly toxic). |                                                                           |
|                                                                       | - In line with chemical toxicity classification schemes (CLP/GHS) which used the lowest validated end point. |                                                                           |
|                                                                       | - Toxicity ranking in LCA is similar to toxicity ranking in regulatory schemes. |                                                                           |
|                                                                       | - Data rich chemicals are penalized (the more a chemical is tested, more likely a lower value will be found). |                                                                           |
|                                                                       | - If based on NOEC or LOEC, statistically weak toxicity end point (values influenced by the test design). |                                                                           |
|                                                                       | - Easy to extract or to calculate from existing databases (e.g REACH) or pesticides properties DB. |                                                                           |
|                                                                       | - Only limited expertise required (value often already calculated by experts) |                                                                           |
| Weighted average of lowest toxicity for 3 trophic levels.⁴          | - all the substances are assessed on the same set of species, avoiding situation in which the substances are in some cases evaluate with a wide number of data points and other with few data points |                                                                           |
|                                                                       | - Ensuring the three basic aquatic trophic level are                   |                                                                           |
|                                                                       | - Weighting set to be tested and further validated                    |                                                                           |
|                                                                       | - Most sensitive species not accounted for                            |                                                                           |
|                                                                       | - Never applied in LCA before                                         |                                                                           |
|                                                                       | - Easy to extract or to calculate from existing databases (e.g. REACH) or pesticides properties DB. |                                                                           |
|                                                                       | - Only limited expertise required (value often already calculated by experts) |                                                                           |

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