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Environmental
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Pellston Mixtures

PROSPECTIVE MIXTURE RISK ASSESSMENT AND MANAGEMENT PRIORITIZATIONS FOR RIVER CATCHMENTS WITH DIVERSE LAND USES

LEO POSTHUMA, COLIN BROWN, DICK DE ZWART, JERRY DIAMOND, SCOTT D. DYER, CHRISTOPHER M. HOLMES, STUART MARSHALL, G. ALLEN BURTON, JR.

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L. Posthuma et al.

Blending mixture exposure scenarios for risk assessment

PROSPECTIVE MIXTURE RISK ASSESSMENT AND MANAGEMENT PRIORITIZATIONS FOR RIVER CATCHMENTS WITH DIVERSE LAND USES

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Abstract:

Ecological risk assessment increasingly focuses on risks from chemical mixtures and multiple stressors, because ecosystems are commonly exposed to a plethora of contaminants and non-chemical stressors. To simplify the task of assessing potential mixture effects we explored three land-use related chemical emission scenarios. We applied a tiered methodology to judge the implications of the emissions of chemicals from agricultural practices, domestic discharges and urban run-off in a quantitative model. Results showed land-use dependent mixture exposures, clearly discriminating downstream effects of land uses, with unique chemical 'signatures' regarding composition, concentration and temporal patterns. Associated risks were characterized in relation to the land use scenarios. Comparisons to measured environmental concentrations and predicted impacts showed relatively good similarity. The results suggest that the land uses imply exceedances of regulatory protective Environmental Quality Standards, varying over time in relation to rain events and associated flow and dilution variation. Higher-tier analyses using ecotoxicological effect criteria confirmed that species assemblages may be affected due to exposures exceeding no-effect levels, and that mixture exposure could be associated with predicted species loss under certain situations. The model outcomes can inform various types of prioritization to support risk management, including a ranking across land uses as a whole, a ranking on characteristics of exposure times and frequencies, and various rankings of the relative role of individual chemicals. Though all results are based on *in silico* assessments, the prospective land-use based approach applied in the present study yields useful insights for simplifying and assessing potential ecological risks of chemical mixtures and can therefore be useful for catchment management decisions. This article is protected by copyright. All rights reserved

Keywords: Chemical mixtures, aquatic risk assessment, watershed management, catchment assessments, exposure scenario, ecological risk assessments

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EDITORS' NOTE

This paper is an output of a SETAC Pellston® workshop "Simplifying environmental mixtures - an aquatic exposure-based approach via exposure scenarios" which was held in March 2015 with the aim of looking at: (1) whether a simplified scenario-based approach could be used to help determine whether mixtures of chemicals posed a risk greater than that identified using single chemical based approaches, and (2), if so, what might be the magnitude and temporal aspects of the exceedances be, so as (3) to determine whether the application of the approach provides insights in mixtures of greatest concern, and the compounds dominating those mixtures (prioritization). The aims of this paper were combine the land use scenarios of the associated manuscripts of the Pellston workshop, references [1], [2] and [3], to investigate these questions for catchments with different combinations of land use.

INTRODUCTION

The goal of various environmental policies in human dominated ecosystems is to achieve a non-toxic environment and sound biological integrity [4]. This status has not been reached in many freshwater and marine systems, based on evidence on the occurrence of a wide array of chemicals in surface waters [5] and organisms' tissues [6], with associated evidence for multiple contaminant risks [7], impacts in bioassays [8], and reduced species biodiversity and abundance in various human dominated systems [9, 10]. Achieving negligible exposures and non-toxic conditions is challenging given the multitude of chemicals associated with human sources such as agricultural practices, treated wastewater and urban runoff. Currently produced chemicals may cause direct species loss, but also effects such as fish intersex and possibly other unknown effects [11], and new chemicals are continuously produced and emitted [12]. Regulatory approaches regarding chemicals presently focus, however, on a relatively small number of

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chemicals for which there are established environmental quality standards (EQS). Less is known about how to assess and reduce the risks and effects of ambient mixtures.

The assessment and management of ecological risk for a highly complex matrix of combinations of chemicals, sites, species, and ecosystems can proceed via various approaches. The traditional approach is based on risk assessment of individual chemicals, using generic protective environmental quality standards (EQS). Those are benchmark concentrations (BM), such as the predicted no-effect concentration (PNEC). A predicted or measured environmental concentration (PEC or MEC) below such a threshold is interpreted as protective of ecosystem structure and function, i.e. the risk quotient (RQ=PEC/BM, or RQ=MEC/BM) is < 1. The origin of these methods dates back to the 1970s and 80s [13, 14]. Since then tailored methods have been defined to serve specific policy goals, such as generic water quality policies and policies to determine the environmental hazards of plant protection products (PPP) for aquatic edge-of-field exposures [15]. Recently, chemical mixture assessment approaches have been recommended for practical application [16]. Many of these mixture approaches evaluate mixture risks by a default approach via aggregation of the individual risk quotients for each chemical in the mixture, such as the Hazard Index ($HI=\sum RQ=\sum[PEC/BM]$), although the expected mixture effects are also quantified via mixture toxic pressures for species assemblages, expressed as multi-substance Potentially Affected Fraction (msPAF) of species [17]. In addition, various methods are available to retrospectively evaluate the ecological risks and impacts of mixtures on the landscape scale [10]. The latter approaches offer an *a posteriori* quantitative risk or impact ranking of sites and stressors of concern (including chemical mixtures).

In this paper we describe a prospective analysis of land-use related emissions, exposures and risks of chemical mixtures. This concerns both the resulting chemical signatures (are there

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land-use specific mixture compositions [1-3]?), as well as the resulting chemical footprints (is there a net risk exported from a catchment to a downstream water body [18, 19]?). Prospective, catchment-scale prioritization of chemical mixture risks can assist decision-making regarding risk mitigation strategies [20-23]. This paper expands on and integrates three detailed analyses of land-use related scenarios, investigating the specific chemical signatures of an agriculture scenario (emissions from agricultural land dictated by rainfall, soils and plant protection product use [1]), a treated domestic wastewater scenario (daily use of household chemicals [2]) and an urban run-off scenario (rainfall-mediated emissions from city surface areas [3]).

The goal of the present study is to develop and test the utility of combining the concepts of continuous exposure of treated domestic wastewater discharge with temporally-variable chemical exposure scenarios associated with urban and agricultural land uses for the purpose of supporting comprehensive mixture risk assessments and environmental management. To achieve this, the following objectives were addressed:

1. Propose and evaluate an approach for deriving a likely chemical signature in a receiving river catchment to
    a. help explain field observations (concentrations and/or impacts)
    b. provide a background against which the toxicity of a new product or a new usage could be assessed
2. Produce an approach balancing pragmatism and simplicity with adequate detail for a scientifically credible outcome;
3. Recognize the complexity of assessing both the exposure and effects of mixtures, and derive generalizations that provide evidence for a reality check of ecological risk assessment; and,

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4. Identify uncertainties and gaps in knowledge requiring further research to refine the prospective assessment of chemical mixtures.

COMBINED SCENARIOS

Overall approach

We integrated risk assessment approaches for three typical human-based emission scenarios (AGR-agriculture; DOM-domestic; URB-urban runoff) and focused on identifying the potential for mixture effects in receiving waters. The scenarios were selected because they commonly occur in human-dominated systems and differ vastly in their chemical emission characteristics. The scenarios were further developed and substantiated as land-use scenarios, whereby DOM and URB are combined as the land use CITY. Further, the land use nature (NAT) was added for demonstrating the influence of water inputs within the catchment where chemical emissions are negligible. The scenarios were combined in a catchment assessment model, with the option to define land uses for between 1 and 10 sub-catchments. Their integration placed the different single land use categories into a landscape-level perspective. This allowed for cross-comparisons and integrated exposure and risk analyses, to evaluate the utility and limitations of land use scenarios for environmental assessment and potential management of chemical mixtures.

Modelling land uses, geography and hydrology

The scenarios AGR, CITY (DOM+URB) and NAT were spatially combined in hypothetical but realistic spatial arrangements to represent either a single-land use scenario in a sub-catchment, or a catchment with multiple land uses and river confluences. A spreadsheet model represented the various catchment layouts. The model included: hydrology, aquatic emissions, concentrations and mixture assessment outcomes for (in its most complex format) a

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catchment of 100 km^2 with 10 sub-catchments of 10 km^2 each, linked within a river network (Figure 1). A sub-catchment was defined to have only one land use. A catchment can have any combination and number of sub-catchments (in our case, up to 10) and assigned land uses. The land uses shown in the Figure define the layout of the modelled MIXED land use scenario, which is just one of many possible catchment layouts.

The characteristics of the separate emission scenarios (AGR, DOM, URB, and NAT) were developed based on literature reviews and by combining hydrological- and ecotoxicological modelling techniques with regulatory judgment criteria (Table 1). Each scenario layout was modelled for 20 years, with daily quantifications of predicted environmental concentrations (PECs) for the each of the studied chemicals. Details are in the Supporting Information (S.I. section 1) and the scenario review papers [1-3].

Modelling concentrations

Emissions of chemicals from AGR, DOM and URB were derived from individual land use studies (details in those papers and the S.I.). The AGR scenario incorporated time dependency of emissions related to PPP use on row crops. A 20-year time period was modelled on a daily basis by using actual pesticide usage application data for a large arable farm in eastern England (see [1]) and actual rain-events from the FOCUS R1 scenario meteorological dataset (used in EU regulatory modeling for PPPs) which is directly applicable to UK agricultural conditions. The selected AGR scenario used a winter wheat exposure scenario, with 13 active ingredients applied on known dates and rates. Accordingly, the scenarios for the other emissions (DOM, URB) were reformulated to enable modelling for the same 20-year period, and combined into the spreadsheet model. Emission data and hydrological data were combined to estimate concentrations for each of the studied chemicals emitted from each of the land uses.

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The spreadsheet model allowed the prediction of concentrations from AGR, DOM and URB emissions separately, as well as their combinations based on the sub-catchment configuration (Figure 1). The model yields 24-hrs PECs for sub-catchment outlets. Large numbers of PECs were calculated using this approach. For example, for AGR the number of PECs equals 94,198 (7246 days, 13 chemicals), and for MIXED 268,102 (7246 days, 37 compounds).

Risk assessment methodologies and prioritizations

The risk patterns associated with the PECs were explored using three approaches: Hazard Indices (HI), Maximum Cumulative Ratios (MCR, [24]), and mixture toxic pressures (multi-substance Potentially Affected Fraction of species, msPAF, [17]). Details are in S.I. section 2.

First, the risks posed by a mixture were determined using individual chemical hazard quotients (HQ) and the net hazard index (HI), in which $HQ_{ij}=PEC_i/BM_{ij}$ (with HQ=Hazard Quotient, and BM=Benchmark concentration, i = substance, j = selected effect endpoint, with j defined as regulatory EQS, chronic-NOEC or acute-EC50, see below), and $HI_j=\Sigma HQ_{ij}$. The Hazard Index is the sum of the individual values of compound-related HQs, implying the use of concentration additivity as default mixture model.

Second, the MCR is the maximum cumulative ratio posed by a combined exposure to multiple chemicals under the assumption of concentration addition divided by the risk of the most toxic compound of the sample. The MCR of a sample expresses whether the net predicted toxicity is driven by multiple components which make a significant contribution to the net mixture toxicity. The MCR-value of a sample was calculated as the ratio of the sample's HI and the highest value of the sample's set of HQ-values: MCR = HI /max(HQ). The combination of

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HI and MCR was used to create subgroupings of the 7246 time samples per scenario in four groups, viz. Group I, Group II, Group IIIA and Group IIIB (Table 2).

The HI-MCR method was applied using different benchmark definitions to derive the HI, representing different tiers and meanings. For Tier 1, HI's were defined by generic, protective regulatory criteria (here: the annual average EQS [AA-EQS] of the European Water Framework Directive). For Tier 2, HI's were defined via the 5th percentile of the SSD of chronic NOECs and the 50th percentile of the SSD of EC50s. For Tier 3, the MCR was plotted against the mixture toxic pressure (msPAF), derived from the SSD models (SSD_{NOEC} and SSD_{EC50}, respectively). In Tier 1, HI>1 indicates regulatory concern, whereby it remains uncertain whether direct ecotoxicological effects are likely, e.g., due to underlying application factors. In Tier 2 and 3, HI>1 is interpreted as a signal for direct chronic or acute effects on species assemblages, while these HI's have no maximum. In Tier 3, additionally, the predicted mixture impacts is maximized to 100% of species affected at a chronic or an acute level, respectively. The MCR-axis is interpreted as to the number of compounds contributing to the mixture risk.

The scenario results were also summarized as chemical footprints [18]. A chemical footprint expresses whether the net emissions in a landscape remain within a pre-set boundary on risks or effects, for example, the mixture exposure level at which 95% of the species is protected against exceedance of their no-effect level for the mixture ($msPAF_{NOEC}<0.05$). Here, the approach is modified to summarize the percentage of days the latter is exceeded at the outflow of a sub-catchment based on the P95 of the $msPAF_{NOEC}$ of all days of a scenario run.

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RESULTS

Rainfall and flow

The natural rainfall varied over time, and resulted in variation in flow. The vast numbers of input data on rain and output data generated on flow (7246 per scenario) are summarized in the S.I. section 3. The outputs show that the variation in flow implied a strong influence on the dilution of emitted chemical loads and domestic discharge effluents. Summarized as the P99.9/P5 flow ratios, the high-low flow ratios were 55, 324, 128 and 94 for the scenarios CITY, AGR, NAT, and MIXED, respectively.

Predicted Environmental Concentrations (PECs)

The temporal variability of PECs is illustrated in Figure 2. The chemical concentrations varied over time due to the sequential use of PPPs combined with rain events (AGR) and rain events passing the runoff threshold of 10.3 mm rain (URB). For DOM, though the per-capita use of chemicals in this scenario was constant over time, the resulting PECs show spatio-temporal variation due to the effects of variations in hydrological conditions.

Predicted and Measured Environmental Concentrations

Predicted environmental concentrations (PECs) were compared to measured values (MECs) obtained from available databases and literature (Figure 3, details in SI section 4). Averaged over the chemicals and as far as represented in the monitoring databases, the fractions of river water samples with measured concentrations higher than the limit of quantification was 1.4% for AGR, 59.8% for DOM and 14.1% for URB chemicals, respectively. For many field samples (frequency for AGR>URB>DOM) the MECs were lower than the limit of quantification. The percentiles of the MEC-distributions (Figure 3) therefore refer to the subset

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of samples with quantifiable concentrations, and those of the PECs to the total set of 7246 predicted values for a compound.

For some chemicals, for example pendimethalin in the AGR-scenario, the upper percentiles of European river water MEC distributions were very similar to the scenario-based PECs. For other chemicals, the highest MEC percentiles were greater (e.g., chlorothanonil), or lower (e.g., caffeine) than the higher PEC percentiles. Given the flow variation, the degree of similarity between detected MEC percentiles and PEC percentiles suggests that the land use scenarios resulted in predicted exposures that may occur in European rivers.

Risk characterization step 1: PECs and exceedance of regulatory endpoints

Tier-1 results show that the regulatory benchmark concentrations were exceeded for various sub-catchment outlet days and for various compounds (HI>1, see SI-Tables 7, 8 and 9). Looking at peak exposures (represented by P95-PEC), the peak PECs of e.g. pendimethalin exceeded the AA-EQS and the MAC-EQS of this compound 8 and 6 times, respectively. For the DOM-scenario, the peak exposure of ethinylestradiol and galaxolide exceeded the AA-EQS 4 and 7.5 times, respectively. For the URB-scenario, the highest exceedance was found for deltamethrin, where the peak exposure was 1171 times the standard. Whether exceedances imply ecotoxic effects depends not only on the magnitude but also on the duration of exposure. This also varied. For example, for 7.3%, 80%, 91% and 5% of the days there was an exceedance of the AA-EQS of pendimethalin (AGR), ethinyl estradiol (DOM), galaxolide (DOM), deltamethrin (URB), respectively. Exposures can thus be shorter or longer, and frequent or incidental. These results suggest, from a regulatory perspective, that the river system at the outlet of a sub-catchment or the whole catchment was not sufficiently protected, although high values may also

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result from high HQ-values resulting from a high AF related to high uncertainty on the benchment (defining a low benchmark due to high data uncertainty).

Risk characterization step 2, characterization of hazard indices of mixtures

The results of Tier 1 were summarized as HI-MCR plots. The MIXED land use (Figure 1) resulted in the plotting of 7246 HI-MCA data points, which partly overlay each other (Figure 4). The figure suggest that the water at the outflow of the catchment often showed HI-values often >>1, which means that the RQs of individual compounds were (far) exceeded, while some of the HI-points (with HI>10,000) are not shown. The latter values were found to be related to chemicals of mainly the URB-scenario, for days after peak rainfall (causing a runoff event), for chemicals with low AA-EQS. The water system is judged to be insufficiently protected for 96% of the days, whereby the MCR remained below 6, with a high frequency of MCR ≅ 3, and many MCR's < 3. The theoretical maximum MCR of the MIXED scenario is 37 (when the 37 compounds considered in this scenario are present at equitoxic concentrations, which is unlikely in nature). The relatively low MCR's suggest that a low number of compounds (always less than 7) induce $HI_{AA\text{-}EQS}>1$). The high frequency of similar MCRs at a single level is attributable to a similar change of HI and the maximum-HQ of a sample with dilution, due to which HI (X) can vary at nearly constant MCR (Y), while the typical HI-MCR pattern in the CITY-scenario related to threshold effects (runoff >10.3 mm rainfall). This threshold contributed to 'forcing' the specific pattern of CITY-MCRs to two key MCR-levels, related to runoff chemicals' effect criteria.

The Tier-2 analyses resulted in modified HI-MCR patterns, slightly shifted left for the criterion based on the 95th percent protection level (Figure 5, upper graphs). Note that both the HI and the MCA of a data point change when the standards underlying the HI change from AA-

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EQS to another effect criterion. A Tier-2 evaluation based on EC50s resulted in a further shift of the data points to the left, so that only few samples were found where PECs have exceeded the EC50 of one or more compounds. Species loss was predicted for those samples, given an earlier observation that $msPAF_{EC50}$ relates to observed species loss in mixture-exposed aquatic systems [25]. Note that defining another Tier-2 HI using, for example, an EC10 or EC25 as benchmark, would result in intermediate shifts (between Figure 5, top and bottom); i.e., between chronic exceedance of NOECs and the earliest onset of effects and species loss.

Exposure frequency and time are important in the process of causing ecotoxic effects. Whereas the data points of Figure 5, bottom, may indicate that peak exposures may induce species loss, the same is not true for the data points of Figure 5, top, as those points predict impacts under the condition that chronic exposure occurs. Investigations showed that the exposure times varied across the land uses. For the acute MIXED scenario, the percentage of days and the maximum number of consecutive days for which the mixture exposure HI>1 is 0.1% and four days, respectively. The period of high exposure at the outflow of the MIXED-scenario is commonly short, but there are a few instances of a few days of exceedance of the mixture-EC50. For AGR, the majority of days where $HI_{NOEC}>1$ were for a single day. Only on 31 days (0.4%) was the exceedance 2 to 3 days, with no periods of 4 or more days with $HI_{NOEC}>1$. In short, there was no chronic exposure. The exposure duration differed vastly for CITY, where the majority of days showed $HI_{NOEC}>1$ (88% of days), and 98% of the exposure lasted at least four consecutive days. The main CITY-emission effects were reflected in the exposure durations of the MIXED-scenario ($HI_{NOEC}>1$ for 93% of days, and 86% of exposures lasting at least four consecutive days).

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Risk characterization step 3 - mixture toxic pressures

The risk characterization in step 3 consisted of expressing the mixture risks as $\text{msPAF}_{\text{NOEC}}$ and plotting these outputs again *vis a vis* the MCAs. The results in Figure 6 suggest that the 95%-protection level is exceeded on 8% of the days for AGR, and 100% of the days for CITY (as well as MIXED, not shown), while these chronic toxic pressure levels are associated most often with a few compounds in the mixtures (judged by the MCR-values). The CITY and MIXED scenarios consisted of exposures of a chronic kind, so that the land use would imply chronic effects for aquatic species assemblages. Acute effects though, quantified via $\text{msPAF}_{\text{EC50}}$, are more restricted. The maximum acute toxic pressure for AGR would affect 8% of the species, whereby 1 out of 1000 species would be affected at the peak exposure days (P95 of $\text{msPAF}_{\text{EC50}} \cong 0.001$). For MIXED these values are 63% of the species at the day of the most toxic mixture outflow, and 10% of the species at P95.

Prioritization

Various prioritization analyses can be made to underpin the choice of an abatement scenario aimed at water quality improvement. While in practice ‘ease of implementation’ of abatement measures will be important too, we consider here various rationales of risk-driven prioritization. Details are in S.I. Section 4.

First, prioritization on the basis of land use showed the rank-order of mixture risks of CITY (DOM+URB) > AGR, for three hazard index definitions (Table 3). A Tier-1 signal for regulatory concern was most frequent (exposure > AA-EQS), followed by the frequency of direct sublethal ecotoxic effects (exposure >NOEC), with a low number of modelled samples with species loss of >50%. In the MIXED scenario, prioritizing of the maximum HI’s using the Tier-2 approach resulted in the mixture risk rank order CITY (URB+DOM) > MIXED >> AGR (Table

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3). The resulting chemical signatures (composition of mixtures and levels of exposure) clearly differ regarding land use.

Second, prioritizations for exposure periods also differ. AGR was characterized by peak exposures (always <2 successive days with mixture HI>1), whereas CITY (URB+DOM) and MIXED were characterized by chronically high HIs. Chemical signatures differed regarding exposure dynamics, and even the constant-emission of DOM appeared highly dynamic related to hydrology. Further examples are in SI Table 10.

Third, the relative importance of chemicals was assessed. Many prioritizations can be made here, e.g., for Tier 1, 2 or 3 evaluations in each scenario, and then on a daily basis (determining the relative importance of each chemical on day=t, 7246 times per scenario) or for the numbers of days where the mixture HI>1. Outcomes are in SI Table 10. It appeared that risk prioritization outcomes depend heavily on the tier and inherent risk characterization method. For AGR, chlorothanonil was for example 6th in rank judged by the AA-EQS definition of HI, but 1st when judged by chronic SSD_{NOEC}-HI. Again, prioritization needs to account for temporal aspects. Chemicals in DOM would have priority when considering the more chronic character of DOM exposures over URB exposures, while the latter contribute more to the risk of mixtures when present after a runoff event. Comparison to the individual scenario studies demonstrated that the prioritizations shown in SI Table 10 are in line with the outcomes of those scenario studies. For AGR in the current study, cypermethrin, pendimethalin and chlorothanonil were found to be important regarding peak exposure levels, ranking 1st, 2nd and 3rd using AA-EQS to define HI. Those were also ranking high in the agriculture study, with RACs as assessment criteria [1]. The rankings according to exposure time also showed similar results. The rankings for chronic ecotoxic effects only (our results) identified chlorothalonil and cypermethrin as 1st

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and 2nd ranking compounds, which is also in line with the earlier study. For the chemicals emitted in the DOM scenario, the outcomes for galaxolide and ethinylestradiol co-rank high, although linear alkylbenzene sulfonate ranked lower in the MIXED scenario analyses than in the earlier scenario study [2]. For URB, the top-ranked chemicals were deltamethrin, bifenthrin, permethrin, copper and zinc, which also rank highly when assessed using landscape scenario analyses [3]. In general, it can be stated that the prioritization options are many, that prioritization outcomes are dynamic in space and time, and – hence – that the problem definition phase should be used to define precisely which ranking information is most valuable for selecting an abatement option. Regulatory prioritization used to prospectively steer preventive policies can thus be different from more realized environmental quality based rankings [26].

Chemical footprints

The land use scenarios were summarized as chemical footprints (CF) for direct, chronic risks for species assemblages. Chemical footprints were quantified using the P95 of the 7246 msPAFNOEC-outputs for each scenario (Table 4). A CF in this definition can be used as management summary information, for example when the P95-msPAF$_{NOEC}$>0.05 this means that for 5 percent of the days the (sub-)catchment outflow is ecotoxic such that the 95%-protection level is exceeded, whereby a higher degree of exceedance of 0.05 implies a higher potential of the mixtures to affect species assemblages in the downstream water body. In other words, the CF=6 for AGR means that the 95%-protection level is exceeded by a factor of six or more for 5% of the outflow days. The ecological implication of that depends on exposure time and downstream water body characteristics, although the CF signals ‘net outflow of toxicity’. In AGR, chronic exposures were not found due to the swift effects of the flow regime. In a real

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system, though, chronic effects related to this CF may occur when chemicals would slowly accumulate in a water body, e.g. in a lentic water body downstream of the outlet.

The CF results ranked the risks of mixtures as CITY > MIXED > AGR, due to higher CF-values and longer exposure durations. An additional scenario – AGR along a river stretch with three 10-km^2 areas with nature downstream (AGR-NAT-NAT-NAT) – implied a reduction of the CF as compared to AGR only. For CITY the same layout did not reduce the CF substantially, related to the fact that the CF for the CITY scenario (0.95) is at the upper end of an exposure-mixture risk model which has a sigmoidal shape (like the underlying SSD model), so that a change in chemical emissions induced an equivalent reduction in CF. As an illustration of the option to evaluate abatement strategies, the bottom lines of the Table show changes in CF following from (imaginary) emission reductions for all chemicals by 25%, 50% or 75%. The latter related to only a 47% lowered CF, but an 80% reduction regarding exposure periods for the number of days with HI>1, and of 90% for the number of days on which HI>1 caused by 1 compound. The 75%-abatement option quantified for the MIXED scenario implied that species assemblages at the catchment outflow experience lower exposure peaks, which are much less frequent and more often attributed to a single chemical.

DISCUSSION

Overview

The large number of chemicals detected in aquatic environments currently implies that there are large uncertainties regarding the question whether or not there is sufficient environmental protection against the adverse effects of individual chemicals and their mixtures. The number and diversity of mixtures in the environment seem to imply an intractable number of combinations of exposures, risks, and associated effects, and a remaining open end to the

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problem. This conundrum is often addressed using simplistic approaches (e.g., focusing on priority chemicals) that focus on protection, but that ignore mixtures, and that use assessment factors to account for the enumerable types of mixtures and uncertainties. However, despite the *in silico* approach of the present study, the present results clearly indicate that the integrated assessment of numerous chemicals with different policy regimes (such as industrial chemicals and plant protection products) and spatial-temporal exposure patterns is tractable. Further, the present study demonstrates an application of a strategic tiered approach, which provides refined ecotoxicological insights into the presence of risks for species assemblages (or even specific taxonomic groups, see [1]). Therefore, the current paper presents a testable framework designed to explore simplification and clarification of the spatio-temporal complexity of exposures and provides an approach for forecasting risks based on scenarios created to capture the major influences on exposure for a given catchment or region. The study was based on three emission scenario assessments, built into a single approach to model emissions and risks at the scale of realistic combinations of sub-catchments and land uses.

Comparison of predicted and observed parameters

A striking feature of the results was that the finding that the PEC variability resembled the observed ranges of the respective measured concentrations in river water samples (EMPODAT), despite considerable variation of modelled and measured data and technical limits regarding measuring compounds in field samples (Figure 3). The most striking observation was that the *in silico* modelled land use scenarios (Figure 4, Figure 5) yielded a HI-MCR plot similar to that from a field study in which 12 to 81 plant protection products were measured per sample [24] (Figure 7), although the field study employed acute risk benchmarks (while we applied chronic ones). The difference between the present study and the agriculture study [1] is caused

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by the use of Regulatory Acceptable Concentrations to define mixture HIs in that study (this includes AFs of 100 to 1000 across compounds). The comparisons between predicted and observed data suggest that many of the findings of the present study can occur in true catchments. Therefore, the key patterns (below) bear relevant insights for assessing and managing complex mixtures n relation to land use.

Key patterns in the data

The similarities of exposures and hazard plots allow deriving some key observations.

First, land use matters. Land use appears to imply a typical chemical signature in receiving water bodies. A signature consists of a typical chemical composition (chemicals, concentrations) and exposure time aspects (durations, frequencies). Attempts to solve existing mixture exposures in aquatic systems could therefore focus on de-coupling land use from aquatic systems, e.g., via buffer zones, waste water treatment, or reduced urban runoff emission events. Such actions would imply a change in emission of suites of chemicals, with those suites including the set of chemicals of high priority within the land use. Abating chemical risks can utilize a suite of options, not solely a chemo-centric approach [27, 28], and it was e.g. shown earlier that an analysis of spatial associations between emission points and water bodies with sensitive functions (drinking water production, protected nature) can be a basis to reduce impacts via smart spatial arrangements [21], and that clever strategies may be utilized to reduce adverse effects of chemicals and other water quality parameters [7, 29]. From upstream to downstream, land use influences on smaller tributaries may be characterized by mixtures with greater exposures and simpler composition, with a 'land-use dilution' effect in the downstream direction [30].

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Second, flow and runoff events matter, related to rain events. Even though it was expected that domestic emissions would result in relatively constant exposures, the opposite is true in the smaller tributaries in our case. The results highlight the importance of rain events and subsequent dilution phenomena. Smaller rivers may be characterized by high temporal variability in chemical concentrations, whether or not there is a constant or an intermittent emission source (DOM *vis a vis* AGR spraying/runoff and URB runoff). Species in flowing aquatic systems can thus be exposed to mixtures that change rapidly in composition. A recent example [31] showed large changes of MECs of untreated waste water emissions in the Danube over the scale of a few kilometers only. Note that the PECs predicted for the sub-catchments (current model) in reality could imply higher exposures at the points where true chemical emissions occur (e.g, edge-of-field exposures for agricultural chemicals, and end-of-pipe exposures at WWTP-outlets and sewer overflows). The spatial and temporal variation we modelled implies challenges for the design of monitoring schemes for flowing waters, and indicates that spatio-temporal variation may disturb a straightforward interpretation of MEC data *vis a vis* the regulatory standards such as AA-EQS [32]. For example, there may be doubts whether the MECs of a set of water samples are ‘representative’ for the system, given spatio-temporal variability that may be order(s) of magnitude. Modelling can help to improve understanding the mixture risks of such systems.

Third, the choice of the assessment benchmarks matters. The integrated scenario analyses differ in this respect from the individual scenario studies [1-3], where various toxicity standards were used (see also Table 1). The uniform use of AA-EQS values in the current study resulted in a large number of days triggering regulatory concern, whilst an inspection of the ecological implications of direct effects of mixture exposures (chronic or acute) showed substantially lower fractions of samples potentially causing direct effects on species assemblages (both related to

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peak exposures as well as non-chronic exposure times). This difference shows that it is important not to over-interpret criteria exceedances, such as the PNEC or the AA-EQS. The exceedance of such a criterion triggers regulatory concern, which should be translated into more specific information on the potential occurrence of direct ecological effects, secondary poisoning effects, or human health concern, or into a trigger to improve the EQS itself when the AF for one or more compounds is high. Avoiding misinterpretations has been proven useful for water quality management [33].

Fourth, prioritization choices matter. Prioritization helps in selecting of cost-effective abatement strategies. A suite of prioritization options can be envisaged, and these result in vastly different lists of compounds for further attention [34]. The current study shows the effects of prioritization choices. Relevant information can be obtained from comparing land uses (clear ranking), exposure types (chronic or intermittent) and chemicals within mixtures. The latter is often used in practice, relating to the current identification of priority hazardous substances and substances prioritized for adoption on a ‘watch list’ (regulatory attention triggered, [35]). The observation of land use specific chemical signatures suggests that chemicals that rank high in priority may serve as surrogates of co-occurring, non-modelled or measured substances [5]. Regulatory priority substances may be indeed prioritized, but may also be of marginal importance for a catchment. Of the modelled compounds cypermethrin is a priority substance for European water policies and ethinyl estradiol is identified as candidate for the watch list [35]. In the present study, we found various substances ranking high in various ways which are not prioritized- or watch list chemicals in the context of current regulation [35], e.g., deltamethrin, permethrin, bifenthrin, galaxolide, sulfomethoxazole, caffeine, carbamazepine, pendimethalin, flufenacet, mesosulfuron-methyl and fluoxastrobin. Regulatory attention may be warranted

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beyond regulatory lists, in line with other categorization options [36]. River basin management is likely served best by a critical application of prioritizations, looking at land use, temporal aspects, chemicals of generic interest (e.g. at the European scale) and chemicals of interest given land use practices. For a sub-catchment, listed priority compounds may pose negligible risks within a given catchment and conversely non-listed compounds may be of high local priority for management. Neglect of compounds because of absence from a central listing can be called a case of unjustified reification. Reification is the process through which concepts (such as 'priority compounds') are increasingly interpreted as facts. Reification fallacies may seriously affect policy making [37, 38]. Unjustified interpretations can induce Type-I errors (risk signals triggering abatement costs, without the signal being related to true impacts [39]) as well as Type-II errors (the potential impacts of many chemicals and their mixtures are neglected or remain unknown due to limitations of current science).

Fifth, the analyses always resulted in a clear identification of some chemicals contributing most to risks in mixtures. This phenomenon seems to be universal in field-related mixture studies, as substantiated a variety of other assessments [18, 24, 40-43] (see also Figure 7). The outputs of our study suggest strong simplifying patterns of risk in highly diverse sets of mixture exposures. Land-use related chemical signatures appear to exist, whereby mixture effects are commonly due to a few chemicals (for a given toxicological endpoint), although those few chemicals differ with land use and time [44].

Sixth, the reporting of findings as chemical footprint information summarizes the data for an area in easily understood metrics: the multiplication factor that mixture toxic stress of a sample exceeds a benchmark, which can be interpreted as a measure of the number of times a sample needs to be diluted before the effects are below the benchmark. In this evaluation, the

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dilution factors needed for the different land use scenarios were 6, 19 and 9 for agriculture (realistic winter wheat scenario), a city (10,000 people/ 10 km^2) and a mixed-land use scenario (Figure 1) to yield 95% of the species protected against NOEC-exceedance due to mixture exposure for 95% of the days. Note that, commonly, various fate processes that we did not model may lower exposures in field conditions, which likely results in lower risks and CFs. The predicted CF-values are in line with other chemical footprint analyses for Europe [18, 19]. In addition, the change in CF can be determined for varying catchment configurations (of URB+DOM, AGR, NAT), and the effects of abatement options on the footprint can be explored (Table 4). Such summaries enable exploratory investigations as to the ecological risk reduction of altering landscape structure, or impacts of alternative chemicals used for specific goals (e.g, choice of PPPs), or of chemical-specific or generically effective abatement strategies, such as buffer zones [45].

Further analyses

Further data analyses are possible; e.g., investigating which taxonomic groups are likely to be most affected by mixtures, or checking time-weighted averaged exposures, the effects of the rainfall threshold causing city runoff, and analyses based on measured efficacies of e.g. buffer zones between human activities and water systems. The refinement for taxonomic groups was already worked out in detail for the water samples of MCR-Group IIIB of the AGR-scenario [1]. Such analyses can refine insights into potentially sensitive groups. As this effect is most prominent for the AGR-scenario, and as the original scenario study presents such outcomes in detail, we refer to that study for details of this kind [1].

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CONCLUSIONS

Based on the conceptual and practical evaluation of an integrative scenario, blending earlier reviewed AGR, DOM and URB scenario data, and acknowledging the limitations of this purely *in silico* study, we conclude:

1. It is possible to create a catchment-oriented approach, encompassing land-use related emissions of chemicals, rain events and hydrological phenomena, to predict likely chemical profiles in receiving river catchments:
    a. The PECs generated by this approach bear a reasonable relationship with measured concentrations of chemicals
    b. The predicted patterns of ecological risks, both regarding their magnitude as well as their maximum cumulative ratios, bear a reasonable resemblance to the pattern based on field data
2. The land-use based approach, with realistic rain events and flow variation, results in highly variable mixture compositions in space and time (composition and concentrations of chemical mixtures), but also in simplified signatures and prioritizations,
3. The outcomes demonstrate spatio-temporal variability of exposure and potential ecological impacts of chemical mixtures in human-dominated systems, but also allowed for simplifying generalizations, such as the potential for various meaningful prioritizations for risk management;
4. The complexity of true catchments and land uses can be addressed through science-based approaches that consider exposure scenarios for a wide-range of ecosystems and land-use types (here dominated by agricultural, urban and domestic wastewater treatment inputs),

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but this requires developing "road map" scenarios with typical exposures for prospective and retrospective risk assessments, and linking to management actions;

5. The varying exposure patterns can be described across ecosystem and land-use types by converting loadings to environmental concentrations in time-varying river flows, and finally ecotoxicologically-relevant endpoints such as hazard quotients and indices and mixture toxic pressures, that can be related in a tiered way to expected net mixture impacts,;
6. The explanation of outcomes of modelled or measured water quality assessments requires specific attention, to avoid over-interpretation of lower-tier methods.
7. The proposed approach for evaluating chemical mixture risks has a wide range of potential regulatory applications where approaches to mixture risk assessment are needed.

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

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Figure 1. The MIXED land use scenario layout, of 10 sub-catchments of similar size (10 km^2 each) in a total catchment of maximally 100 km^2. Water flows from the top of the figure to the bottom. Top (Table): scenario definition table, defining the catchment, with land use and associated emission types. Bottom: resulting catchment map with position codes (related to lag times of flow, left) and land use codes (right) as defined in the scenario definition table. The different color intensities of the sub-catchments indicate various hydrological travel times to reach the main catchment outlet, which enables modelling of time-dependent chemical fate processes. Other scenarios can be defined via entering codes for the lag-times of the land uses in the scenario definition table.

Figure 2. Illustration of the temporal variability of PECs, for two substances of the AGR-scenario (boscalid and pendimethalin), one for the DOM-scenario (ibuprofen) and one for the URB-scenario (fluoranthene).

Figure 3. Comparison of measured environmental concentrations (MECs) of surface water systems, summarized as P5, P50, P95 and P99.9 of samples with a detectable concentration (>LOQ in the monitoring data), and predicted environmental concentrations (PECs), summarized as P95 at the outflow of a sub-catchment. Grey bars: MECs (darkening grey tones from low to high percentiles of detectable MECs); white and black bars: PECs of sub-catchments with a single land use and the MIXED scenario, respectively. The P99.9 percentiles are added to demonstrate the magnitude of peak concentrations within the series of 7246 daily PECs per scenario. The flow P99.9/P5 ratio is added to illustrate the magnitude of dilution (PEC) variation related to flow.

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Figure 4. Judgment of the 7246 HI-MCR data points for predicted mixtures at the outlet of the whole 100 km^2 catchment, according to the land use scenario depicted in Figure 1, evaluated by a generically protective regulatory criterion, the AA-EQS, to define the HI. Left: for all dates, right: for a single (randomly selected) year. Note: in the left sub-figure some extremely high HI data points are not shown (see text).

Figure 5. Judgment of the 7246 mixtures at the outlet of the whole 100 km^2 catchment, according to the land use scenario depicted in Figure 1, judged by compound-specific HQs derived from the 5th percentile of SSD-NOEC's (top) and the 50th percentile of SSD-EC50's (bottom). Left: for all dates, right: for a single (randomly selected) year.

Figure 6. Tier-3 analyses of mixture impacts in scenarios with (left) land use AGR and CITY (DOM+URB) and (right) the MIXED scenario of Figure 1. The dotted line at $msPAF_{NOEC}=0.05$ is the 95%-protection criterion which was originally used in the derivation of PNECs for individual compounds. Water samples positioned right of the dotted line are mixture exposures at a level that, if exposure is indeed chronic, induces chronic effects to the fraction of species indicated on the X-axis.

Figure 7. Overlay of the HI-MCR plots of 4380 measured concentrations of plant protection products in U.S. watersheds [24] and of 7246 daily samples with associated PECs from the AGR sub-catchment. HI's were based on acute aquatic benchmarks for ecotoxicological effects and on the 5th percentiles of the SSD_{NOEC} respectively, with the latter representing a more sensitive endpoint. Black dots: field data, white dots: current model results.

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Table 1. Characteristics of the original land use scenario studies [1-3], and evaluations based on a sub-catchment area of 10-km^2. PPP=Plant protection product. PNEC = Predicted No Effect Concentration, utilized in generic protective chemical regulations. RAC = Regulatory Acceptable Concentrations for edge-of-field water bodies, utilized in PPP-regulations. RCR = Risk Characterization Ratio (similar to HI in the present study). $msPAF_{EC50}$ = multi-substance Potentially Affected Fraction of species exposed beyond their EC50. [1] In the present paper a PEC-benchmark ratio is generally referred to as Hazard Index, HI.

Scenario →	**AGR - agriculture**	**DOM - Domestic**	**URBAN – urban run off**	**Nature**
Emissions	Rain-event and PPP-use related (discontinuous, PPP-use related to crop type)	Household-related (continuous, household chemicals, WWTP-chemical removal efficacies in SI Table 1)	Rain-event related (discontinuous, from wearing of buildings, brake pads, oils, etc.)	None
Emissions Source	13 PPP's applied annually to winter wheat	Typical # people / area, (10,000 inhabitants) Water use 200 L / person.day Effluent flow 0.0231 m^3/sec	Runoff, occurring when >10.3 mm rainfall per day (P95 of rainfall)	None
Chemicals	Boscalid (A-BOS) Chlorothalonil (A-CHLOR) Cypermethrin (A-CYP) Epoxiconazole (A-EPOX) Flufenacet (A-FLUF) Fluoxastrobin (A-FLUO) Iodosulfuron-methyl	1-OH-Benzotriazole (D-BTZ) Acesulfame (D-ACS) Benzalkonium chloride (D-BAC) Caffeine (D-CAF) Carbamazepin (D-CMZ) Erythromycin Sulfomethoxazole (D-SMX) Ethinylestradiol (D-EE2)	Aluminium (U-ALU) Benz(a)anthracene (U-BaA) Bifenthrin (U-BIF) Copper (dissolved) (U-CU) Deltamethrin (U-DELTA) Fluoranthene (U-FLUO) Iron (dissolved) (U-FE) Nonylfenolmonoethoxylate (U-NP1EO) Permethrin (U-PER)	

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	(A-IODO) Mesosulfuron-methyl (A-MESO) Pendimethalin (A-PEND) Prochloraz (A-PROCH) Proquinazid (A-PROQ) Prothioconazole (A-PROT) Pyraclostrobin (A-PYRA)	HHCB (Galaxolide) (D-HHCB) Ibuprofen (D-IBU) LAS (D-LAS) Methylisothiazolinone (D-MI) TiO (D-TiO) Zinc acetate (D-ZnA) ZnO (D-ZnO)	Zinc (dissolved) (U-ZN)	
Benchmark for PECs	Tier 1: RAC Tier-2: RAC-species groups	Tier 1: PNEC Tier 2: PNEC of species groups	Tier 1: median-EC50 (all species)	
Assessing mixtures	∑PEC/RAC [1]	∑RCR [1]	∑RCR [1] $\text{msPAF}_{\text{EC50}}$	
Reference	[1]	[2]	[3]	

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Table 2. Definition of sample subgroups at the outlet of the (sub-)catchment, characterized by grouping the Maximum Cumulative Ratios [24].

Group	**Mixture risk (HI)**	**Individual risk (HQ)**	**MCR**	**Meaning**
I	HI>1	Max HQ>1		Mixture presents potential risk already based on individual compounds
II	HI<1	Max HQ<1		The assessment does not identify a concern
IIIA	HI>1	Max HQ<1	MCR<2	Mixture risk arises only from summing individual substance risk, although the majority of the mixture risk is driven by one substance
IIIB	HI>1	Max HQ<1	MCR>2	Mixture risk arises only from summing individual substance risk, with overall risk driven by multiple components

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Table 3. Prioritizations on land use, based on various options to define the mixture-HI.

SCENARIO	Mixture-HI definition	Signal of	Group I	Group IIIA	Group IIIB	Σ Dates with mixture-HI>1
AGR	AA-EQS	Regulatory concern	634	66	14	714
	5th percentile SSD_{NOEC}	Sublethal effects	110	46	35	191
CITY (URB+DOM)	AA-EQS	Regulatory concern	6836	0	355	7191
	5th percentile SSD_{NOEC}	Sublethal effects	6577	0	617	7194
MIXED	AA-EQS	Regulatory concern	4236	4	2710	6950
	5th percentile SSD_{NOEC}	Sublethal effects	2442	8	4261	6711
	50th percentile SSD-EC50	Species loss	0	0	7	7

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Table 4. Scenarios summarized as chemical footprint indicators.

Scenario	**P95 msPAF$_{NOEC}$**	**Chemical footprint (multiplication factor the 95%-protection level is exceeded)**
AGR	0.30	6.0
AGR-NAT-NAT-NAT	0.14	2.8
CITY	0.95	19.0
CITY-NAT-NAT-NAT	0.93	18.8
MIXED	0.46	9.1
MIXED-Abatement 25%	0.40	8.0
MIXED-Abatement 50%	0.33	6.6
MIXED-Abatement 75%	0.22	4.3

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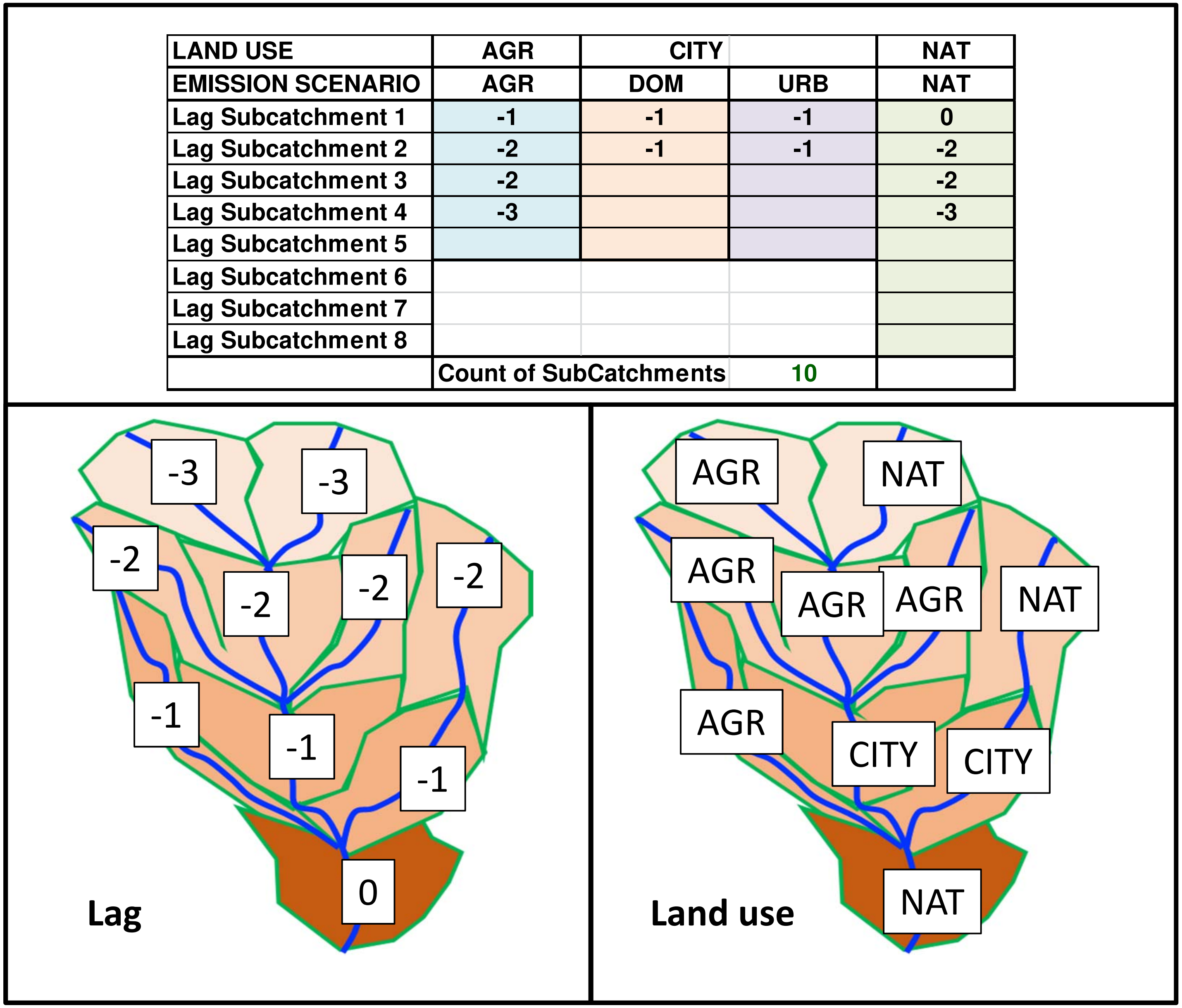

LAND USE	AGR	CITY		NAT
EMISSION SCENARIO	AGR	DOM	URB	NAT
Lag Subcatchment 1	-1	-1	-1	0
Lag Subcatchment 2	-2	-1	-1	-2
Lag Subcatchment 3	-2			-2
Lag Subcatchment 4	-3			-3
Lag Subcatchment 5				
Lag Subcatchment 6				
Lag Subcatchment 7				
Lag Subcatchment 8				
	Count of SubCatchments		10	

Figure 1_Posthuma et al_Pellston Mixtures Integration

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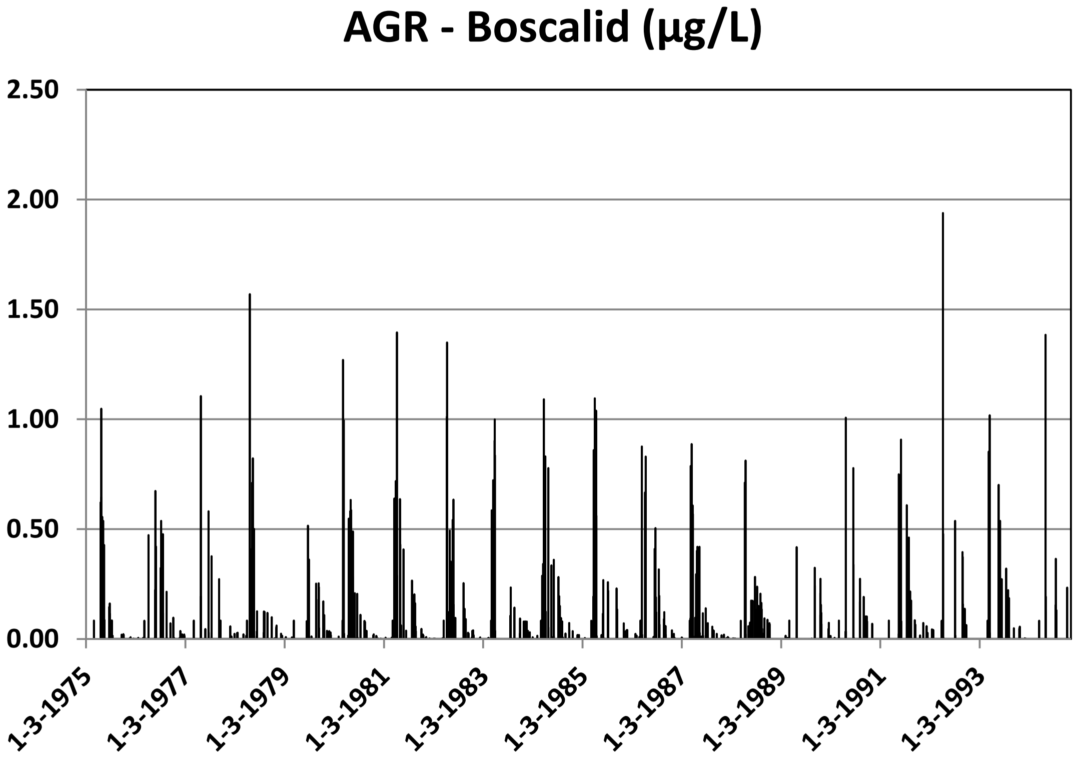

Figure 2_(top left) Posthuma et al_Pellston Mixtures Integration

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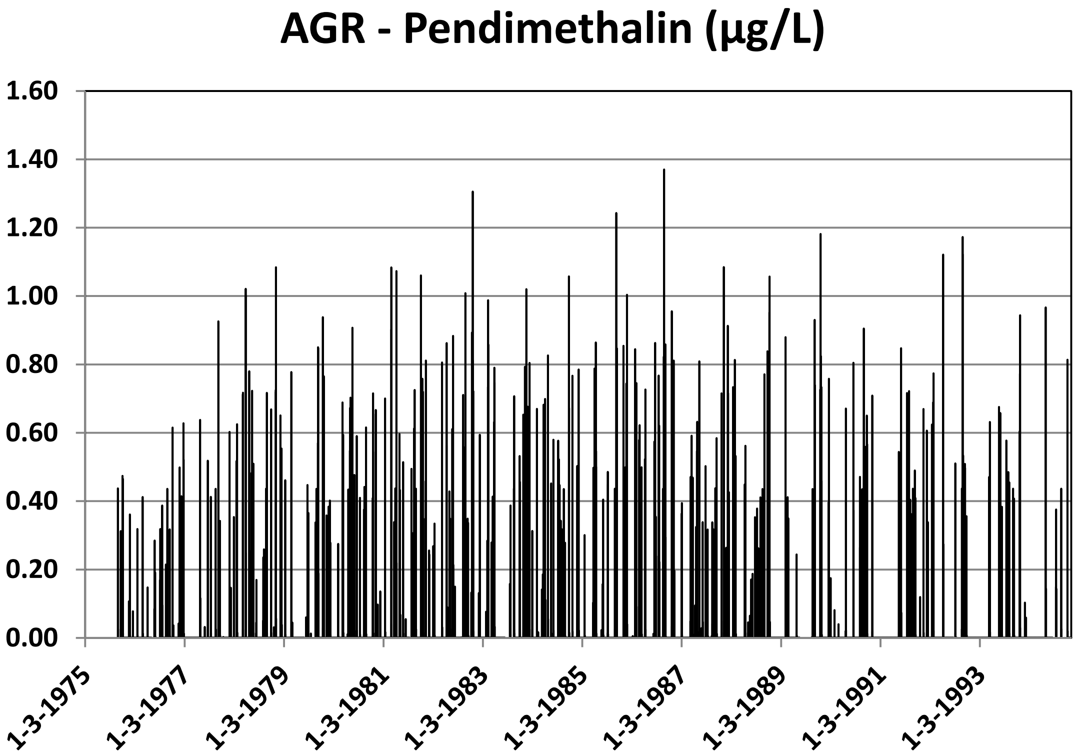

Figure 2_(top right) Posthuma et al_Pellston Mixtures Integration

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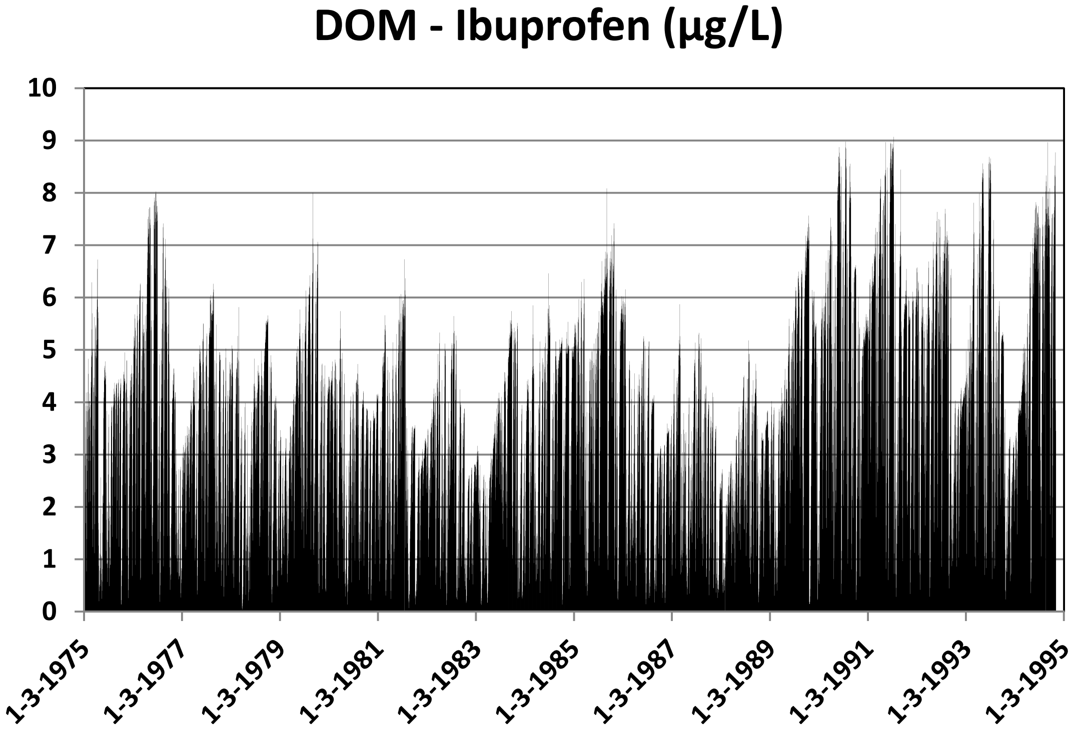

Figure 2_(bottom left) Posthuma et al_Pellston Mixtures Integration

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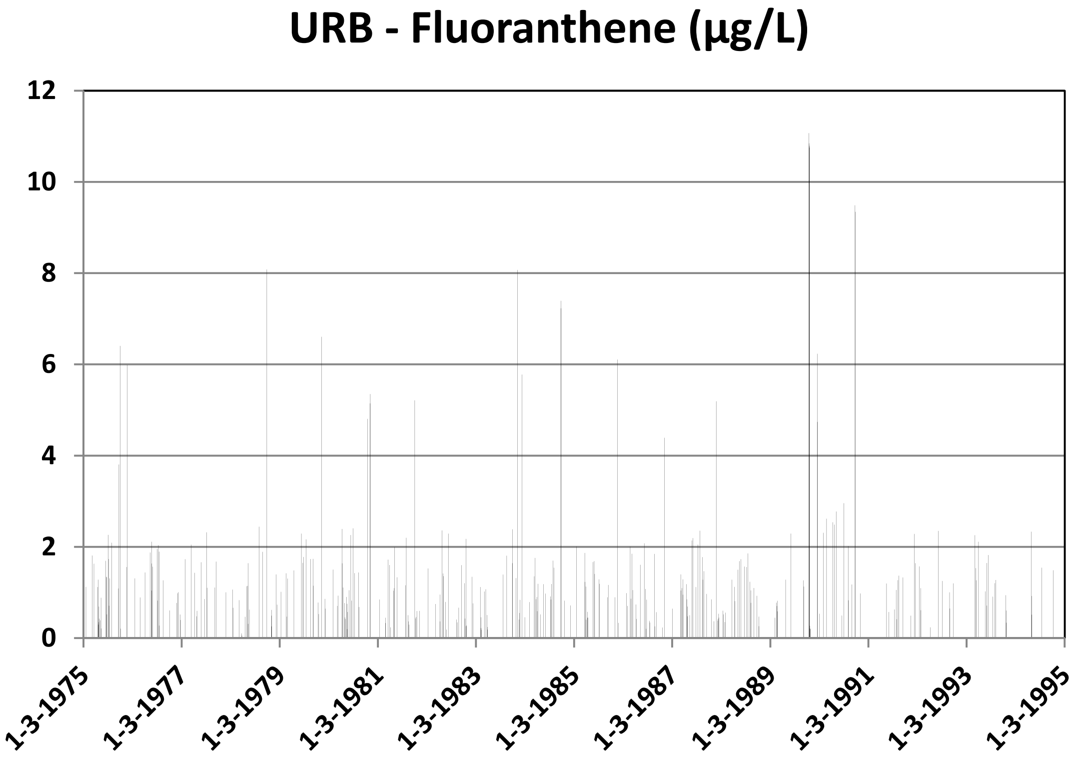

Figure 2_(bottom right) Posthuma et al_Pellston Mixtures Integration

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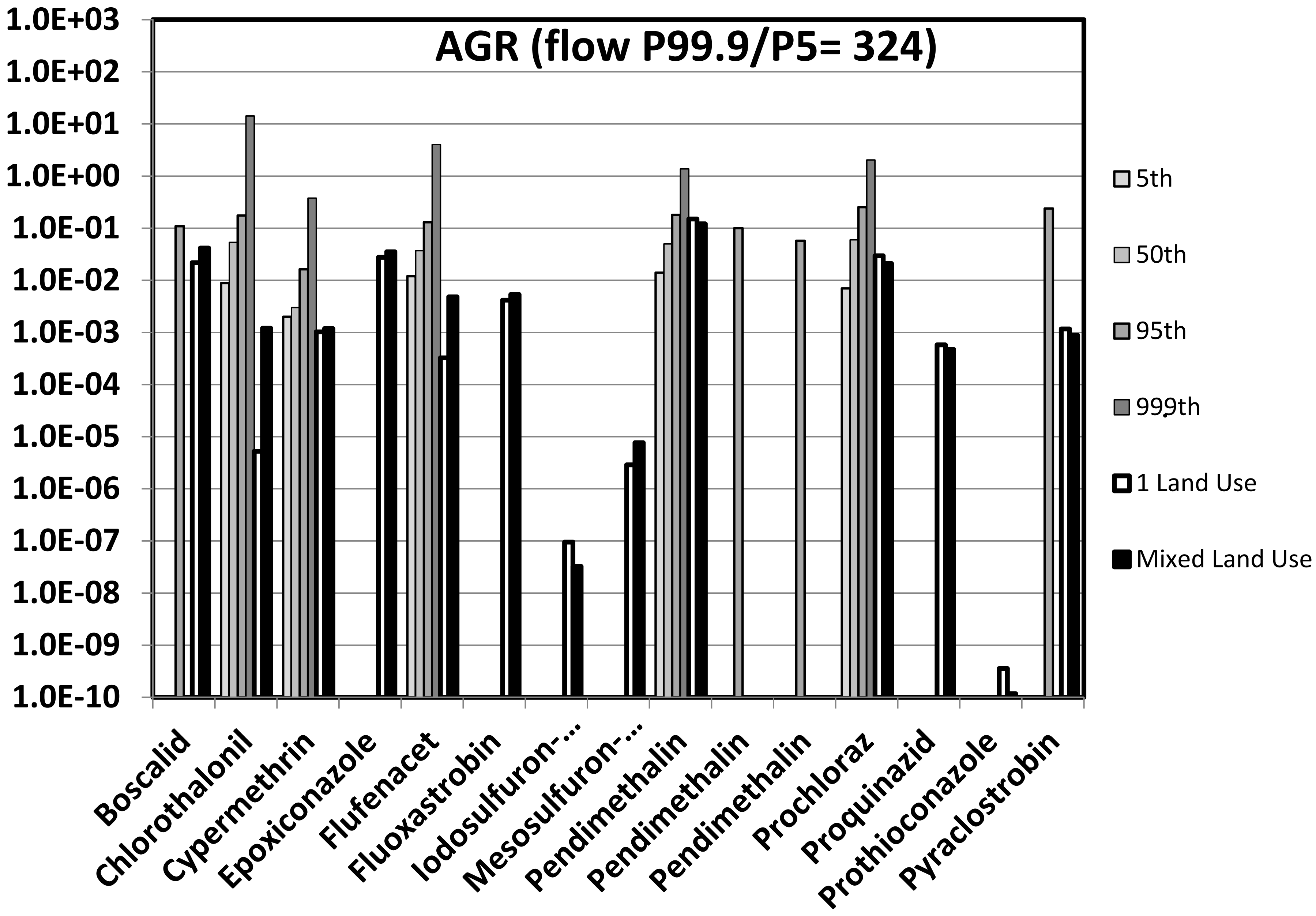

Figure 3_Left_Posthuma et al_Pellston Mixtures Integration

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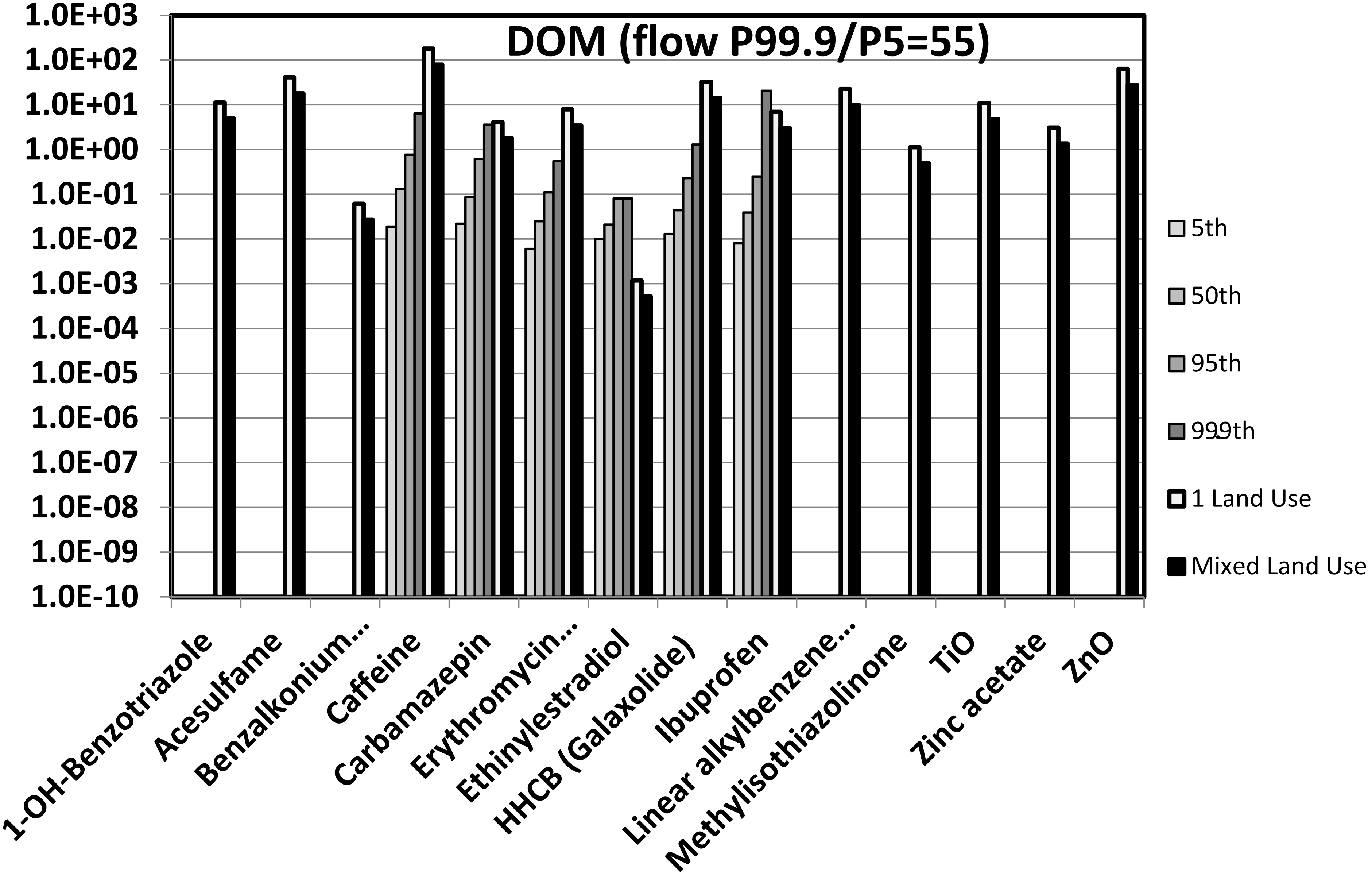

Figure 3_Middle_Posthuma et al_Pellston Mixtures Integration

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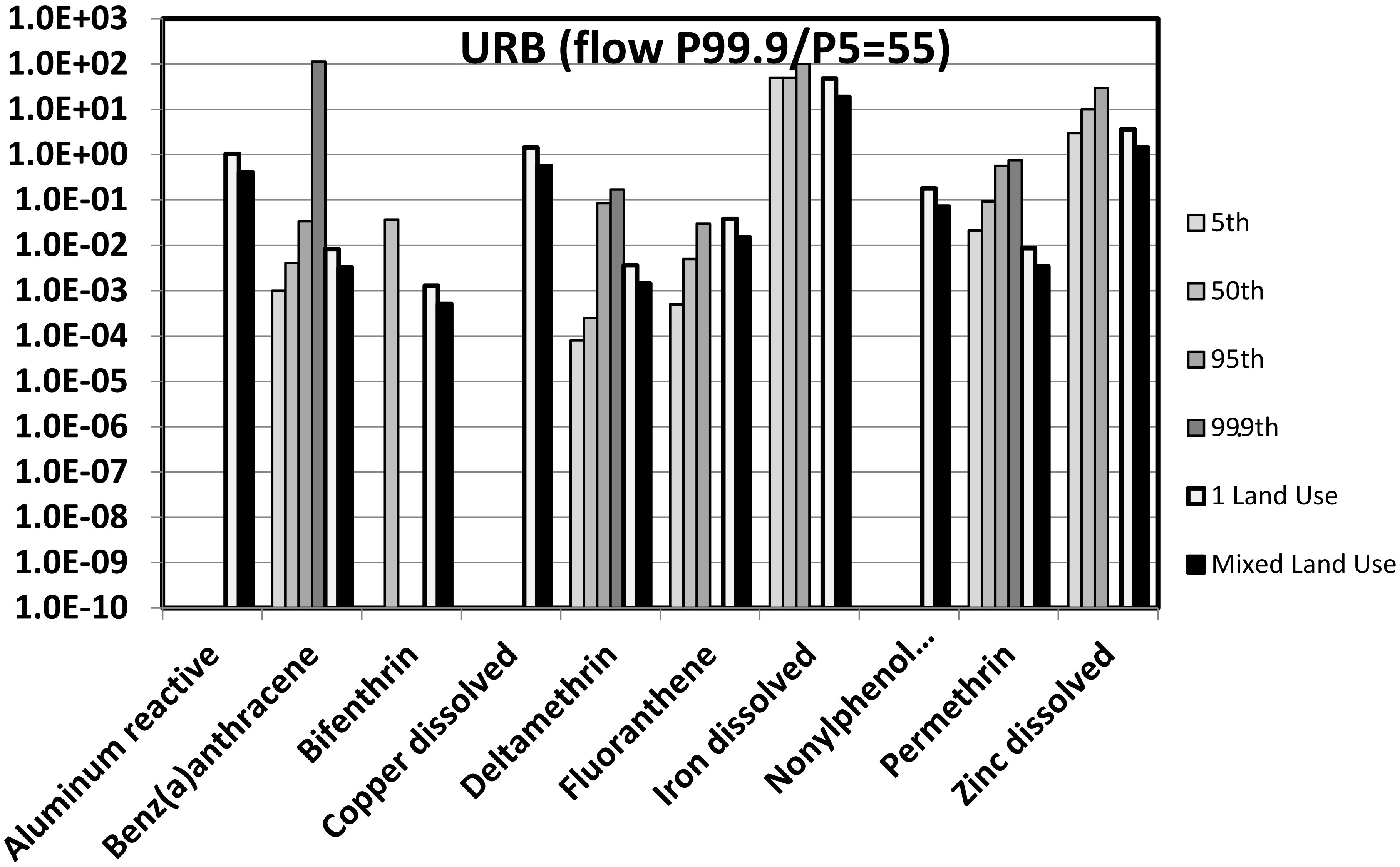

Figure 3_Right_Posthuma et al_Pellston Mixtures Integration

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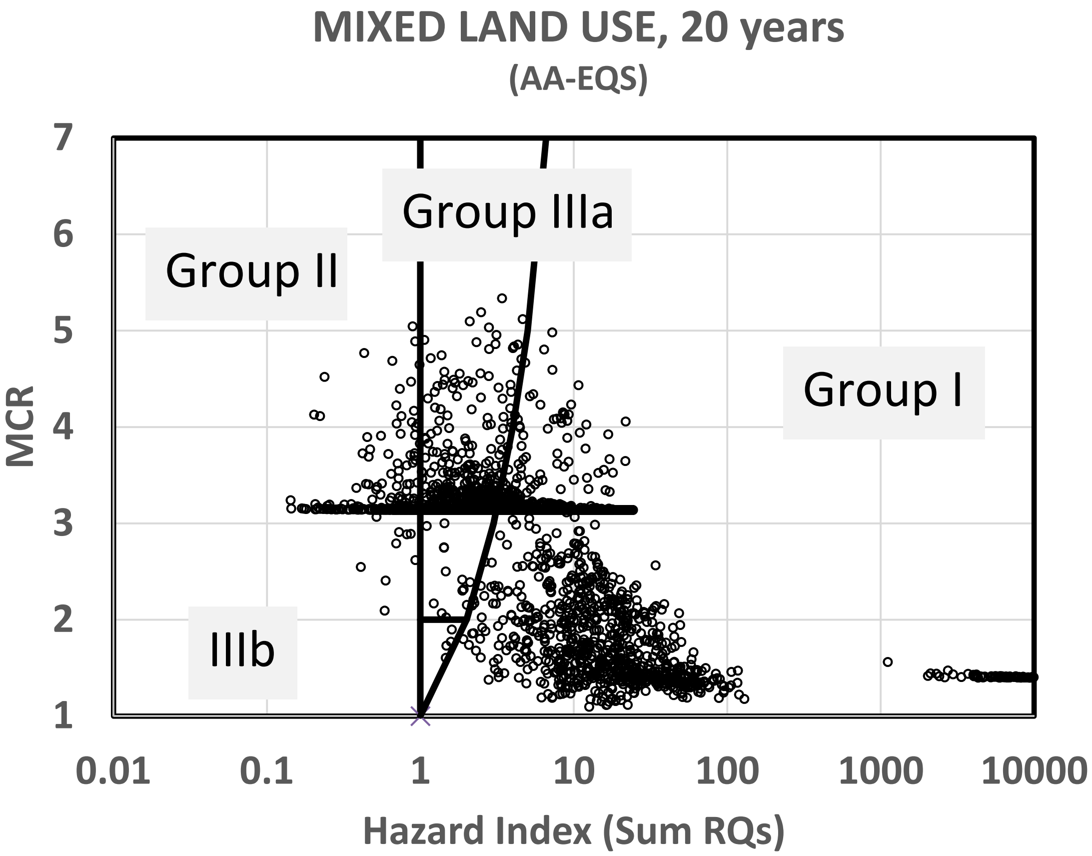

Figure 4_left_Posthuma et al_Pellston Mixtures Integration

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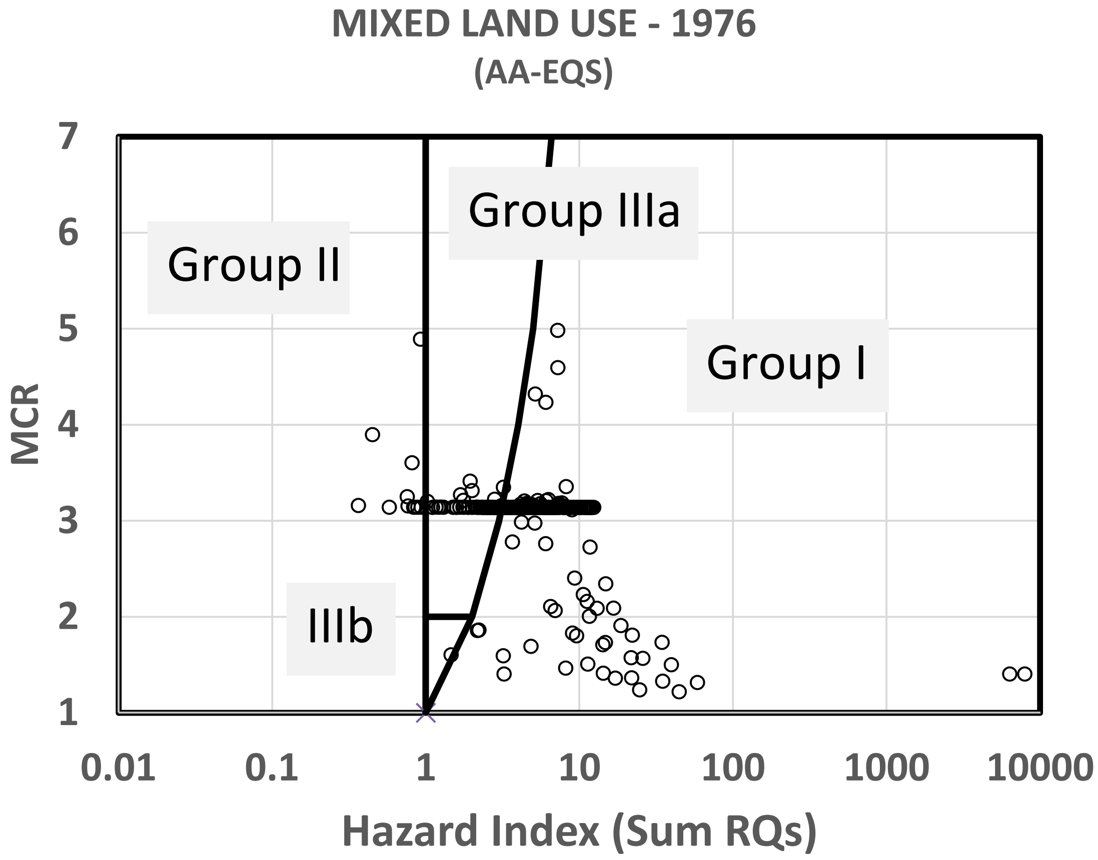

Figure 4_right_Posthuma et al_Pellston Mixtures Integration

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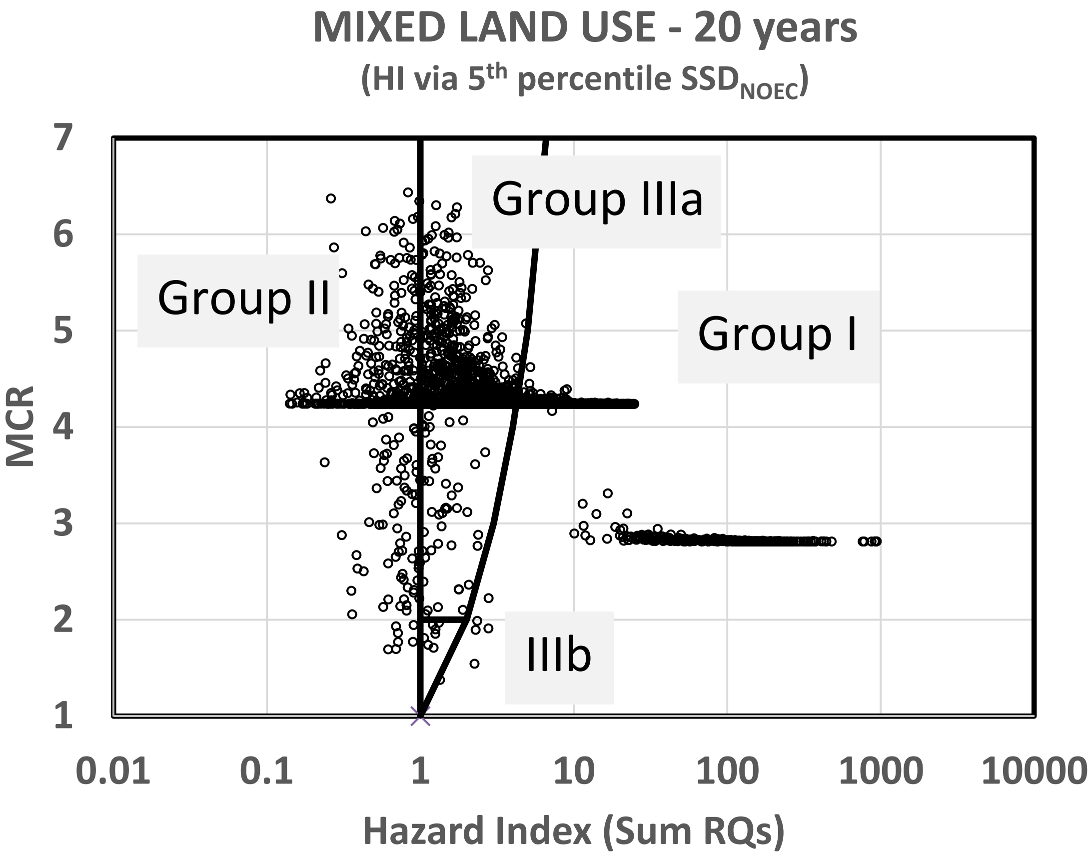

Figure 5_Upper left_Posthuma et al_Pellston Mixtures Integration

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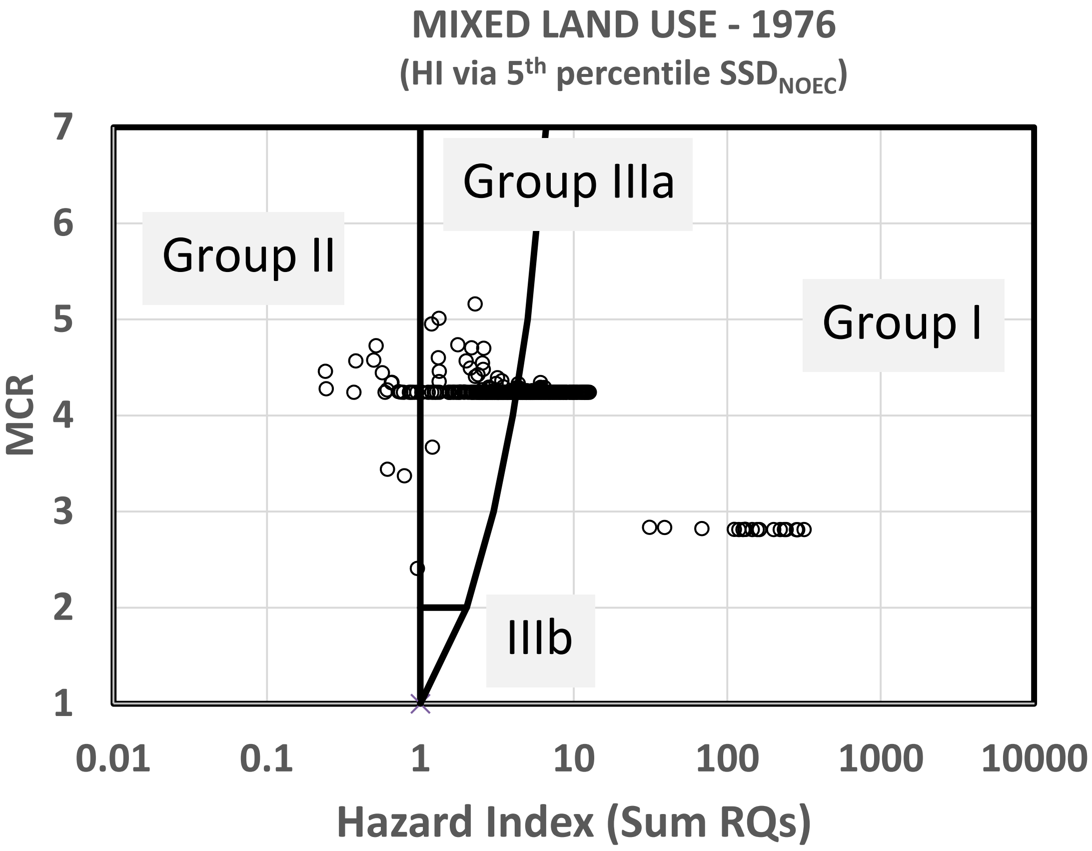

Figure 5_Upper right_Posthuma et al_Pellston Mixtures Integration

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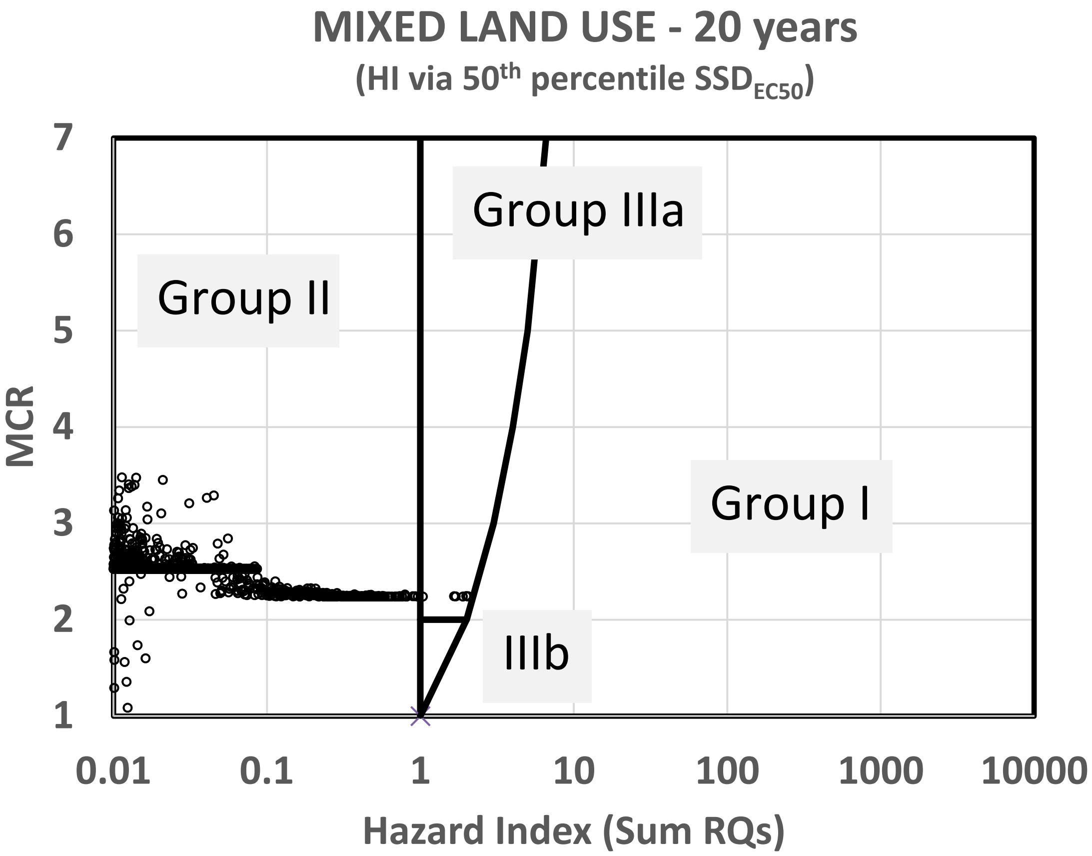

Figure _5 Bottom_left_Posthuma et al_Pellston Mixtures Integration

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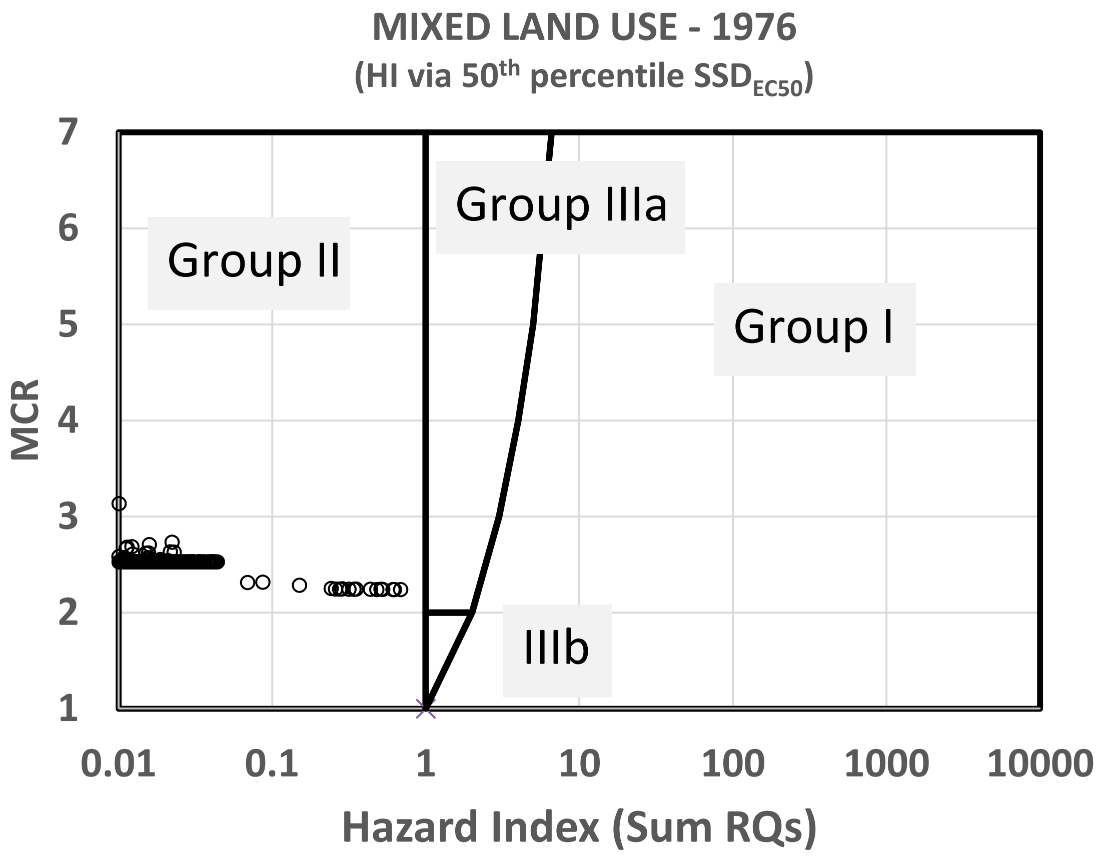

Figure 5_Bottom, right_Posthuma et al_Pellston Mixtures Integration

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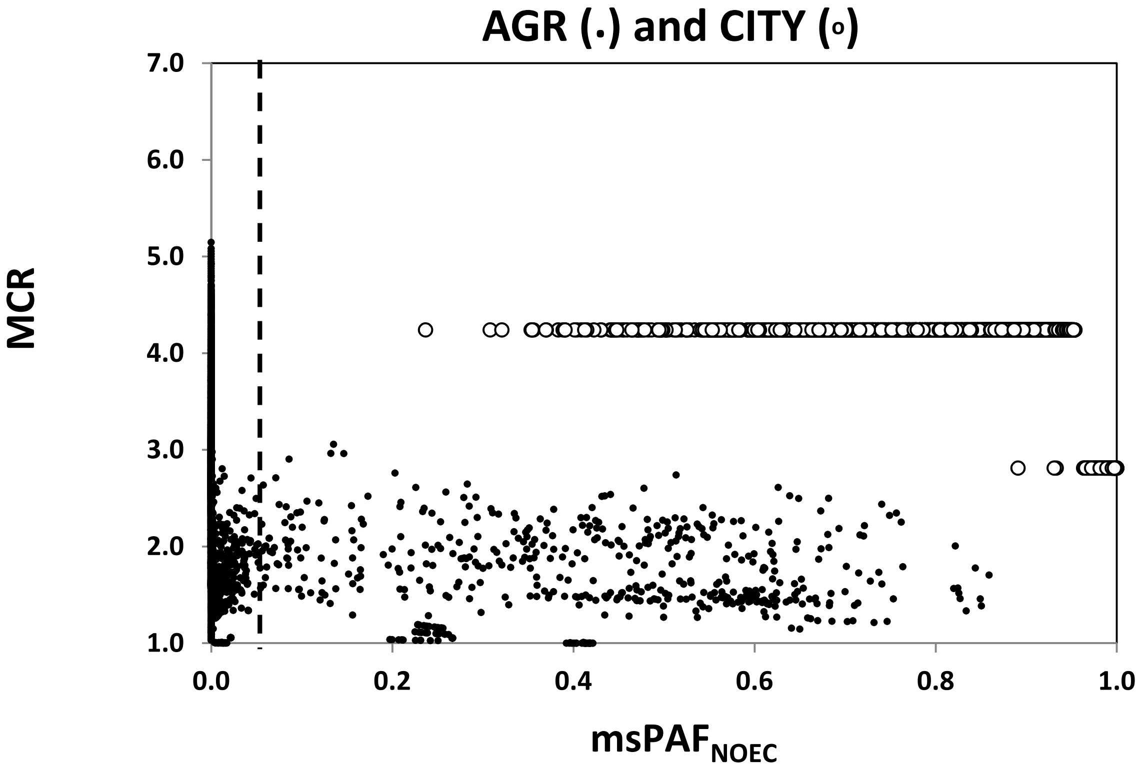

Figure 6_Left_Posthuma et al_Pellston Mixtures Integration

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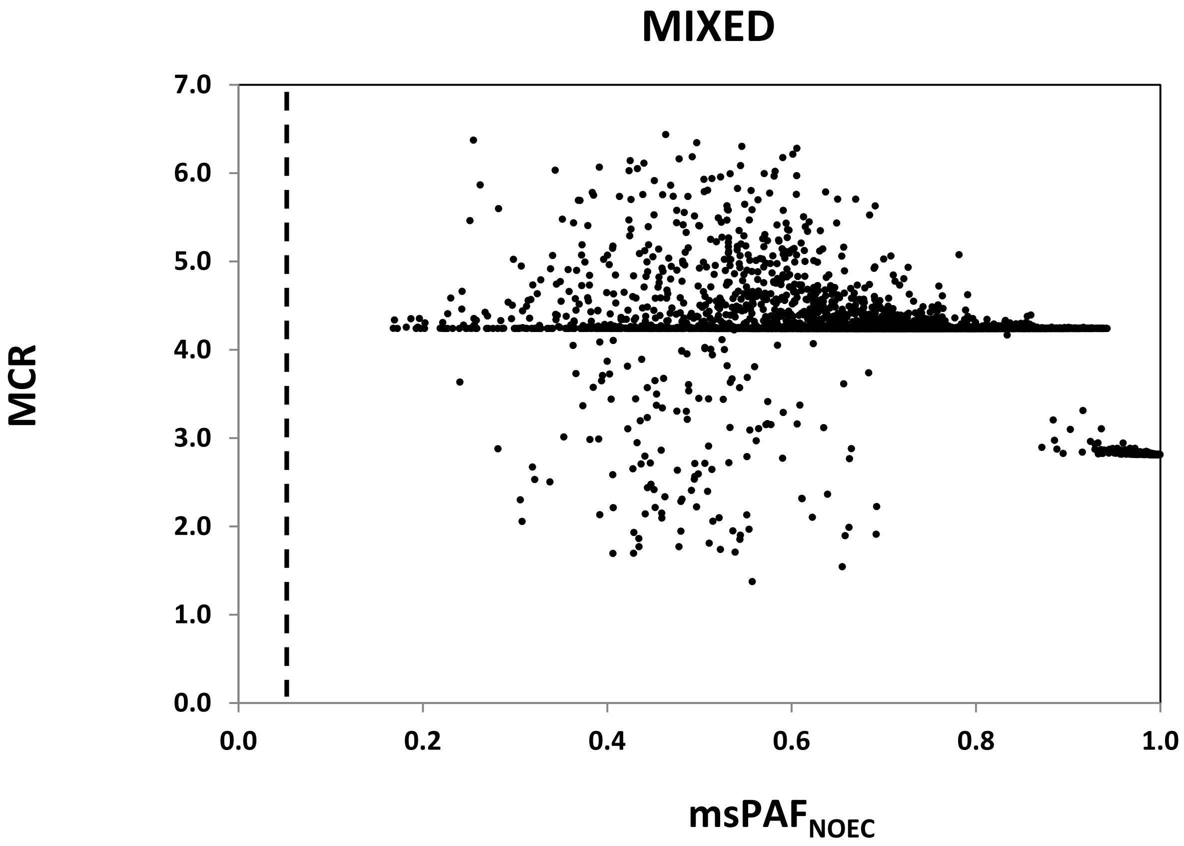

Figure 6_Right_Posthuma et al_Pellston Mixtures Integration

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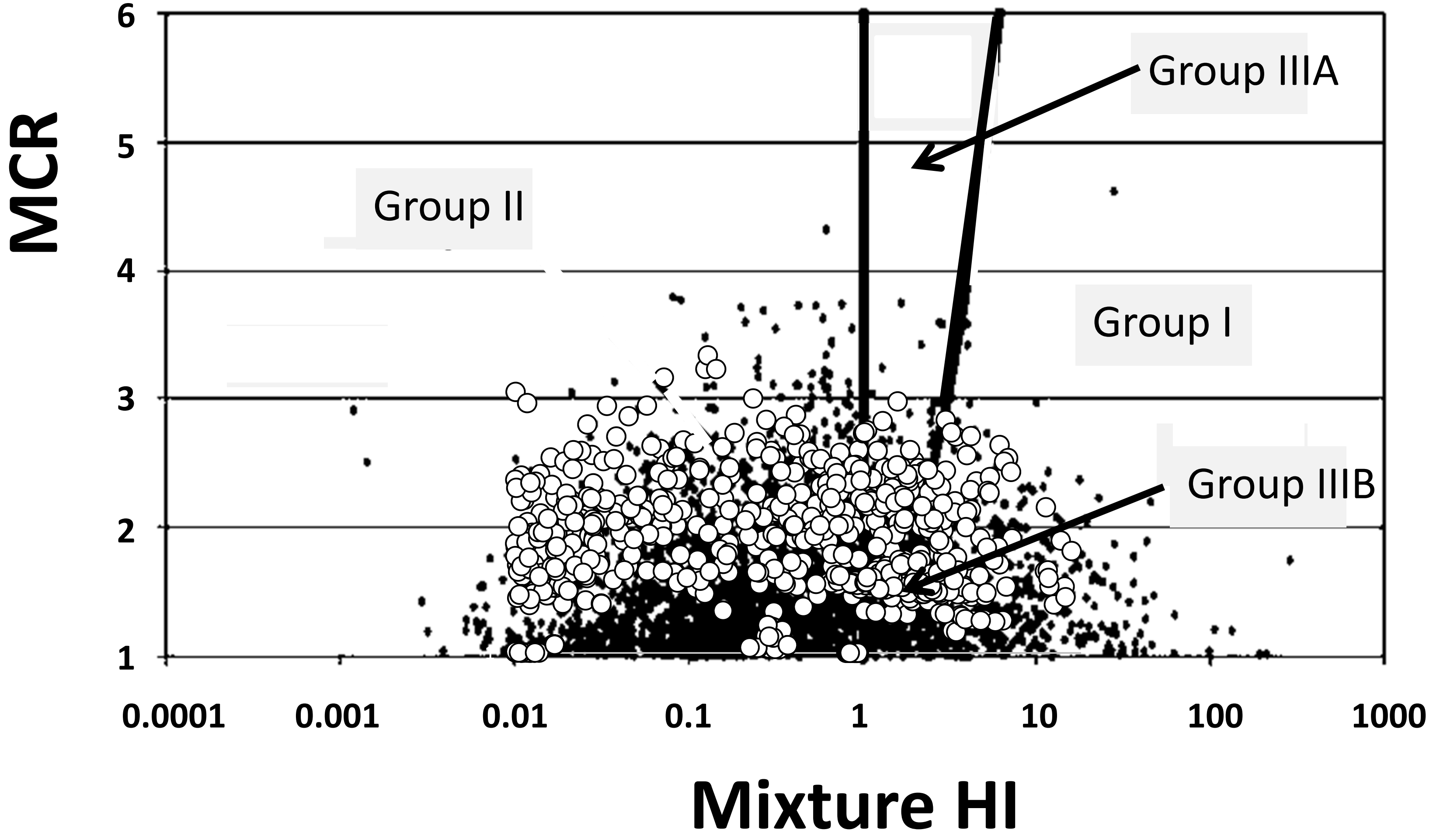

Figure 7_Posthuma et al_Pellston Mixtures Integration

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