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Environmental Toxicology  
& Chemistry

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

Journal:	<i>Environmental Toxicology and Chemistry</i>
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Mandatory Keywords:	mixtures, risk assessment, risk ranking, water quality, water quality criteria
Additional Keywords (Optional):	exposure scenario, land use
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

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	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.

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Manuscripts

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2 **PROSPECTIVE MIXTURE RISK ASSESSMENT AND MANAGEMENT**  
3 **PRIORITIZATIONS FOR RIVER CATCHMENTS WITH DIVERSE LAND**  
4 **USES**

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6 Christopher M. Holmes<sup>†</sup>, Stuart Marshall<sup>⊕</sup>, G. Allen Burton, Jr.<sup>◇</sup>

7 **Running Head:**

8 Blending mixture exposure scenarios for risk assessment

9

10 **Supplemental Data—The Supplemental Data are available on the Wiley**  
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12

13 **Word count: 8050 words.**

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3 29 **Abstract:** Ecological risk assessment increasingly focuses on risks from chemical  
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5 30 mixtures and multiple stressors, because ecosystems are commonly exposed to a  
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7 31 plethora of contaminants and non-chemical stressors. To simplify the task of assessing  
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9 32 potential mixture effects we explored three land-use related chemical emission  
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11 33 scenarios. We applied a tiered methodology to judge the implications of the emissions  
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13 34 of chemicals from agricultural practices, domestic discharges and urban run-off in a  
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15 35 quantitative model. Results showed land-use dependent mixture exposures, clearly  
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17 36 discriminating downstream effects of land uses, with unique chemical ‘signatures’  
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19 37 regarding composition, concentration and temporal patterns. Associated risks were  
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21 38 characterized in relation to the land use scenarios. Comparisons to measured  
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23 39 environmental concentrations and predicted impacts showed relatively good  
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25 40 similarity. The results suggest that the land uses imply exceedances of regulatory  
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27 41 protective Environmental Quality Standards, varying over time in relation to rain  
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29 42 events and associated flow and dilution variation. Higher-tier analyses using  
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31 43 ecotoxicological effect criteria confirmed that species assemblages may be affected  
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37 46 can inform various types of prioritization to support risk management, including a  
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39 47 ranking across land uses as a whole, a ranking on characteristics of exposure times  
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41 48 and frequencies, and various rankings of the relative role of individual chemicals.  
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43 49 Though all results are based on *in silico* assessments, the prospective land-use based  
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45 50 approach applied in the present study yields useful insights for simplifying and  
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47 51 assessing potential ecological risks of chemical mixtures and can therefore be useful  
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49 52 for catchment management decisions.  
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54 **Key words:** Chemical mixtures, aquatic risk assessment, watershed management,  
55 catchment assessments, exposure scenario, ecological risk assessments

**56 Editors' note**

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

67



## 68 INTRODUCTION

69 The goal of various environmental policies in human dominated ecosystems is to achieve a non-  
70 toxic environment and sound biological integrity [4]. This status has not been reached in many  
71 freshwater and marine systems, based on evidence on the occurrence of a wide array of  
72 chemicals in surface waters [5] and organisms' tissues [6], with associated evidence for multiple  
73 contaminant risks [7], impacts in bioassays [8], and reduced species biodiversity and abundance  
74 in various human dominated systems [9, 10]. Achieving negligible exposures and non-toxic  
75 conditions is challenging given the multitude of chemicals associated with human sources such  
76 as agricultural practices, treated wastewater and urban runoff. Currently produced chemicals may  
77 cause direct species loss, but also effects such as fish intersex and possibly other unknown  
78 effects [11], and new chemicals are continuously produced and emitted [12]. Regulatory  
79 approaches regarding chemicals presently focus, however, on a relatively small number of  
80 chemicals for which there are established environmental quality standards (EQS). Less is known  
81 about how to assess and reduce the risks and effects of ambient mixtures.

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83 The assessment and management of ecological risk for a highly complex matrix of combinations  
84 of chemicals, sites, species, and ecosystems can proceed via various approaches. The traditional  
85 approach is based on risk assessment of individual chemicals, using generic protective  
86 environmental quality standards (EQS). Those are benchmark concentrations (BM), such as the  
87 predicted no-effect concentration (PNEC). A predicted or measured environmental concentration  
88 (PEC or MEC) below such a threshold is interpreted as protective of ecosystem structure and  
89 function, i.e. the risk quotient ( $RQ=PEC/BM$ , or  $RQ=MEC/BM$ ) is  $< 1$ . The origin of these  
90 methods dates back to the 1970s and 80s [13, 14]. Since then tailored methods have been defined

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3 91 to serve specific policy goals, such as generic water quality policies and policies to determine the  
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5 92 environmental hazards of plant protection products (PPP) for aquatic edge-of-field exposures  
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8 93 [15]. Recently, chemical mixture assessment approaches have been recommended for practical  
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10 94 application [16]. Many of these mixture approaches evaluate mixture risks by a default approach  
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12 95 via aggregation of the individual risk quotients for each chemical in the mixture, such as the  
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14 96 Hazard Index ( $HI = \sum RQ = \sum [PEC/BM]$ ), although the expected mixture effects are also quantified  
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16 97 via mixture toxic pressures for species assemblages, expressed as multi-substance Potentially  
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18 98 Affected Fraction (msPAF) of species [17]. In addition, various methods are available to  
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20 99 retrospectively evaluate the ecological risks and impacts of mixtures on the landscape scale [10].  
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22 100 The latter approaches offer an *a posteriori* quantitative risk or impact ranking of sites and  
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24 101 stressors of concern (including chemical mixtures).  
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32 103 In this paper we describe a prospective analysis of land-use related emissions, exposures and  
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34 104 risks of chemical mixtures. This concerns both the resulting chemical signatures (are there land-  
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36 105 use specific mixture compositions [1-3]?), as well as the resulting chemical footprints (is there a  
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38 106 net risk exported from a catchment to a downstream water body [18, 19]?). Prospective,  
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40 107 catchment-scale prioritization of chemical mixture risks can assist decision-making regarding  
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42 108 risk mitigation strategies [20-23]. This paper expands on and integrates three detailed analyses  
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44 109 of land-use related scenarios, investigating the specific chemical signatures of an agriculture  
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46 110 scenario (emissions from agricultural land dictated by rainfall, soils and plant protection product  
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48 111 use [1]), a treated domestic wastewater scenario (daily use of household chemicals [2]) and an  
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50 112 urban run-off scenario (rainfall-mediated emissions from city surface areas [3]).  
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3 113 The goal of the present study is to develop and test the utility of combining the concepts of  
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5 114 continuous exposure of treated domestic wastewater discharge with temporally-variable  
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8 115 chemical exposure scenarios associated with urban and agricultural land uses for the purpose of  
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10 116 supporting comprehensive mixture risk assessments and environmental management. To achieve  
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12 117 this, the following objectives were addressed:  
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17 119 1. Propose and evaluate an approach for deriving a likely chemical signature in a receiving  
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20 120 river catchment to
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22 121 a. help explain field observations (concentrations and/or impacts)
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24 122 b. provide a background against which the toxicity of a new product or a new usage  
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26 123 could be assessed
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28 124 2. Produce an approach balancing pragmatism and simplicity with adequate detail for a  
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30 125 scientifically credible outcome;
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32 126 3. Recognize the complexity of assessing both the exposure and effects of mixtures, and  
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34 127 derive generalizations that provide evidence for a reality check of ecological risk  
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36 128 assessment; and,
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38 129 4. Identify uncertainties and gaps in knowledge requiring further research to refine the  
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40 130 prospective assessment of chemical mixtures.  
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## 47 48 49 132 **COMBINED SCENARIOS**

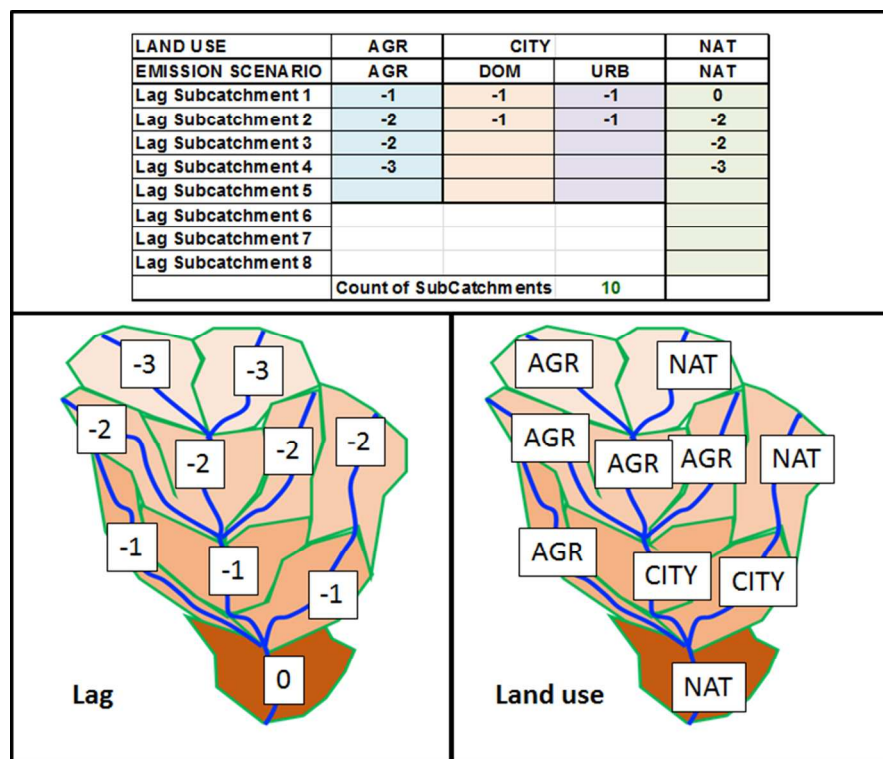
### 50 51 52 133 ***Overall approach***

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55 134 We integrated risk assessment approaches for three typical human-based emission scenarios  
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57 135 (AGR-agriculture; DOM-domestic; URB-urban runoff) and focused on identifying the potential  
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3 136 for mixture effects in receiving waters. The scenarios were selected because they commonly  
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6 137 occur in human-dominated systems and differ vastly in their chemical emission characteristics.  
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8 138 The scenarios were further developed and substantiated as land-use scenarios, whereby DOM  
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10 139 and URB are combined as the land use CITY. Further, the land use nature (NAT) was added for  
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12 140 demonstrating the influence of water inputs within the catchment where chemical emissions are  
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14 141 negligible. The scenarios were combined in a catchment assessment model, with the option to  
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16 142 define land uses for between 1 and 10 sub-catchments. Their integration placed the different  
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18 143 single land use categories into a landscape-level perspective. This allowed for cross-comparisons  
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20 144 and integrated exposure and risk analyses, to evaluate the utility and limitations of land use  
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22 145 scenarios for environmental assessment and potential management of chemical mixtures.  
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### 28 29 147 ***Modelling land uses, geography and hydrology***

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32 148 The scenarios AGR, CITY (DOM+URB) and NAT were spatially combined in hypothetical but  
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34 149 realistic spatial arrangements to represent either a single-land use scenario in a sub-catchment, or  
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36 150 a catchment with multiple land uses and river confluences. A spreadsheet model represented the  
37  
38 151 various catchment layouts. The model included: hydrology, aquatic emissions, concentrations  
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40 152 and mixture assessment outcomes for (in its most complex format) a catchment of 100 km<sup>2</sup> with  
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42 153 10 sub-catchments of 10 km<sup>2</sup> each, linked within a river network (Figure 1). A sub-catchment  
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44 154 was defined to have only one land use. A catchment can have any combination and number of  
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46 155 sub-catchments (in our case, up to 10) and assigned land uses. The land uses shown in the Figure  
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48 156 define the layout of the modelled MIXED land use scenario, which is just one of many possible  
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51 157 catchment layouts.  
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160 **Figure 1. The MIXED land use scenario layout, of 10 sub-catchments of similar size (10**  
 161 **km<sup>2</sup> each) in a total catchment of maximally 100 km<sup>2</sup>. Water flows from the top of the**  
 162 **figure to the bottom. Top (Table): scenario definition table, defining the catchment, with**  
 163 **land use and associated emission types. Bottom: resulting catchment map with position**  
 164 **codes (related to lag times of flow, left) and land use codes (right) as defined in the scenario**  
 165 **definition table. The different color intensities of the sub-catchments indicate various**  
 166 **hydrological travel times to reach the main catchment outlet, which enables modelling of**  
 167 **time-dependent chemical fate processes. Other scenarios can be defined via entering codes**  
 168 **for the lag-times of the land uses in the scenario definition table.**

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

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4 177 **Table 1. Characteristics of the original land use scenario studies [1-3], and evaluations**  
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6 178 **based on a sub-catchment area of 10-km<sup>2</sup>. PPP=Plant protection product. PNEC =**  
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8 179 **Predicted No Effect Concentration, utilized in generic protective chemical regulations.**  
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10 180 **RAC = Regulatory Acceptable Concentrations for edge-of-field water bodies, utilized in**  
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12 181 **PPP-regulations. RCR = Risk Characterization Ratio (similar to HI in the present study).**  
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15 182 **msPAF<sub>EC50</sub> = multi-substance Potentially Affected Fraction of species exposed beyond their**  
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17 183 **EC50.<sup>1</sup> In the present paper a PEC-benchmark ratio is generally referred to as Hazard**  
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20 184 **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 <sup>3</sup> /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 (A-iodo) Mesosulfuron-methyl (A-MESO) Pendimethalin (A-PEND) Prochloraz (A-PROCH) Proquinazid (A-PROQ) Prothioconazole (A-PROT) Pyraclostrobin (A-PYRA)	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) HHCB (Galaxolide) (D-HHCB) Ibuprofen (D-IBU) LAS (D-LAS) Methylisothiazolinone (D-MI) TiO (D-TiO)	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) Nonylphenolmonoethoxylate (U-NP1EO) Permethrin (U-PER) Zinc (dissolved) (U-ZN)	

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		Zinc acetate (D-ZnA) ZnO (D-ZnO)		
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	$\sum \text{PEC/RAC}^{-1}$	$\sum \text{RCR}^{-1}$	$\sum \text{RCR}^{-1}$ $\text{msPAF}_{\text{EC50}}$	
Reference	[1]	[2]	[3]	

185

186 ***Modelling concentrations***

187 Emissions of chemicals from AGR, DOM and URB were derived from individual land use  
 188 studies (details in those papers and the S.I.). The AGR scenario incorporated time dependency of  
 189 emissions related to PPP use on row crops. A 20-year time period was modelled on a daily basis  
 190 by using actual pesticide usage application data for a large arable farm in eastern England (see  
 191 [1]) and actual rain-events from the FOCUS R1 scenario meteorological dataset (used in EU  
 192 regulatory modeling for PPPs) which is directly applicable to UK agricultural conditions. The  
 193 selected AGR scenario used a winter wheat exposure scenario, with 13 active ingredients applied  
 194 on known dates and rates. Accordingly, the scenarios for the other emissions (DOM, URB) were  
 195 reformulated to enable modelling for the same 20-year period, and combined into the spreadsheet  
 196 model. Emission data and hydrological data were combined to estimate concentrations for each  
 197 of the studied chemicals emitted from each of the land uses.

198

199 The spreadsheet model allowed the prediction of concentrations from AGR, DOM and URB  
 200 emissions separately, as well as their combinations based on the sub-catchment configuration  
 201 (Figure 1). The model yields 24-hrs PECs for sub-catchment outlets. Large numbers of PECs

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3 202 were calculated using this approach. For example, for AGR the number of PECs equals 94,198  
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5 203 (7246 days, 13 chemicals), and for MIXED 268,102 (7246 days, 37 compounds).  
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11 205 ***Risk assessment methodologies and prioritizations***

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13 206 The risk patterns associated with the PECs were explored using three approaches: Hazard Indices  
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15 207 (HI), Maximum Cumulative Ratios (MCR, [24]), and mixture toxic pressures (multi-substance  
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17 208 Potentially Affected Fraction of species, msPAF, [17]). Details are in S.I. section 2.  
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23 210 First, the risks posed by a mixture were determined using individual chemical hazard quotients  
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25 211 (HQ) and the net hazard index (HI), in which  $HQ_{ij} = PEC_i / BM_{ij}$  (with HQ=Hazard Quotient, and  
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27 212 BM=Benchmark concentration,  $i$  = substance,  $j$  = selected effect endpoint, with  $j$  defined as  
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29 213 regulatory EQS, chronic-NOEC or acute-EC50, see below), and  $HI_j = \sum HQ_{ij}$ . The Hazard Index is  
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31 214 the sum of the individual values of compound-related HQs, implying the use of concentration  
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33 215 additivity as default mixture model.  
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39 217 Second, the MCR is the maximum cumulative ratio posed by a combined exposure to multiple  
40  
41 218 chemicals under the assumption of concentration addition divided by the risk of the most toxic  
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43 219 compound of the sample. The MCR of a sample expresses whether the net predicted toxicity is  
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45 220 driven by multiple components which make a significant contribution to the net mixture toxicity.  
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48 221 The MCR-value of a sample was calculated as the ratio of the sample's HI and the highest value  
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50 222 of the sample's set of HQ-values:  $MCR = HI / \max(HQ)$ . The combination of HI and MCR was  
51  
52 223 used to create subgroupings of the 7246 time samples per scenario in four groups, viz. Group I,  
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54 224 Group II, Group IIIA and Group IIIB (Table 2).  
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226 **Table 2. Definition of sample subgroups at the outlet of the (sub-)catchment, characterized**  
 227 **by grouping the Maximum Cumulative Ratios [24].**  
 228

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

229

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

242

243 The scenario results were also summarized as chemical footprints [18]. A chemical footprint  
 244 expresses whether the net emissions in a landscape remain within a pre-set boundary on risks or

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3 245 effects, for example, the mixture exposure level at which 95% of the species is protected against  
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5 246 exceedance of their no-effect level for the mixture ( $msPAF_{NOEC} < 0.05$ ). Here, the approach is  
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7  
8 247 modified to summarize the percentage of days the latter is exceeded at the outflow of a sub-  
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10 248 catchment based on the P95 of the  $msPAF_{NOEC}$  of all days of a scenario run.  
11  
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## 16 250 **RESULTS**

### 19 251 *Rainfall and flow*

22 252 The natural rainfall varied over time, and resulted in variation in flow. The vast numbers of input  
23  
24 253 data on rain and output data generated on flow (7246 per scenario) are summarized in the S.I.  
25  
26  
27 254 section 3. The outputs show that the variation in flow implied a strong influence on the dilution  
28  
29 255 of emitted chemical loads and domestic discharge effluents. Summarized as the P99.9/P5 flow  
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31 256 ratios, the high-low flow ratios were 55, 324, 128 and 94 for the scenarios CITY, AGR, NAT,  
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34 257 and MIXED, respectively.  
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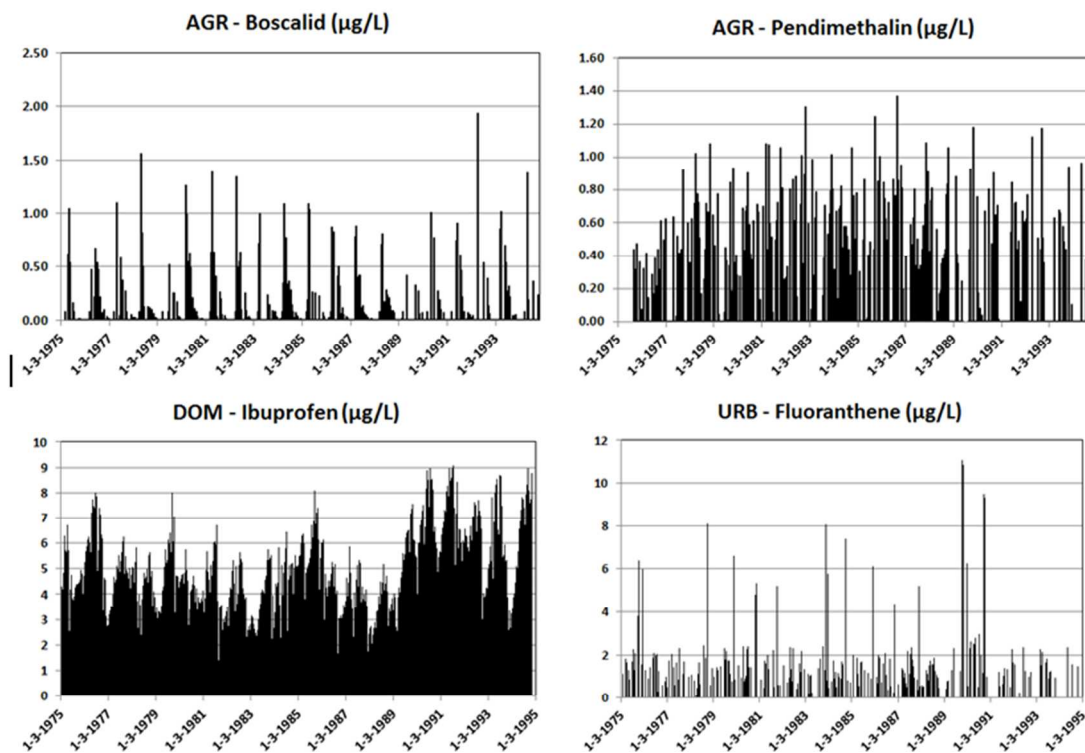
### 39 259 *Predicted Environmental Concentrations (PECs)*

41 260 The temporal variability of PECs is illustrated in Figure 2. The chemical concentrations varied  
42  
43 261 over time due to the sequential use of PPPs combined with rain events (AGR) and rain events  
44  
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46 262 passing the runoff threshold of 10.3 mm rain (URB). For DOM, though the per-capita use of  
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48 263 chemicals in this scenario was constant over time, the resulting PECs show spatio-temporal  
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50 264 variation due to the effects of variations in hydrological conditions.  
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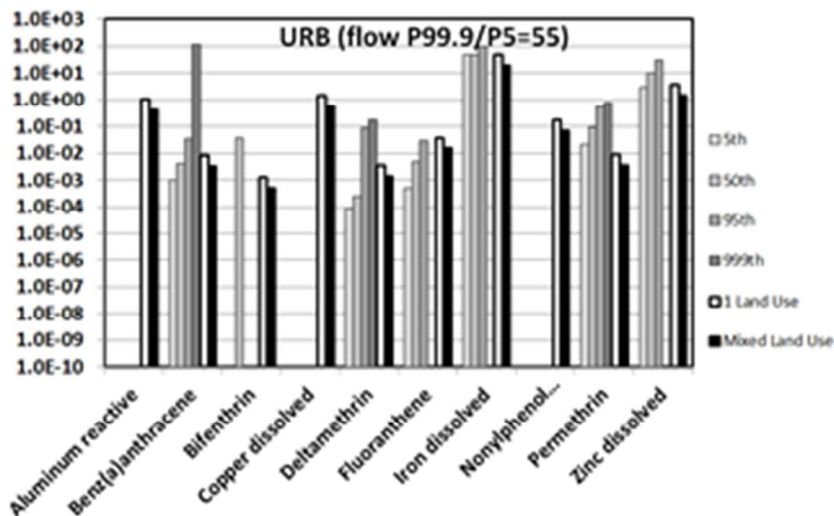
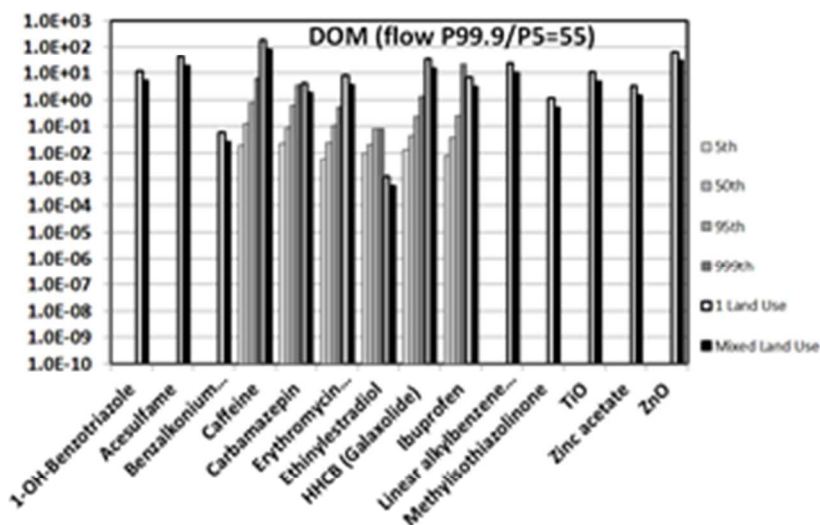
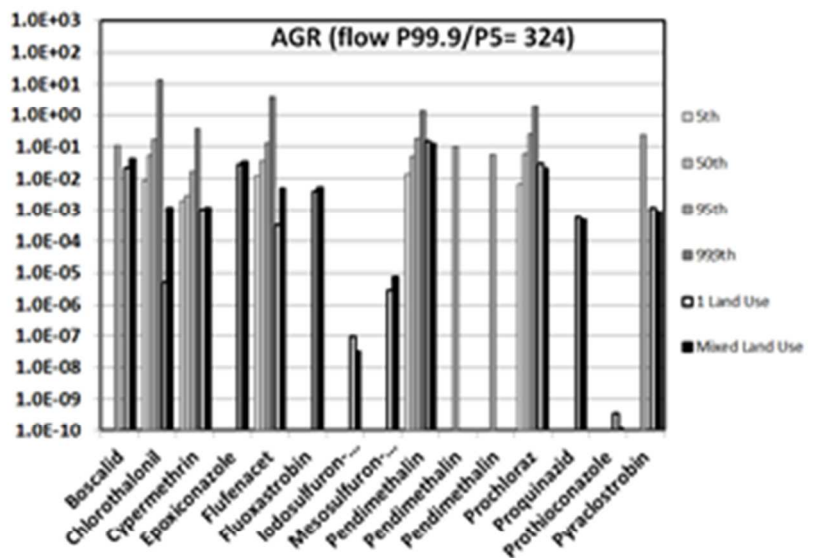
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**Figure 2. Illustration of the temporal variability of PECs, for two substances of the AGR-scenario (boscalid and pendimethalin), one for the DOM-scenario (ibuprofen) and one for the URB-scenario (fluoranthene).**

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



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3 282 **Figure 3. Comparison of measured environmental concentrations (MECs) of surface water**  
4 283 **systems, summarized as P5, P50, P95 and P99.9 of samples with a detectable concentration**  
5 284 **(>LOQ in the monitoring data), and predicted environmental concentrations (PECs),**  
6 285 **summarized as P95 at the outflow of a sub-catchment. Grey bars: MECs (darkening grey**  
7 286 **tones from low to high percentiles of detectable MECs); white and black bars: PECs of**  
8 287 **sub-catchments with a single land use and the MIXED scenario, respectively. The P99.9**  
9 288 **percentiles are added to demonstrate the magnitude of peak concentrations within the**  
10 289 **series of 7246 daily PECs per scenario. The flow P99.9/P5 ratio is added to illustrate the**  
11 290 **magnitude of dilution (PEC) variation related to flow.**  
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16 292 For some chemicals, for example pendimethalin in the AGR-scenario, the upper percentiles of  
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18 293 European river water MEC distributions were very similar to the scenario-based PECs. For other  
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20 294 chemicals, the highest MEC percentiles were greater (e.g., chlorothanoni), or lower (e.g.,  
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22 295 caffeine) than the higher PEC percentiles. Given the flow variation, the degree of similarity  
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24 296 between detected MEC percentiles and PEC percentiles suggests that the land use scenarios  
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26 297 resulted in predicted exposures that may occur in European rivers.  
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### 32 33 299 ***Risk characterization step 1: PECs and exceedance of regulatory endpoints***

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35 300 Tier-1 results show that the regulatory benchmark concentrations were exceeded for various sub-  
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37 301 catchment outlet days and for various compounds (HI>1, see SI-Tables 7, 8 and 9). Looking at  
38  
39 302 peak exposures (represented by P95-PEC), the peak PECs of e.g. pendimethalin exceeded the  
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41 303 AA-EQS and the MAC-EQS of this compound 8 and 6 times, respectively. For the DOM-  
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43 304 scenario, the peak exposure of ethinylestradiol and galaxolide exceeded the AA-EQS 4 and 7.5  
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45 305 times, respectively. For the URB-scenario, the highest exceedance was found for deltamethrin,  
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47 306 where the peak exposure was 1171 times the standard. Whether exceedances imply ecotoxic  
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49 307 effects depends not only on the magnitude but also on the duration of exposure. This also varied.  
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51 308 For example, for 7.3%, 80%, 91% and 5% of the days there was an exceedance of the AA-EQS  
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53 309 of pendimethalin (AGR), ethinyl estradiol (DOM), galaxolide (DOM), deltamethrin (URB),  
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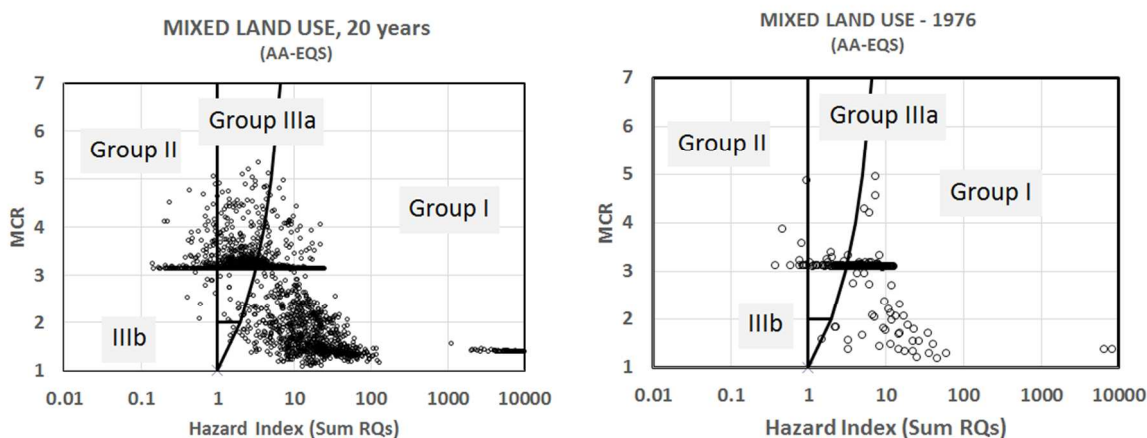
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3 310 respectively. Exposures can thus be shorter or longer, and frequent or incidental. These results  
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6 311 suggest, from a regulatory perspective, that the river system at the outlet of a sub-catchment or  
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8 312 the whole catchment was not sufficiently protected, although high values may also result from  
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10 313 high HQ-values resulting from a high AF related to high uncertainty on the benchmark (defining  
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12 314 a low benchmark due to high data uncertainty).  
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18 316 ***Risk characterization step 2, characterization of hazard indices of mixtures***

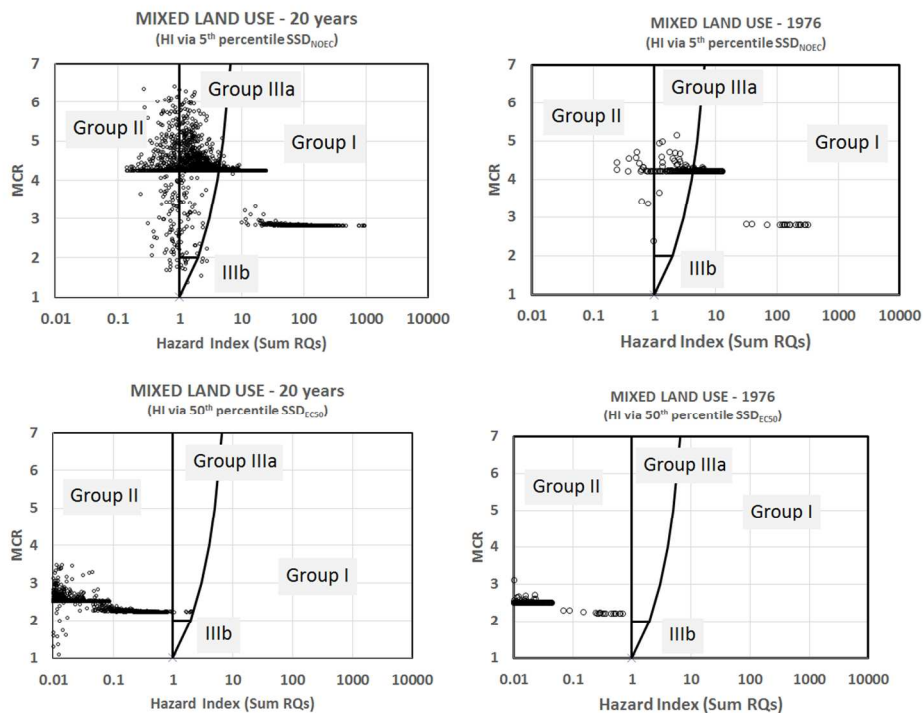
19  
20 317 The results of Tier 1 were summarized as HI-MCR plots. The MIXED land use (Figure 1)  
21  
22 318 resulted in the plotting of 7246 HI-MCA data points, which partly overlay each other (Figure 4).  
23  
24  
25 319 The figure suggest that the water at the outflow of the catchment often showed HI-values often  
26  
27 320  $\gg 1$ , which means that the RQs of individual compounds were (far) exceeded, while some of the  
28  
29 321 HI-points (with  $HI > 10,000$ ) are not shown. The latter values were found to be related to  
30  
31 322 chemicals of mainly the URB-scenario, for days after peak rainfall (causing a runoff event), for  
32  
33 323 chemicals with low AA-EQS. The water system is judged to be insufficiently protected for 96%  
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35 324 of the days, whereby the MCR remained below 6, with a high frequency of  $MCR \cong 3$ , and many  
36  
37 325  $MCR's < 3$ . The theoretical maximum MCR of the MIXED scenario is 37 (when the 37  
38  
39 326 compounds considered in this scenario are present at equitoxic concentrations, which is unlikely  
40  
41 327 in nature). The relatively low MCR's suggest that a low number of compounds (always less than  
42  
43 328 7) induce  $HI_{AA-EQS} > 1$ ). The high frequency of similar MCRs at a single level is attributable to a  
44  
45 329 similar change of HI and the maximum-HQ of a sample with dilution, due to which HI (X) can  
46  
47 330 vary at nearly constant MCR (Y), while the typical HI-MCR pattern in the CITY-scenario related  
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49 331 to threshold effects (runoff  $> 10.3$  mm rainfall). This threshold contributed to 'forcing' the  
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332 specific pattern of CITY-MCRs to two key MCR-levels, related to runoff chemicals' effect  
 333 criteria.  
 334



335  
 336 **Figure 4. Judgment of the 7246 HI-MCR data points for predicted mixtures at the outlet of**  
 337 **the whole 100 km<sup>2</sup> catchment, according to the land use scenario depicted in Figure 1,**  
 338 **evaluated by a generically protective regulatory criterion, the AA-EQS, to define the HI.**  
 339 **Left: for all dates, right: for a single (randomly selected) year. Note: in the left sub-figure**  
 340 **some extremely high HI data points are not shown (see text).**  
 341

342 The Tier-2 analyses resulted in modified HI-MCR patterns, slightly shifted left for the criterion  
 343 based on the 95<sup>th</sup> percent protection level (Figure 5, upper graphs). Note that both the HI and the  
 344 MCA of a data point change when the standards underlying the HI change from AA-EQS to  
 345 another effect criterion. A Tier-2 evaluation based on EC50s resulted in a further shift of the data  
 346 points to the left, so that only few samples were found where PECs have exceeded the EC50 of  
 347 one or more compounds. Species loss was predicted for those samples, given an earlier  
 348 observation that  $msPAF_{EC50}$  relates to observed species loss in mixture-exposed aquatic systems  
 349 [25]. Note that defining another Tier-2 HI using, for example, an EC10 or EC25 as benchmark,  
 350 would result in intermediate shifts (between Figure 5, top and bottom); i.e., between chronic  
 351 exceedance of NOECs and the earliest onset of effects and species loss.  
 352



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

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



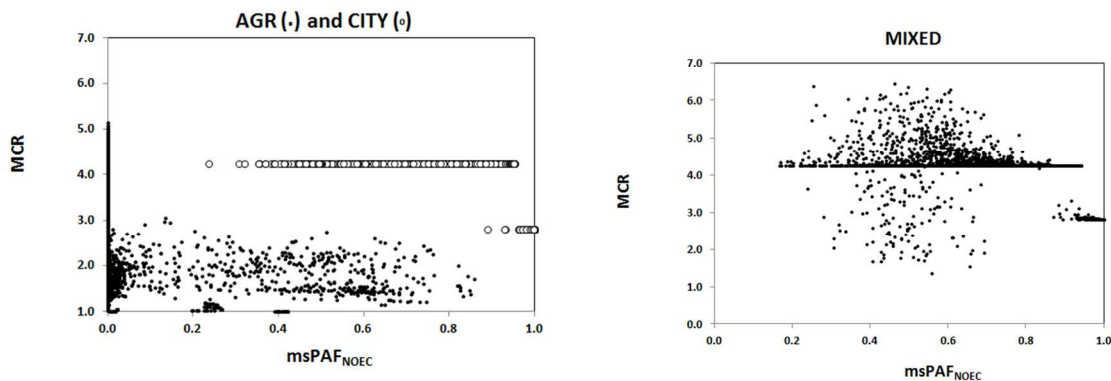
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3 370 chronic exposure. The exposure duration differed vastly for CITY, where the majority of days  
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5  
6 371 showed  $HI_{NOEC} > 1$  (88% of days), and 98% of the exposure lasted at least four consecutive days.  
7  
8 372 The main CITY-emission effects were reflected in the exposure durations of the MIXED-  
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10 373 scenario ( $HI_{NOEC} > 1$  for 93% of days, and 86% of exposures lasting at least four consecutive  
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12 374 days).

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18 376 ***Risk characterization step 3 - mixture toxic pressures***

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20 377 The risk characterization in step 3 consisted of expressing the mixture risks as  $msPAF_{NOEC}$  and  
21  
22 378 plotting these outputs again *vis a vis* the MCAs. The results in Figure 6 suggest that the 95%-  
23  
24 379 protection level is exceeded on 8% of the days for AGR, and 100% of the days for CITY (as well  
25  
26 380 as MIXED, not shown), while these chronic toxic pressure levels are associated most often with  
27  
28 381 a few compounds in the mixtures (judged by the MCR-values). The CITY and MIXED scenarios  
29  
30 382 consisted of exposures of a chronic kind, so that the land use would imply chronic effects for  
31  
32 383 aquatic species assemblages. Acute effects though, quantified via  $msPAF_{EC50}$ , are more  
33  
34 384 restricted. The maximum acute toxic pressure for AGR would affect 8% of the species, whereby  
35  
36 385 1 out of 1000 species would be affected at the peak exposure days ( $P95$  of  $msPAF_{EC50} \cong 0.001$ ).  
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38 386 For MIXED these values are 63% of the species at the day of the most toxic mixture outflow,  
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40 387 and 10% of the species at  $P95$ .

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390 **Figure 6. Tier-3 analyses of mixture impacts in scenarios with (left) land use AGR and**  
391 **CITY (DOM+URB) and (right) the MIXED scenario of Figure 1. The dotted line at**  
392 **msPAF<sub>NOEC</sub>=0.05 is the 95%-protection criterion which was originally used in the**  
393 **derivation of PNECs for individual compounds. Water samples positioned right of the**  
394 **dotted line are mixture exposures at a level that, if exposure is indeed chronic, induces**  
395 **chronic effects to the fraction of species indicated on the X-axis.**  
396

### 397 *Prioritization*

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

402  
403 First, prioritization on the basis of land use showed the rank-order of mixture risks of CITY  
404 (DOM+URB) > AGR, for three hazard index definitions (Table 3). A Tier-1 signal for regulatory  
405 concern was most frequent (exposure > AA-EQS), followed by the frequency of direct sublethal  
406 ecotoxic effects (exposure > NOEC), with a low number of modelled samples with species loss of  
407 >50%. In the MIXED scenario, prioritizing of the maximum HI’s using the Tier-2 approach  
408 resulted in the mixture risk rank order CITY (URB+DOM) > MIXED >> AGR (Table 3). The  
409 resulting chemical signatures (composition of mixtures and levels of exposure) clearly differ  
410 regarding land use.

411

412 **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
	5 <sup>th</sup> percentile SSD <sub>NOEC</sub>	Sublethal effects	110	46	35	191
CITY (URB+DOM)	AA-EQS	Regulatory concern	6836	0	355	7191
	5 <sup>th</sup> percentile SSD <sub>NOEC</sub>	Sublethal effects	6577	0	617	7194
MIXED	AA-EQS	Regulatory concern	4236	4	2710	6950
	5 <sup>th</sup> percentile SSD <sub>NOEC</sub>	Sublethal effects	2442	8	4261	6711
	50 <sup>th</sup> percentile SSD-EC50	Species loss	0	0	7	7

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

418  
 419 Third, the relative importance of chemicals was assessed. Many prioritizations can be made here,  
 420 e.g., for Tier 1, 2 or 3 evaluations in each scenario, and then on a daily basis (determining the  
 421 relative importance of each chemical on day=t, 7246 times per scenario) or for the numbers of  
 422 days where the mixture HI>1. Outcomes are in SI Table 10. It appeared that risk prioritization  
 423 outcomes depend heavily on the tier and inherent risk characterization method. For AGR,  
 424 chlorothanonil was for example 6<sup>th</sup> in rank judged by the AA-EQS definition of HI, but 1<sup>st</sup> when  
 425 judged by chronic SSD<sub>NOEC</sub>-HI. Again, prioritization needs to account for temporal aspects.  
 426 Chemicals in DOM would have priority when considering the more chronic character of DOM  
 427 exposures over URB exposures, while the latter contribute more to the risk of mixtures when

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3 428 present after a runoff event. Comparison to the individual scenario studies demonstrated that the  
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5 429 prioritizations shown in SI Table 10 are in line with the outcomes of those scenario studies. For  
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7 430 AGR in the current study, cypermethrin, pendimethalin and chlorothalonil were found to be  
8  
9 431 important regarding peak exposure levels, ranking 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> using AA-EQS to define HI.  
10  
11 432 Those were also ranking high in the agriculture study, with RACs as assessment criteria [1]. The  
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13 433 rankings according to exposure time also showed similar results. The rankings for chronic  
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15 434 ecotoxic effects only (our results) identified chlorothalonil and cypermethrin as 1<sup>st</sup> and 2<sup>nd</sup>  
16  
17 435 ranking compounds, which is also in line with the earlier study. For the chemicals emitted in the  
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19 436 DOM scenario, the outcomes for galaxolide and ethinylestradiol co-rank high, although linear  
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21 437 alkylbenzene sulfonate ranked lower in the MIXED scenario analyses than in the earlier  
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23 438 scenario study [2]. For URB, the top-ranked chemicals were deltamethrin, bifenthrin, permethrin,  
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25 439 copper and zinc, which also rank highly when assessed using landscape scenario analyses [3]. In  
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27 440 general, it can be stated that the prioritization options are many, that prioritization outcomes are  
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29 441 dynamic in space and time, and – hence – that the problem definition phase should be used to  
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31 442 define precisely which ranking information is most valuable for selecting an abatement option.  
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33 443 Regulatory prioritization used to prospectively steer preventive policies can thus be different  
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35 444 from more realized environmental quality based rankings [26].  
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### 446 ***Chemical footprints***

447 The land use scenarios were summarized as chemical footprints (CF) for direct, chronic risks for  
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49 448 species assemblages. Chemical footprints were quantified using the P95 of the 7246  
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51 449 msPAFNOEC-outputs for each scenario (Table 4). A CF in this definition can be used as  
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53 450 management summary information, for example when the  $P95\text{-msPAF}_{\text{NOEC}} > 0.05$  this means that  
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3 451 for 5 percent of the days the (sub-)catchment outflow is ecotoxic such that the 95%-protection  
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6 452 level is exceeded, whereby a higher degree of exceedance of 0.05 implies a higher potential of  
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8 453 the mixtures to affect species assemblages in the downstream water body. In other words, the  
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10 454 CF=6 for AGR means that the 95%-protection level is exceeded by a factor of six or more for  
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12 455 5% of the outflow days. The ecological implication of that depends on exposure time and  
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14 456 downstream water body characteristics, although the CF signals 'net outflow of toxicity'. In  
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16 457 AGR, chronic exposures were not found due to the swift effects of the flow regime. In a real  
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18 458 system, though, chronic effects related to this CF may occur when chemicals would slowly  
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20 459 accumulate in a water body, e.g. in a lentic water body downstream of the outlet.  
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22 460 The CF results ranked the risks of mixtures as CITY > MIXED > AGR, due to higher CF-values  
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24 461 and longer exposure durations. An additional scenario – AGR along a river stretch with three 10-  
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26 462 km<sup>2</sup> areas with nature downstream (AGR-NAT-NAT-NAT) – implied a reduction of the CF as  
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28 463 compared to AGR only. For CITY the same layout did not reduce the CF substantially, related to  
29  
30 464 the fact that the CF for the CITY scenario (0.95) is at the upper end of an exposure-mixture risk  
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32 465 model which has a sigmoidal shape (like the underlying SSD model), so that a change in  
33  
34 466 chemical emissions induced an equivalent reduction in CF. As an illustration of the option to  
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36 467 evaluate abatement strategies, the bottom lines of the Table show changes in CF following from  
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38 468 (imaginary) emission reductions for all chemicals by 25%, 50% or 75%. The latter related to  
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40 469 only a 47% lowered CF, but an 80% reduction regarding exposure periods for the number of  
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42 470 days with HI>1, and of 90% for the number of days on which HI>1 caused by 1 compound. The  
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44 471 75%-abatement option quantified for the MIXED scenario implied that species assemblages at  
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46 472 the catchment outflow experience lower exposure peaks, which are much less frequent and more  
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48 473 often attributed to a single chemical.  
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474  
475**Table 4. Scenarios summarized as chemical footprint indicators.**

Scenario	P95 msPAF <sub>NOEC</sub>	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

476

477

## 478 DISCUSSION

### 479 *Overview*

480 The large number of chemicals detected in aquatic environments currently implies that there are  
481 large uncertainties regarding the question whether or not there is sufficient environmental  
482 protection against the adverse effects of individual chemicals and their mixtures. The number  
483 and diversity of mixtures in the environment seem to imply an intractable number of  
484 combinations of exposures, risks, and associated effects, and a remaining open end to the  
485 problem. This conundrum is often addressed using simplistic approaches (e.g., focusing on  
486 priority chemicals) that focus on protection, but that ignore mixtures, and that use assessment  
487 factors to account for the enumerable types of mixtures and uncertainties. However, despite the  
488 *in silico* approach of the present study, the present results clearly indicate that the integrated  
489 assessment of numerous chemicals with different policy regimes (such as industrial chemicals

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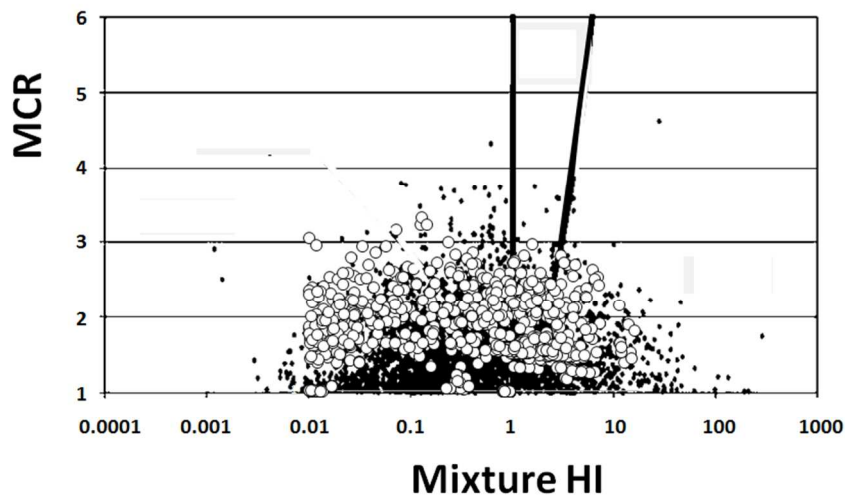
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3 490 and plant protection products) and spatial-temporal exposure patterns is tractable. Further, the  
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5 491 present study demonstrates an application of a strategic tiered approach, which provides refined  
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8 492 ecotoxicological insights into the presence of risks for species assemblages (or even specific  
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10 493 taxonomic groups, see [1]). Therefore, the current paper presents a testable framework designed  
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12 494 to explore simplification and clarification of the spatio-temporal complexity of exposures and  
13  
14 495 provides an approach for forecasting risks based on scenarios created to capture the major  
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16 496 influences on exposure for a given catchment or region. The study was based on three emission  
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18 497 scenario assessments, built into a single approach to model emissions and risks at the scale of  
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20 498 realistic combinations of sub-catchments and land uses.  
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### 27 500 ***Comparison of predicted and observed parameters***

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29 501 A striking feature of the results was that the finding that the PEC variability resembled the  
30  
31 502 observed ranges of the respective measured concentrations in river water samples (EMPODAT),  
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33 503 despite considerable variation of modelled and measured data and technical limits regarding  
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35 504 measuring compounds in field samples (Figure 3). The most striking observation was that the *in*  
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37 505 *silico* modelled land use scenarios (Figure 4, Figure 5) yielded a HI-MCR plot similar to that  
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39 506 from a field study in which 12 to 81 plant protection products were measured per sample [24]  
40  
41 507 (Figure 7), although the field study employed acute risk benchmarks (while we applied chronic  
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43 508 ones). The difference between the present study and the agriculture study [1] is caused by the use  
44  
45 509 of Regulatory Acceptable Concentrations to define mixture HIs in that study (this includes AFs  
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47 510 of 100 to 1000 across compounds). The comparisons between predicted and observed data  
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49 511 suggest that many of the findings of the present study can occur in true catchments. Therefore,  
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3 512 the key patterns (below) bear relevant insights for assessing and managing complex mixtures n  
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6 513 relation to land use.  
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26 514  
27 515 **Figure 7. Overlay of the HI-MCR plots of 4380 measured concentrations of plant**  
28 516 **protection products in U.S. watersheds [24] and of 7246 daily samples with associated**  
29 517 **PECs from the AGR sub-catchment. HI's were based on acute aquatic benchmarks for**  
30 518 **ecotoxicological effects and on the 5<sup>th</sup> percentiles of the SSD<sub>NOEC</sub> respectively, with the**  
31 519 **latter representing a more sensitive endpoint. Black dots: field data, white dots: current**  
32 520 **model results.**  
33 521

34 522 ***Key patterns in the data***

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36 523 The similarities of exposures and hazard plots allow deriving some key observations.  
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41 525 First, land use matters. Land use appears to imply a typical chemical signature in receiving water  
42 526 bodies. A signature consists of a typical chemical composition (chemicals, concentrations) and  
43 527 exposure time aspects (durations, frequencies). Attempts to solve existing mixture exposures in  
44 528 aquatic systems could therefore focus on de-coupling land use from aquatic systems, e.g., via  
45 529 buffer zones, waste water treatment, or reduced urban runoff emission events. Such actions  
46 530 would imply a change in emission of suites of chemicals, with those suites including the set of  
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3 531 chemicals of high priority within the land use. Abating chemical risks can utilize a suite of  
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6 532 options, not solely a chemo-centric approach [27, 28], and it was e.g. shown earlier that an  
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8 533 analysis of spatial associations between emission points and water bodies with sensitive  
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10 534 functions (drinking water production, protected nature) can be a basis to reduce impacts via  
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12 535 smart spatial arrangements [21], and that clever strategies may be utilized to reduce adverse  
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15 536 effects of chemicals and other water quality parameters [7, 29]. From upstream to downstream,  
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17 537 land use influences on smaller tributaries may be characterized by mixtures with greater  
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19 538 exposures and simpler composition, with a 'land-use dilution' effect in the downstream direction  
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22 539 [30].  
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27 541 Second, flow and runoff events matter, related to rain events. Even though it was expected that  
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29 542 domestic emissions would result in relatively constant exposures, the opposite is true in the  
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31 543 smaller tributaries in our case. The results highlight the importance of rain events and subsequent  
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33 544 dilution phenomena. Smaller rivers may be characterized by high temporal variability in  
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35 545 chemical concentrations, whether or not there is a constant or an intermittent emission source  
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37 546 (DOM *vis a vis* AGR spraying/runoff and URB runoff). Species in flowing aquatic systems can  
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39 547 thus be exposed to mixtures that change rapidly in composition. A recent example [31] showed  
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41 548 large changes of MECs of untreated waste water emissions in the Danube over the scale of a few  
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43 549 kilometers only. Note that the PECs predicted for the sub-catchments (current model) in reality  
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45 550 could imply higher exposures at the points where true chemical emissions occur (e.g, edge-of-  
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47 551 field exposures for agricultural chemicals, and end-of-pipe exposures at WWTP-outlets and  
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49 552 sewer overflows). The spatial and temporal variation we modelled implies challenges for the  
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51 553 design of monitoring schemes for flowing waters, and indicates that spatio-temporal variation  
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3 554 may disturb a straightforward interpretation of MEC data *vis a vis* the regulatory standards such  
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5 555 as AA-EQS [32]. For example, there may be doubts whether the MECs of a set of water samples  
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7 556 are ‘representative’ for the system, given spatio-temporal variability that may be order(s) of  
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9 557 magnitude. Modelling can help to improve understanding the mixture risks of such systems.  
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15 559 Third, the choice of the assessment benchmarks matters. The integrated scenario analyses differ  
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17 560 in this respect from the individual scenario studies [1-3], where various toxicity standards were  
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19 561 used (see also Table 1). The uniform use of AA-EQS values in the current study resulted in a  
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21 562 large number of days triggering regulatory concern, whilst an inspection of the ecological  
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23 563 implications of direct effects of mixture exposures (chronic or acute) showed substantially lower  
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25 564 fractions of samples potentially causing direct effects on species assemblages (both related to  
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27 565 peak exposures as well as non-chronic exposure times). This difference shows that it is important  
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29 566 not to over-interpret criteria exceedances, such as the PNEC or the AA-EQS. The exceedance of  
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31 567 such a criterion triggers regulatory concern, which should be translated into more specific  
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33 568 information on the potential occurrence of direct ecological effects, secondary poisoning effects,  
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35 569 or human health concern, or into a trigger to improve the EQS itself when the AF for one or  
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37 570 more compounds is high. Avoiding misinterpretations has been proven useful for water quality  
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39 571 management [33].  
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573 Fourth, prioritization choices matter. Prioritization helps in selecting of cost-effective abatement  
574 strategies. A suite of prioritization options can be envisaged, and these result in vastly different  
575 lists of compounds for further attention [34]. The current study shows the effects of prioritization  
576 choices. Relevant information can be obtained from comparing land uses (clear ranking),

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3 577 exposure types (chronic or intermittent) and chemicals within mixtures. The latter is often used  
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6 578 in practice, relating to the current identification of priority hazardous substances and substances  
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8 579 prioritized for adoption on a 'watch list' (regulatory attention triggered, [35]). The observation of  
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10 580 land use specific chemical signatures suggests that chemicals that rank high in priority may serve  
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12 581 as surrogates of co-occurring, non-modelled or measured substances [5]. Regulatory priority  
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14 582 substances may be indeed prioritized, but may also be of marginal importance for a catchment.  
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16 583 Of the modelled compounds cypermethrin is a priority substance for European water policies and  
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18 584 ethinyl estradiol is identified as candidate for the watch list [35]. In the present study, we found  
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20 585 various substances ranking high in various ways which are not prioritized- or watch list  
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22 586 chemicals in the context of current regulation [35], e.g., deltamethrin, permethrin, bifenthrin,  
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24 587 galaxolide, sulfomethoxazole, caffeine, carbamazepine, pendimethalin, flufenacet, mesosulfuron-  
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26 588 methyl and fluoxastrobin. Regulatory attention may be warranted beyond regulatory lists, in line  
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28 589 with other categorization options [36]. River basin management is likely served best by a critical  
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30 590 application of prioritizations, looking at land use, temporal aspects, chemicals of generic interest  
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32 591 (e.g. at the European scale) and chemicals of interest given land use practices. For a sub-  
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34 592 catchment, listed priority compounds may pose negligible risks within a given catchment and  
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36 593 conversely non-listed compounds may be of high local priority for management. Neglect of  
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38 594 compounds because of absence from a central listing can be called a case of unjustified  
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40 595 reification. Reification is the process through which concepts (such as 'priority compounds') are  
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42 596 increasingly interpreted as facts. Reification fallacies may seriously affect policy making [37,  
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44 597 38]. Unjustified interpretations can induce Type-I errors (risk signals triggering abatement costs,  
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46 598 without the signal being related to true impacts [39]) as well as Type-II errors (the potential  
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3 599 impacts of many chemicals and their mixtures are neglected or remain unknown due to  
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6 600 limitations of current science).

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10 602 Fifth, the analyses always resulted in a clear identification of some chemicals contributing most  
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12 603 to risks in mixtures. This phenomenon seems to be universal in field-related mixture studies, as  
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15 604 substantiated a variety of other assessments [18, 24, 40-43] (see also Figure 7). The outputs of  
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17 605 our study suggest strong simplifying patterns of risk in highly diverse sets of mixture exposures.  
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20 606 Land-use related chemical signatures appear to exist, whereby mixture effects are commonly due  
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22 607 to a few chemicals (for a given toxicological endpoint), although those few chemicals differ with  
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24 608 land use and time [44].

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29 610 Sixth, the reporting of findings as chemical footprint information summarizes the data for an area  
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31 611 in easily understood metrics: the multiplication factor that mixture toxic stress of a sample  
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33 612 exceeds a benchmark, which can be interpreted as a measure of the number of times a sample  
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35 613 needs to be diluted before the effects are below the benchmark. In this evaluation, the dilution  
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37 614 factors needed for the different land use scenarios were 6, 19 and 9 for agriculture (realistic  
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39 615 winter wheat scenario), a city (10,000 people/ 10 km<sup>2</sup>) and a mixed-land use scenario (Figure 1)  
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41 616 to yield 95% of the species protected against NOEC-exceedance due to mixture exposure for  
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43 617 95% of the days. Note that, commonly, various fate processes that we did not model may lower  
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45 618 exposures in field conditions, which likely results in lower risks and CFs. The predicted CF-  
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47 619 values are in line with other chemical footprint analyses for Europe [18, 19]. In addition, the  
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49 620 change in CF can be determined for varying catchment configurations (of URB+DOM, AGR,  
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51 621 NAT), and the effects of abatement options on the footprint can be explored (Table 4). Such  
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3 622 summaries enable exploratory investigations as to the ecological risk reduction of altering  
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5 623 landscape structure, or impacts of alternative chemicals used for specific goals (e.g, choice of  
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8 624 PPPs), or of chemical-specific or generically effective abatement strategies, such as buffer zones  
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10 625 [45].  
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15 627 ***Further analyses***  
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18 628 Further data analyses are possible; e.g., investigating which taxonomic groups are likely to be  
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20 629 most affected by mixtures, or checking time-weighted averaged exposures, the effects of the  
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22 630 rainfall threshold causing city runoff, and analyses based on measured efficacies of e.g. buffer  
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24 631 zones between human activities and water systems. The refinement for taxonomic groups was  
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26 632 already worked out in detail for the water samples of MCR-Group IIIB of the AGR-scenario [1].  
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28 633 Such analyses can refine insights into potentially sensitive groups. As this effect is most  
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30 634 prominent for the AGR-scenario, and as the original scenario study presents such outcomes in  
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32 635 detail, we refer to that study for details of this kind [1].  
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38 636 **CONCLUSIONS**  
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41 637 Based on the conceptual and practical evaluation of an integrative scenario, blending earlier  
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43 638 reviewed AGR, DOM and URB scenario data, and acknowledging the limitations of this purely  
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45 639 *in silico* study, we conclude:  
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48 640 1. It is possible to create a catchment-oriented approach, encompassing land-use related  
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50 641 emissions of chemicals, rain events and hydrological phenomena, to predict likely  
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52 642 chemical profiles in receiving river catchments:  
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55 643 a. The PECs generated by this approach bear a reasonable relationship with  
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57 644 measured concentrations of chemicals  
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3 645           b. The predicted patterns of ecological risks, both regarding their magnitude as well  
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6 646           as their maximum cumulative ratios, bear a reasonable resemblance to the pattern  
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8 647           based on field data  
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11 648        2. The land-use based approach, with realistic rain events and flow variation, results in  
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13 649        highly variable mixture compositions in space and time (composition and concentrations  
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15 650        of chemical mixtures), but also in simplified signatures and prioritizations,  
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17 651        3. The outcomes demonstrate spatio-temporal variability of exposure and potential  
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19 652        ecological impacts of chemical mixtures in human-dominated systems, but also allowed  
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21 653        for simplifying generalizations, such as the potential for various meaningful  
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23 654        prioritizations for risk management;  
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27 655        4. The complexity of true catchments and land uses can be addressed through science-based  
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29 656        approaches that consider exposure scenarios for a wide-range of ecosystems and land-use  
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31 657        types (here dominated by agricultural, urban and domestic wastewater treatment inputs),  
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33 658        but this requires developing "road map" scenarios with typical exposures for prospective  
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35 659        and retrospective risk assessments, and linking to management actions;  
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39 660        5. The varying exposure patterns can be described across ecosystem and land-use types by  
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41 661        converting loadings to environmental concentrations in time-varying river flows, and  
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43 662        finally ecotoxicologically-relevant endpoints such as hazard quotients and indices and  
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45 663        mixture toxic pressures, that can be related in a tiered way to expected net mixture  
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47 664        impacts,;  
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51 665        6. The explanation of outcomes of modelled or measured water quality assessments requires  
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53 666        specific attention, to avoid over-interpretation of lower-tier methods.  
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3 667 7. The proposed approach for evaluating chemical mixture risks has a wide range of  
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6 668 potential regulatory applications where approaches to mixture risk assessment are  
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8 669 needed.  
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## 14 671 **SUPPLEMENTAL DATA**

17 672 The Supplemental Data are available on the Wiley Online Library at DOI: @@@.

19 673

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49  
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51  
52 688 database (<http://www.norman-network.net/empodat/>).  
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689  
690 **Disclaimer** – The opinions expressed in the present study are those of the authors and not  
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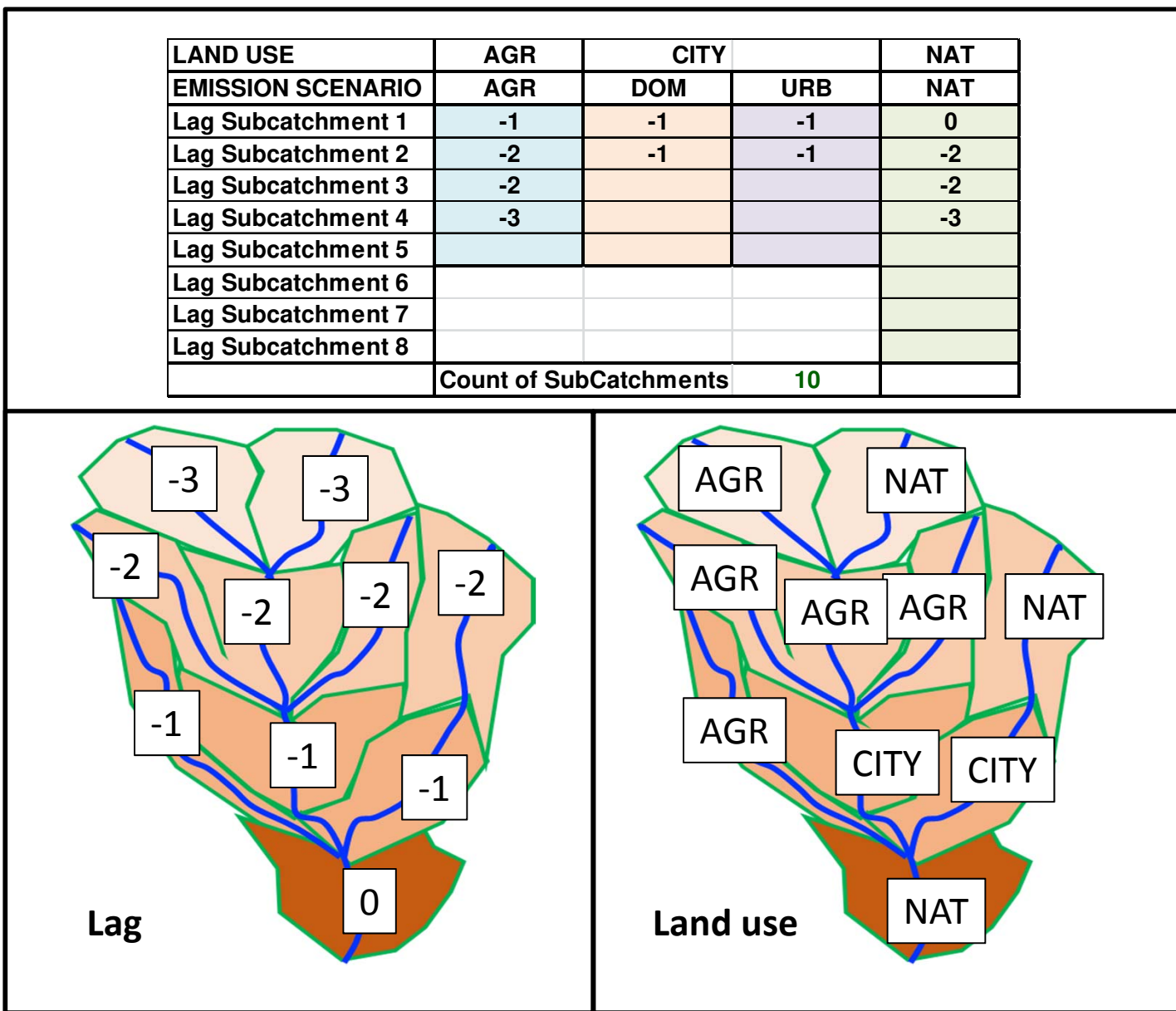
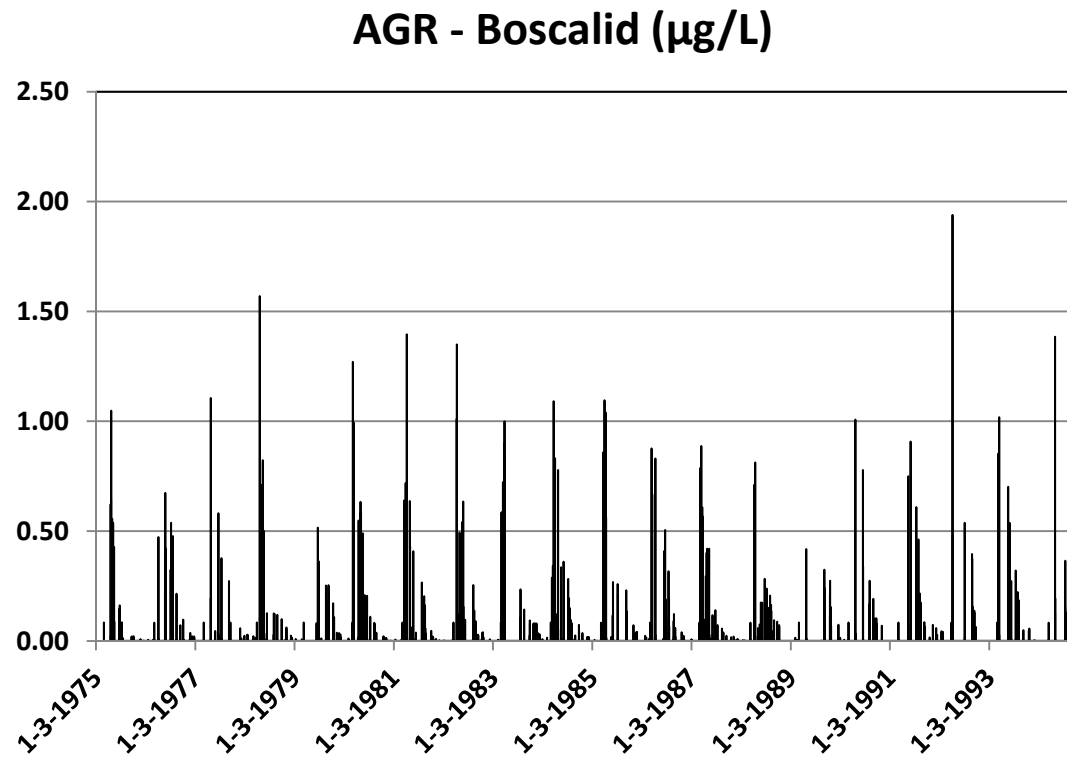


Figure 1\_Posthuma et al\_Pellston Mixtures Integration



**Figure 2\_(top left)** Posthuma et al\_Pellston Mixtures Integration

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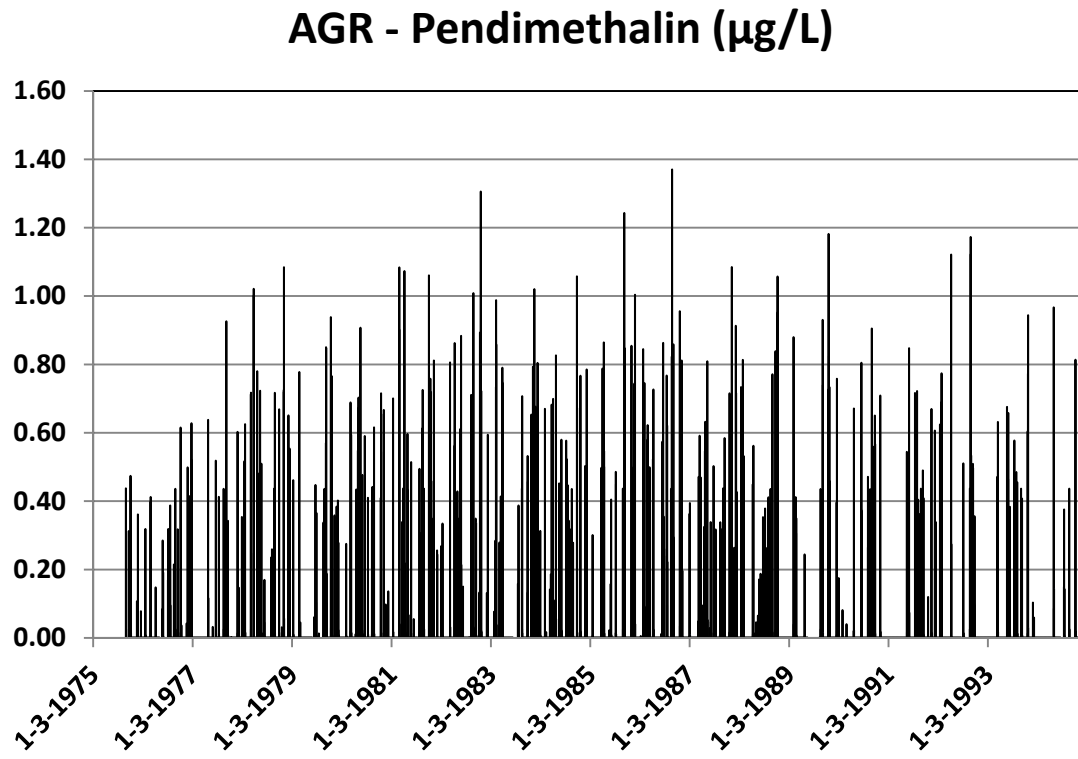
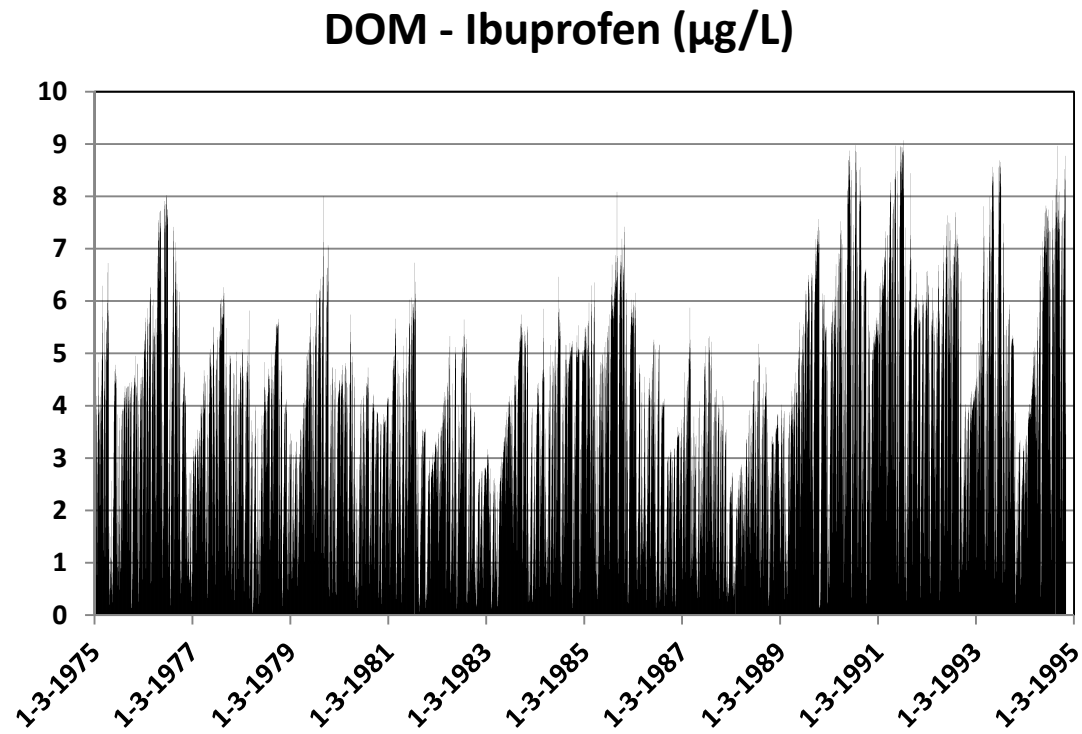
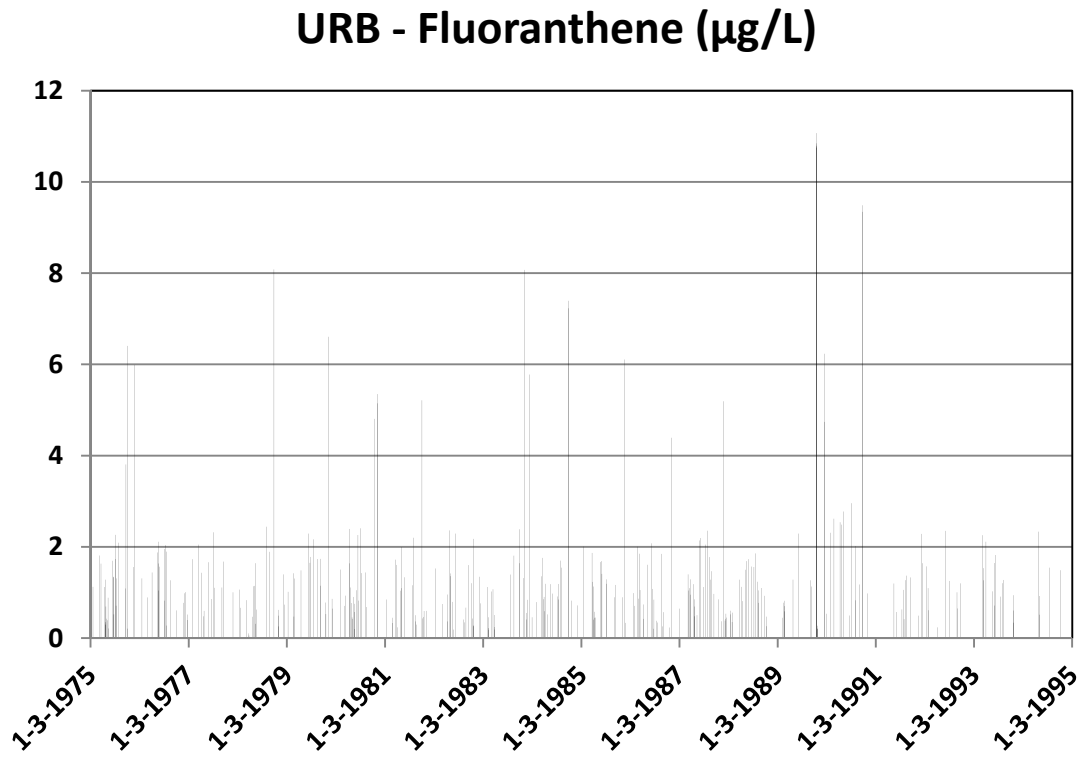


Figure 2\_(top right) Posthuma et al\_Pellston Mixtures Integration



**Figure 2\_**(bottom left) Posthuma et al\_Pellston Mixtures Integration

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**Figure 2\_**(bottom right) Posthuma et al\_Pellston Mixtures Integration

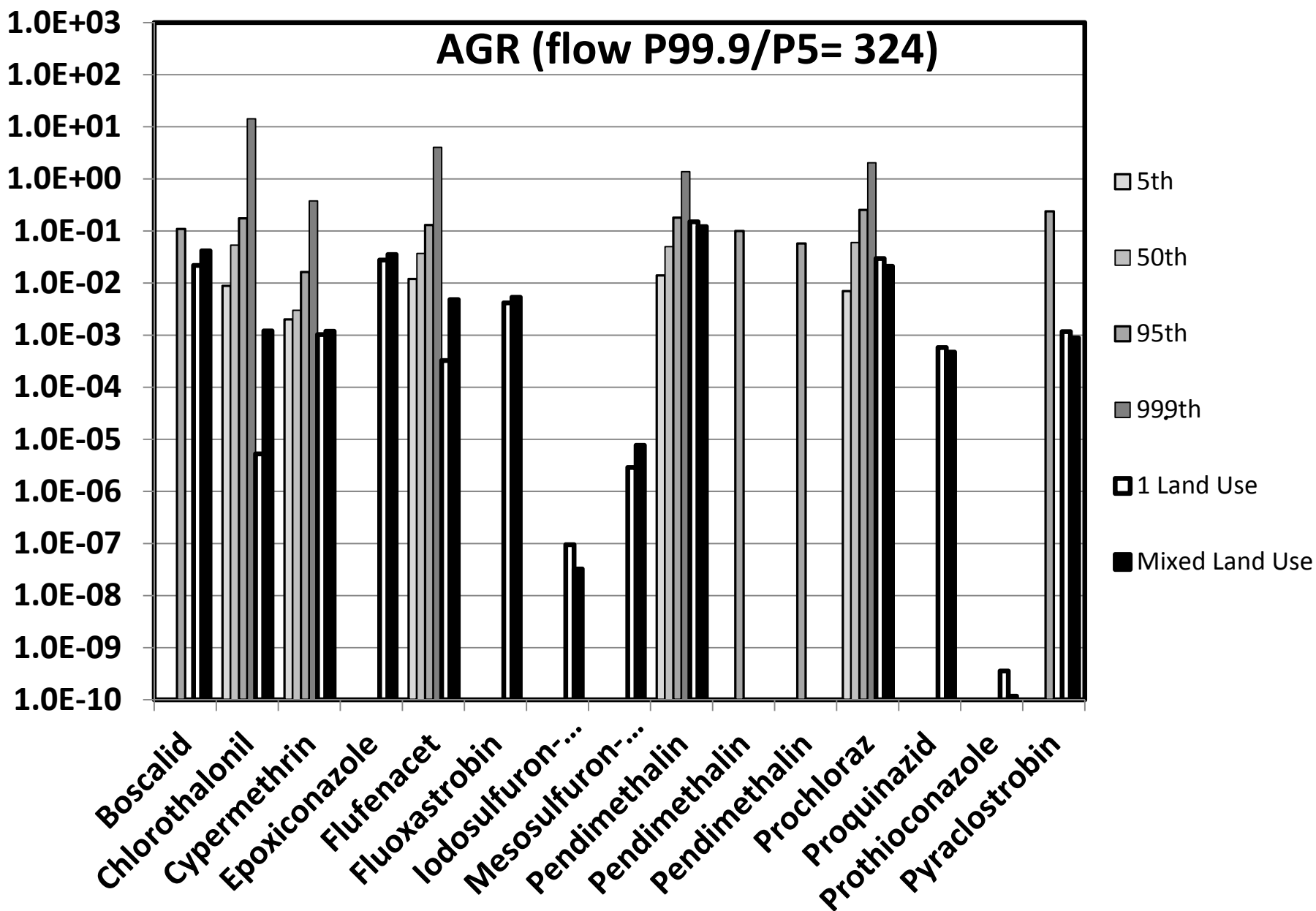


Figure 3\_Left\_Posthuma et al\_Pellston Mixtures Integration

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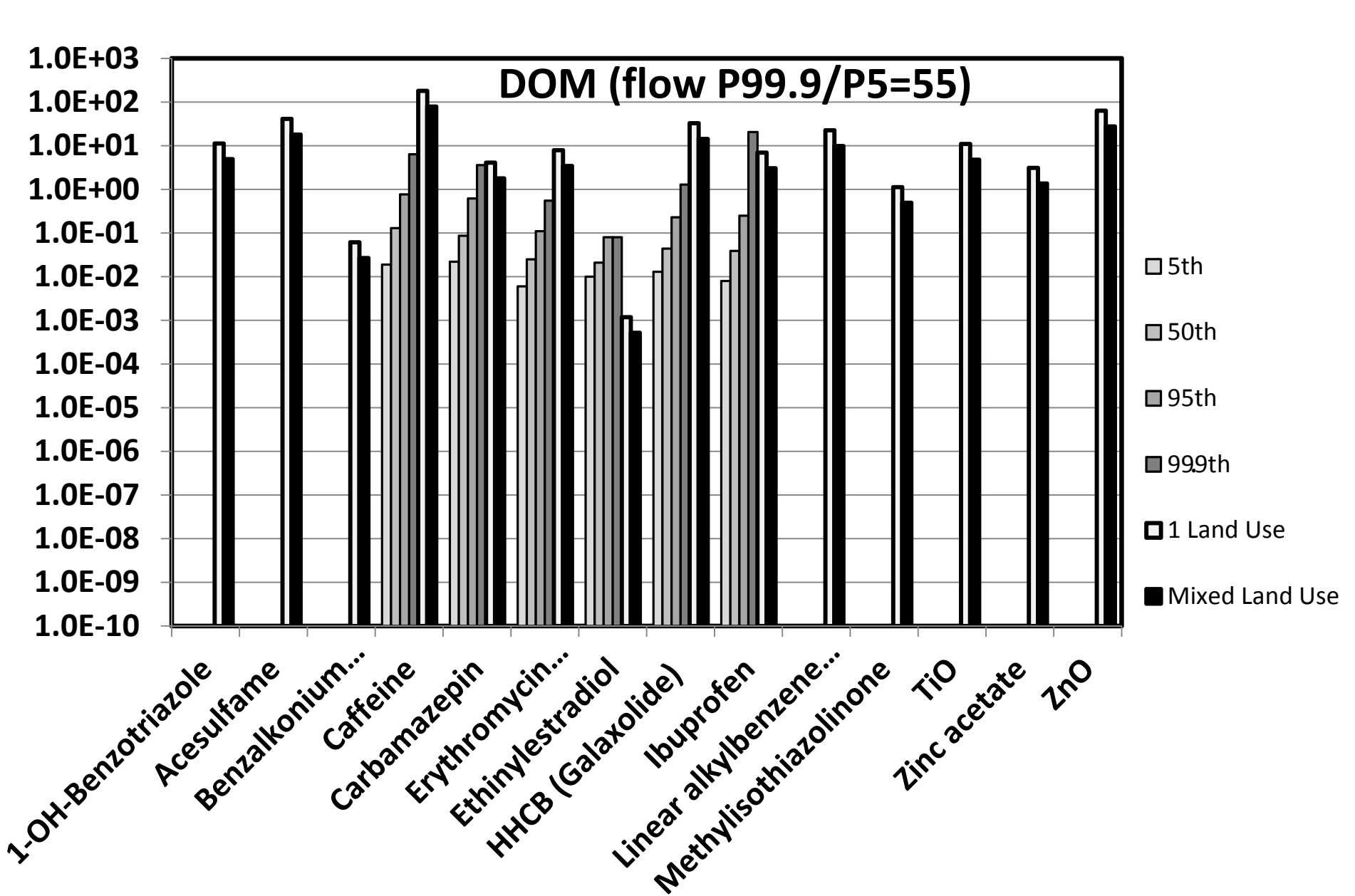


Figure 3\_Middle\_Posthuma et al\_Pellston Mixtures Integration

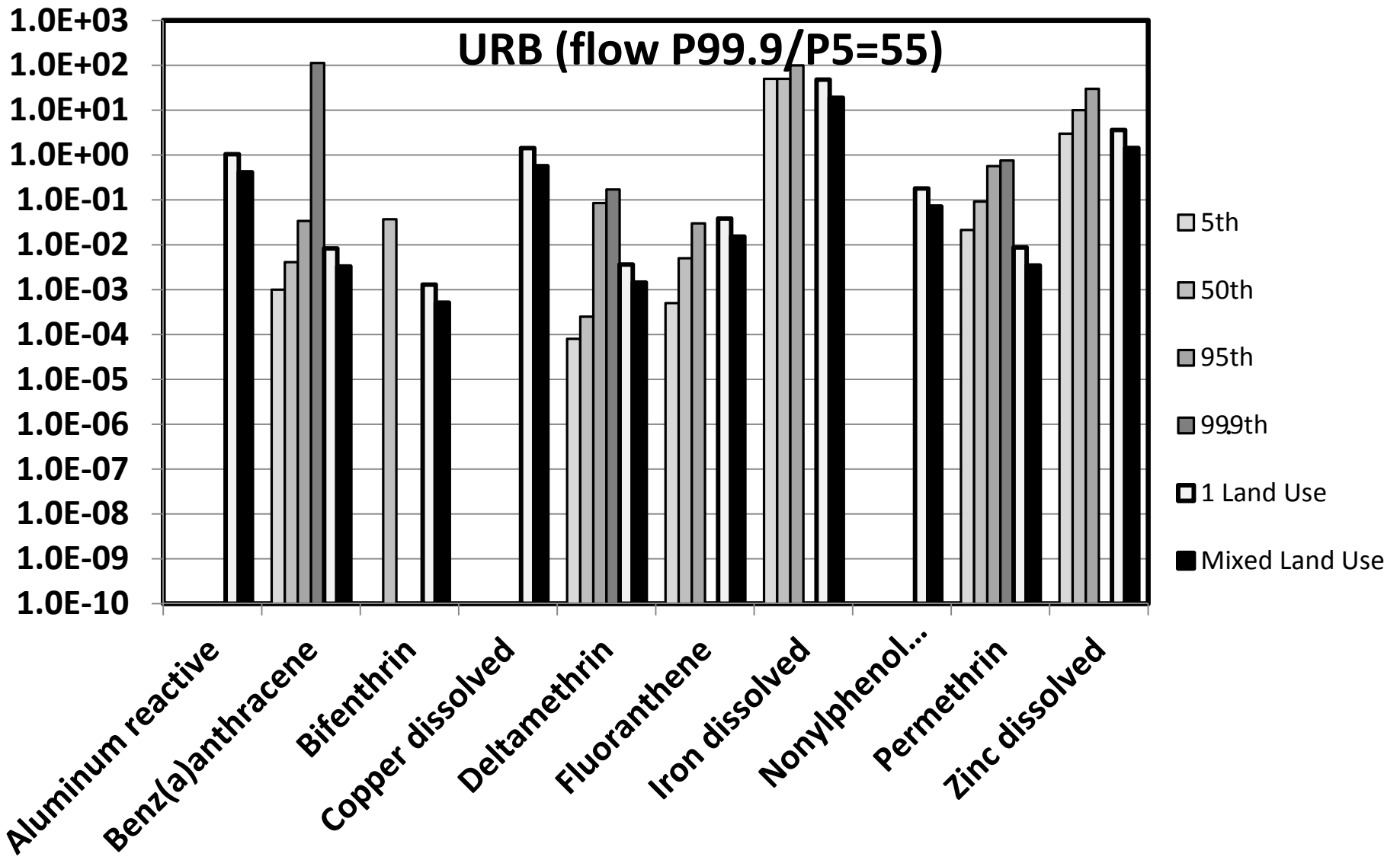


Figure 3\_Right\_Posthuma et al\_Pellston Mixtures Integration

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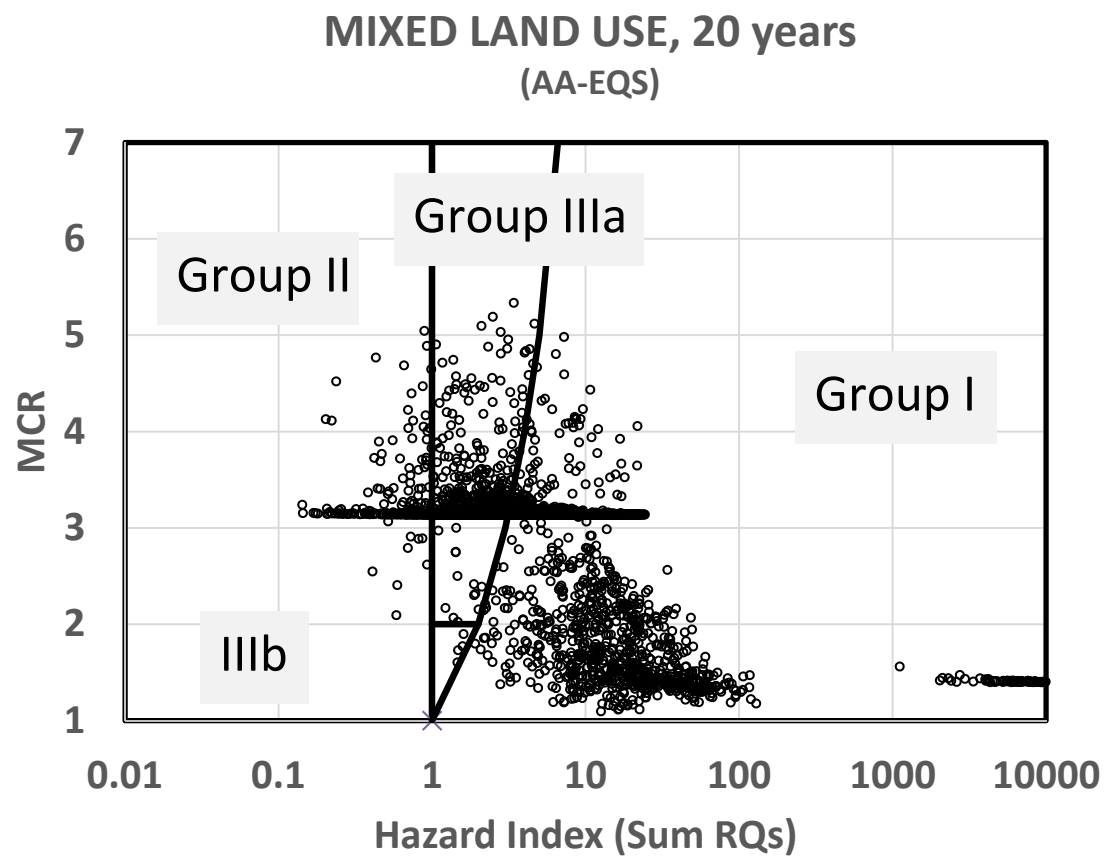


Figure 4\_left\_Posthuma et al\_Pellston Mixtures Integration

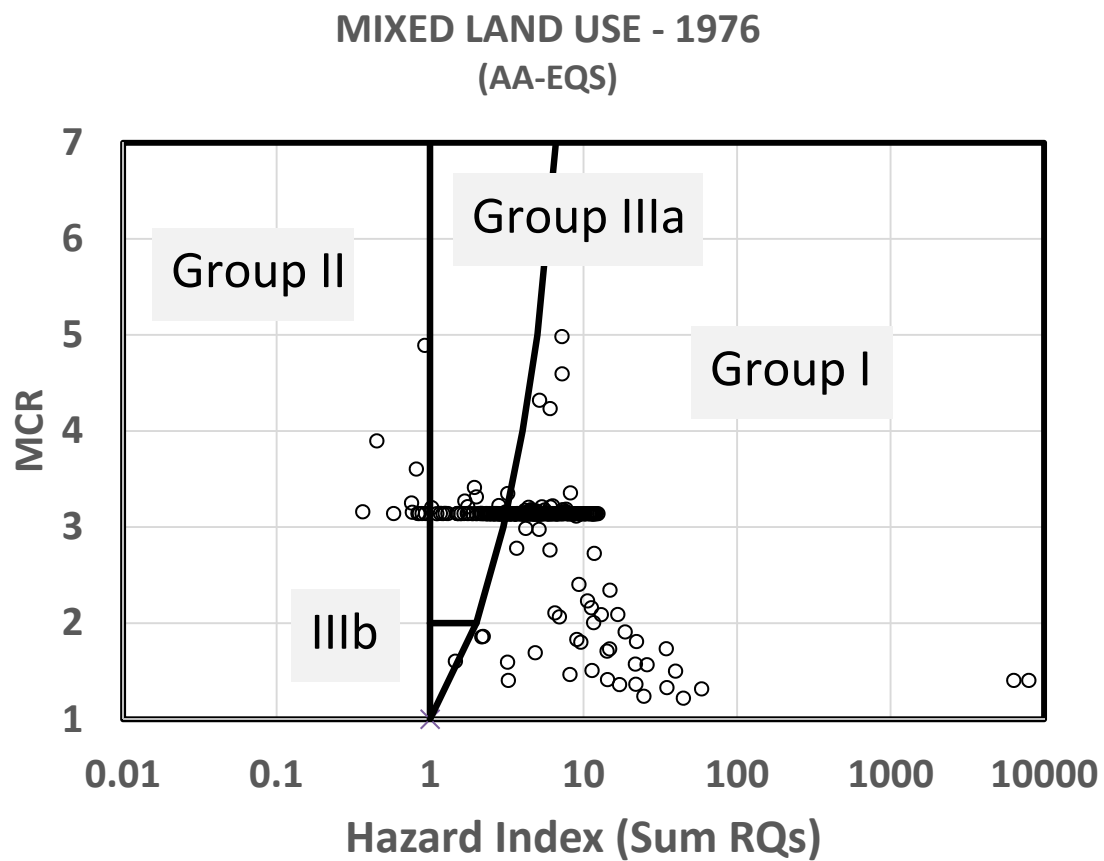


Figure 4\_right\_Posthuma et al\_Pellston Mixtures Integration

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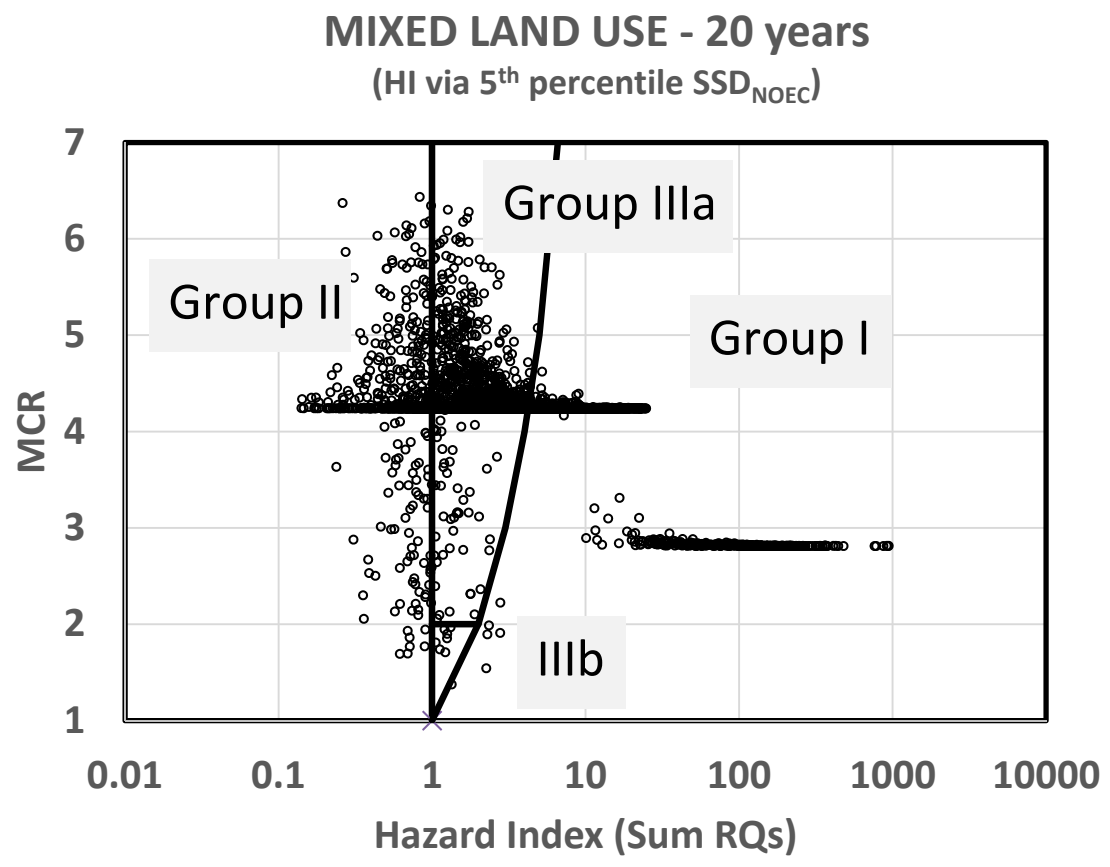


Figure 5\_Upper left\_Posthuma et al\_Pellston Mixtures Integration

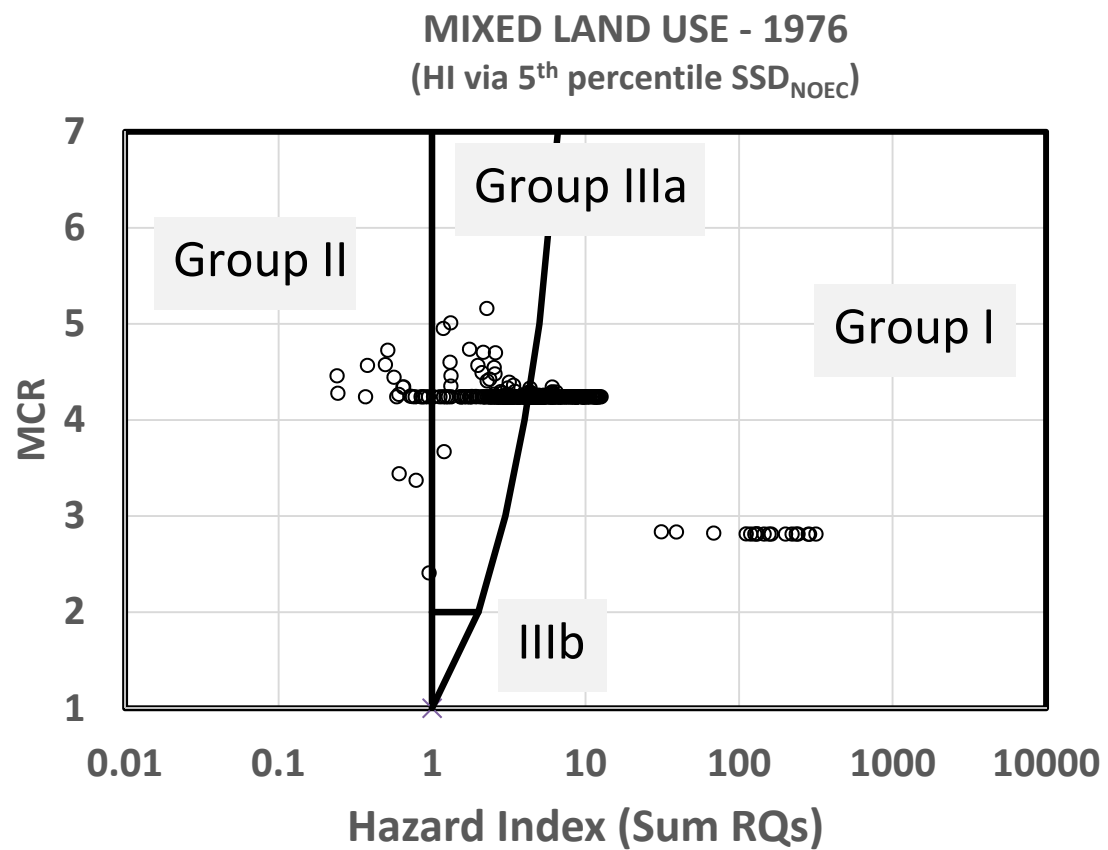
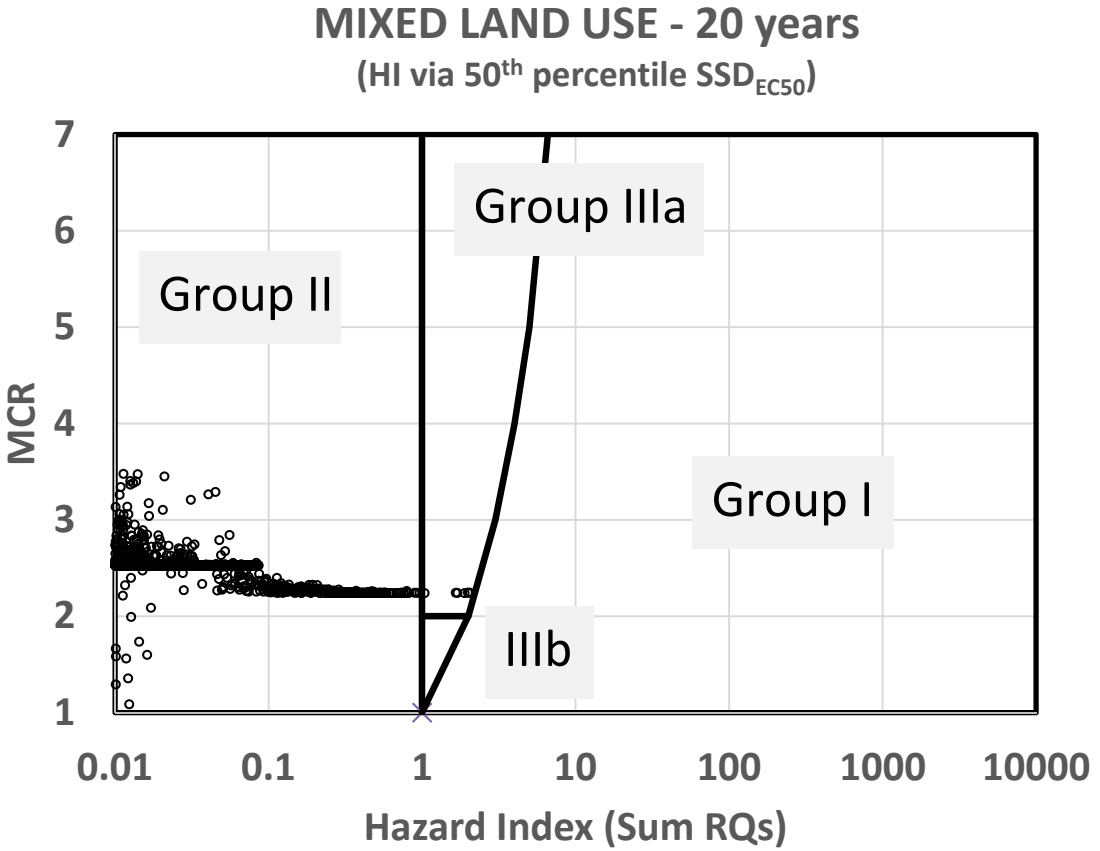


Figure 5\_Upper right\_Posthuma et al\_Pellston Mixtures Integration

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**Figure\_5 Bottom\_left\_Posthuma et al\_Pellston Mixtures Integration**

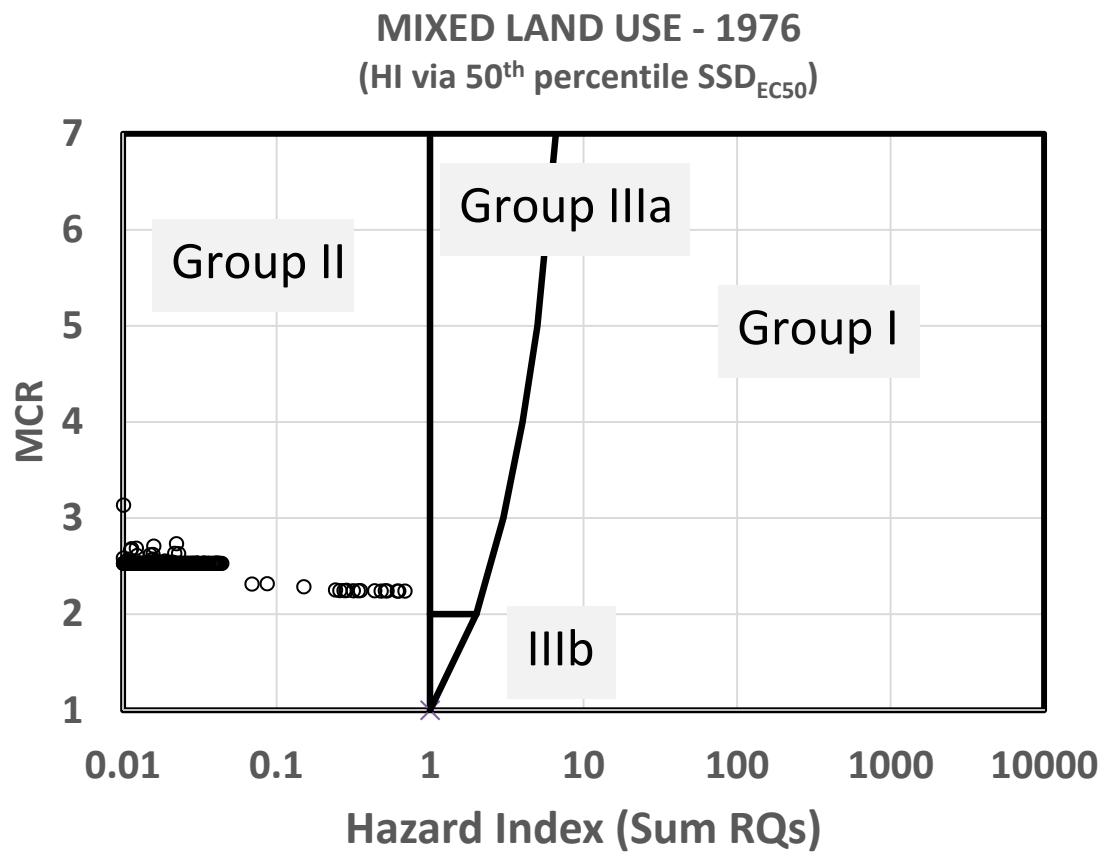


Figure 5\_Bottom, right\_Posthuma et al\_Pellston Mixtures Integration



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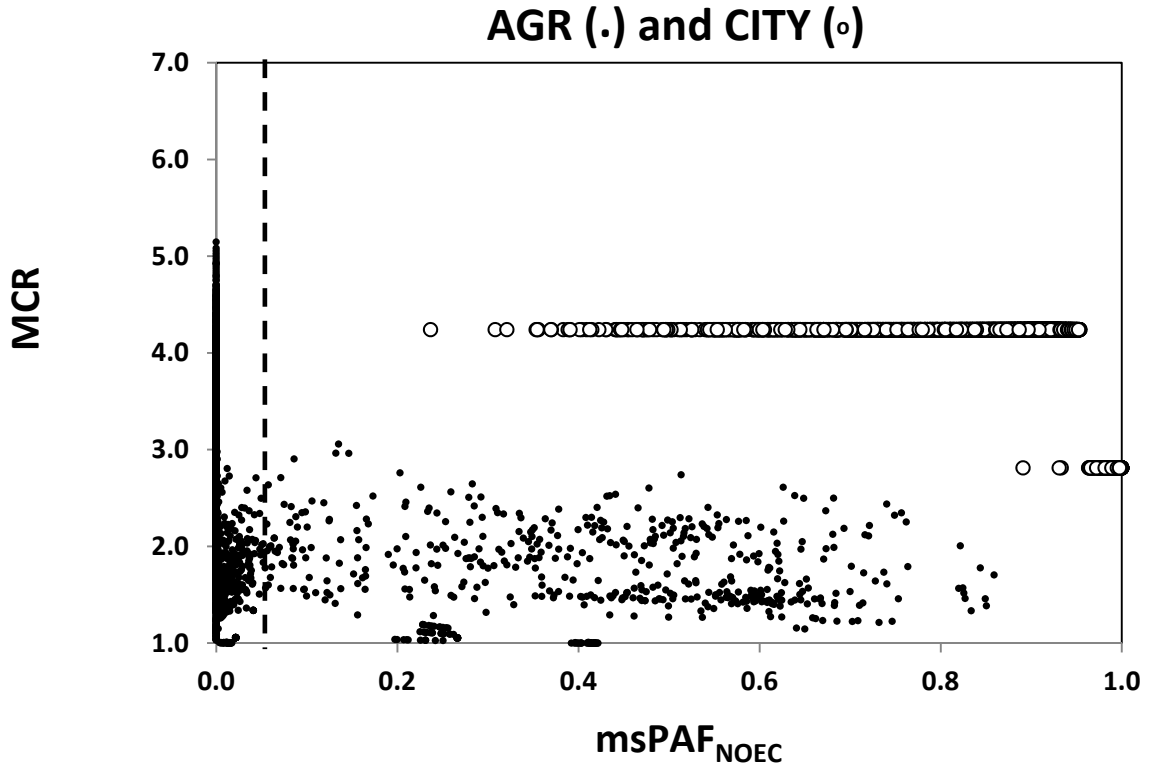
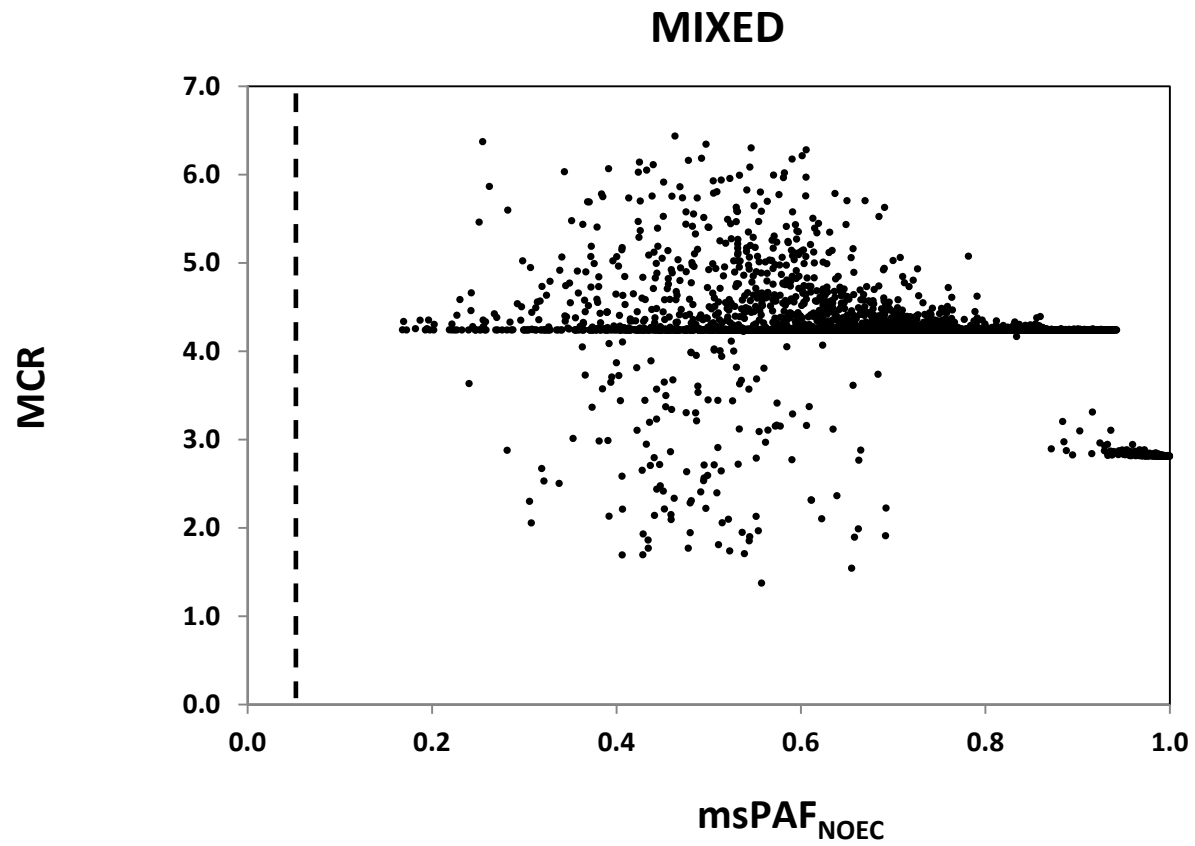


Figure 6\_Left\_Posthuma et al\_Pellston Mixtures Integration



**Figure 6\_Right\_Posthuma et al\_Pellston Mixtures Integration**

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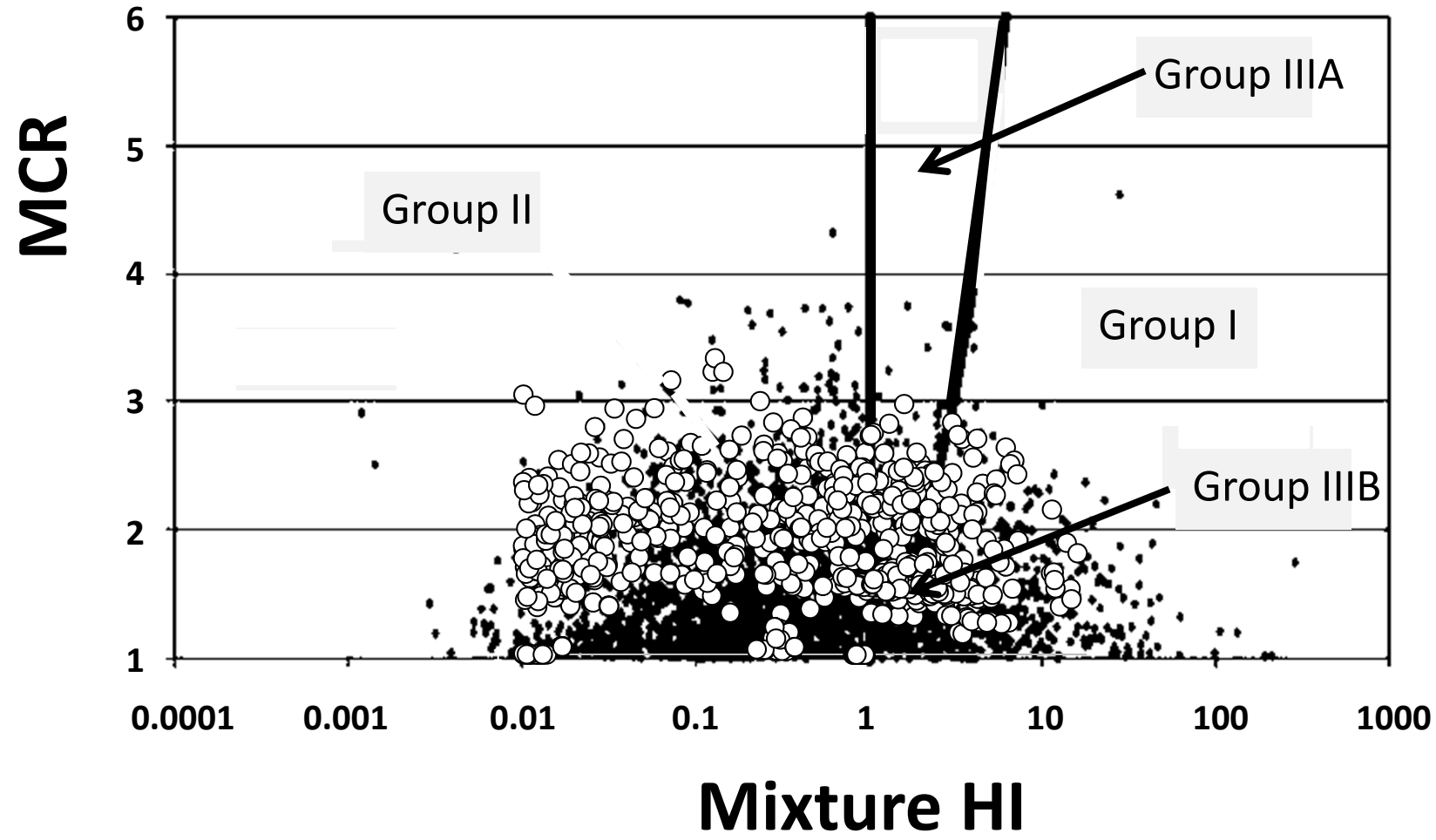
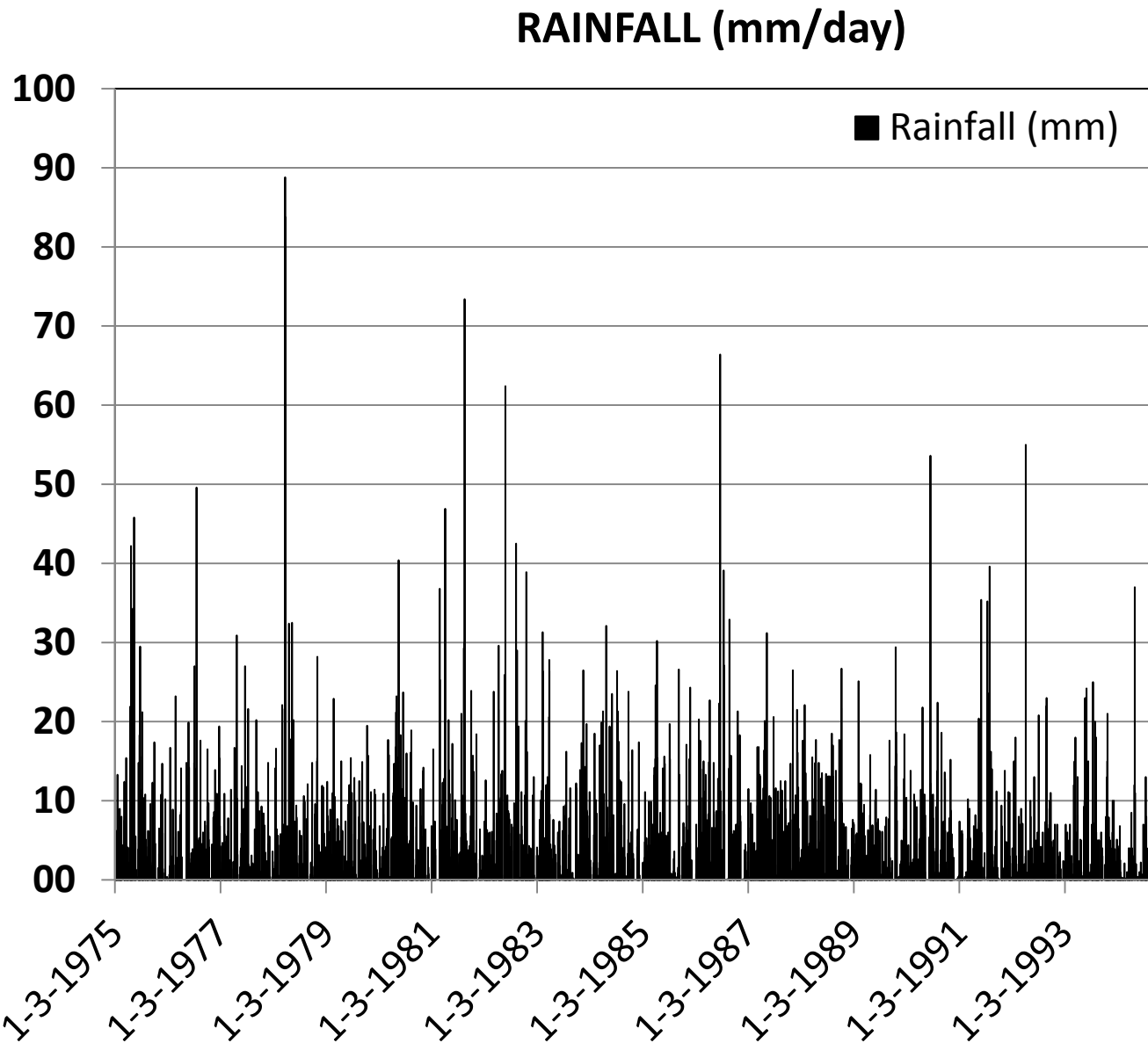


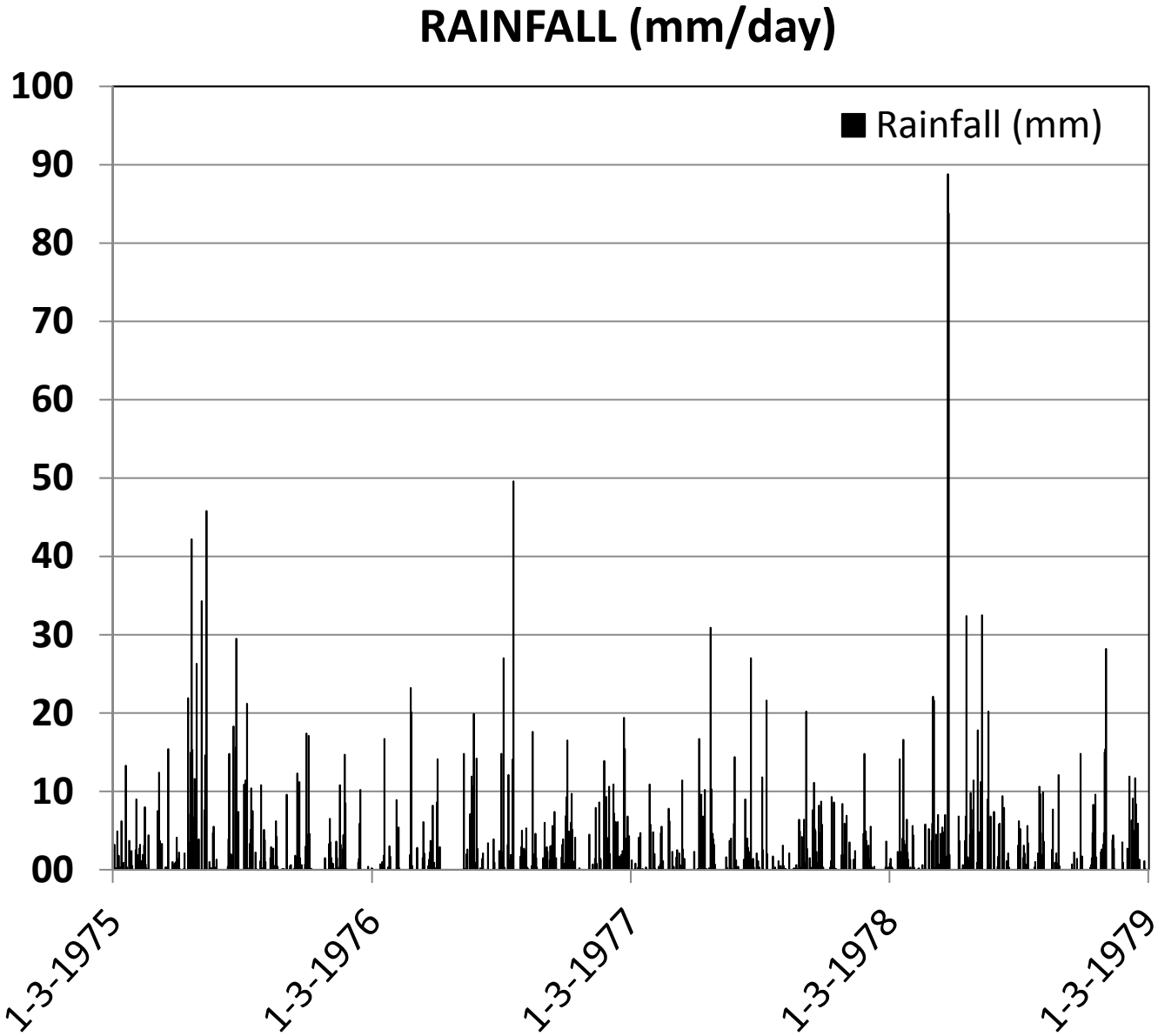
Figure 7\_Posthuma et al\_Pellston Mixtures Integration



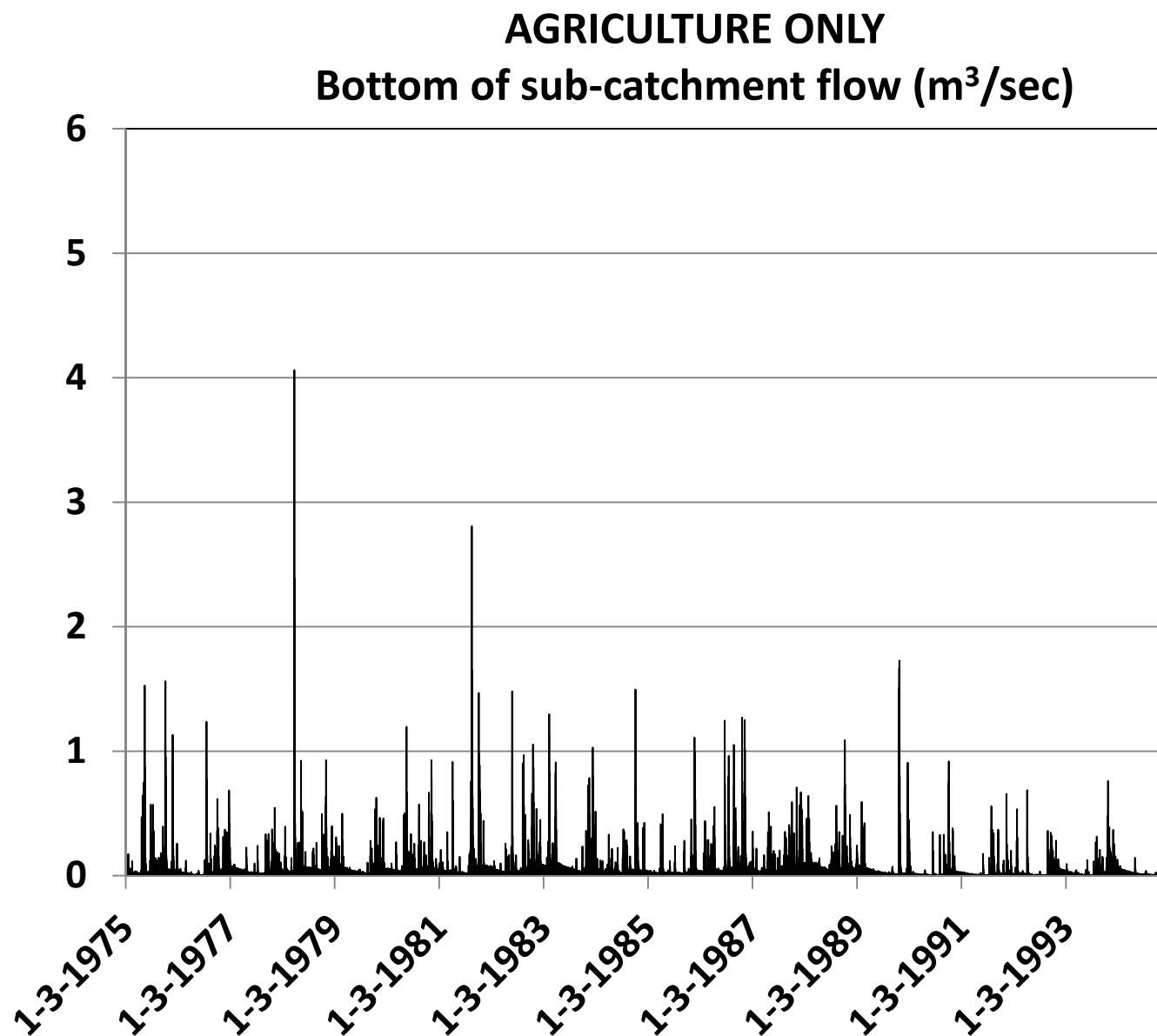
Supporting Information Figure 1 (left)\_Posthuma et al

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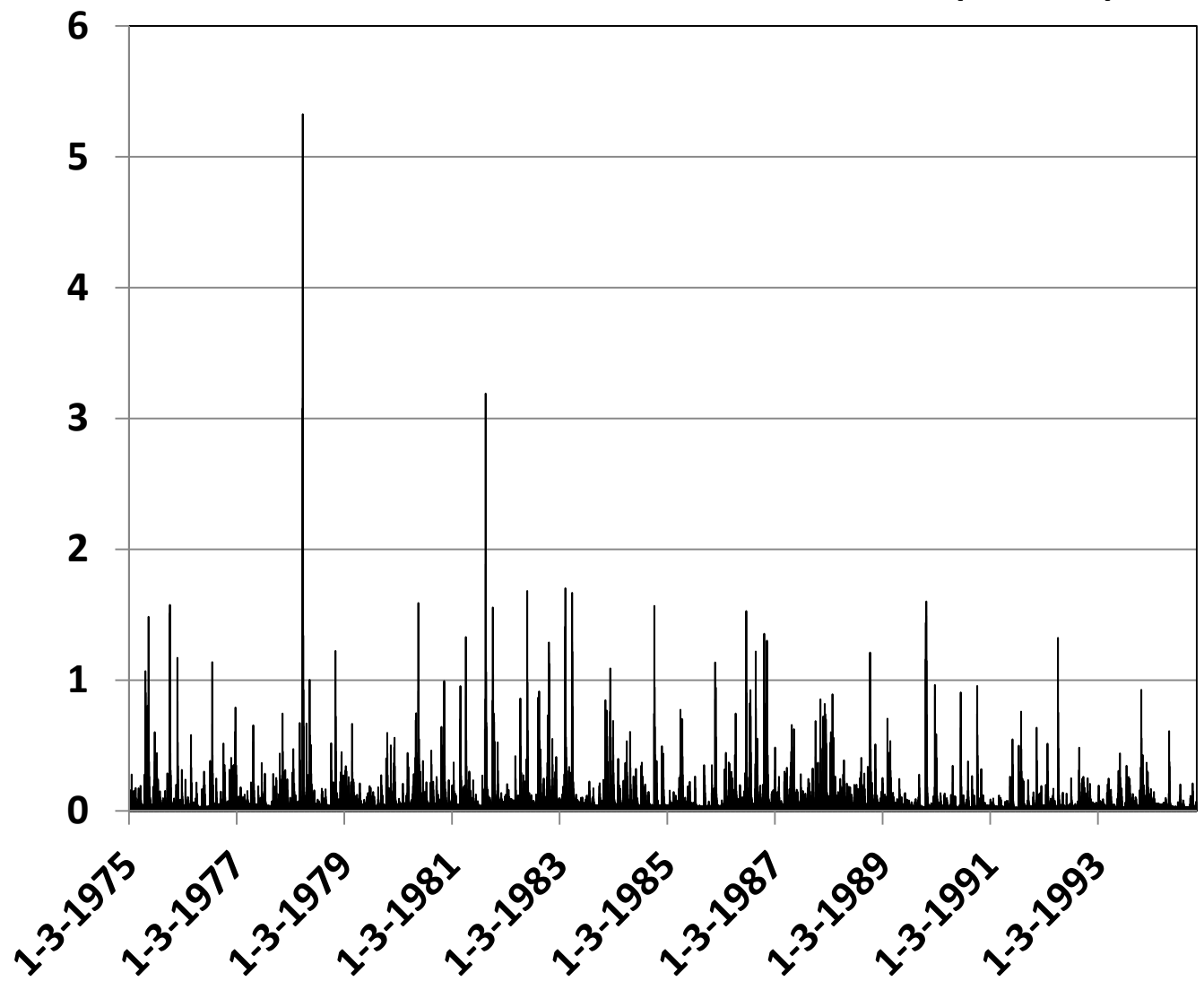
Supporting Information Figure 1 (right)\_Posthuma et al



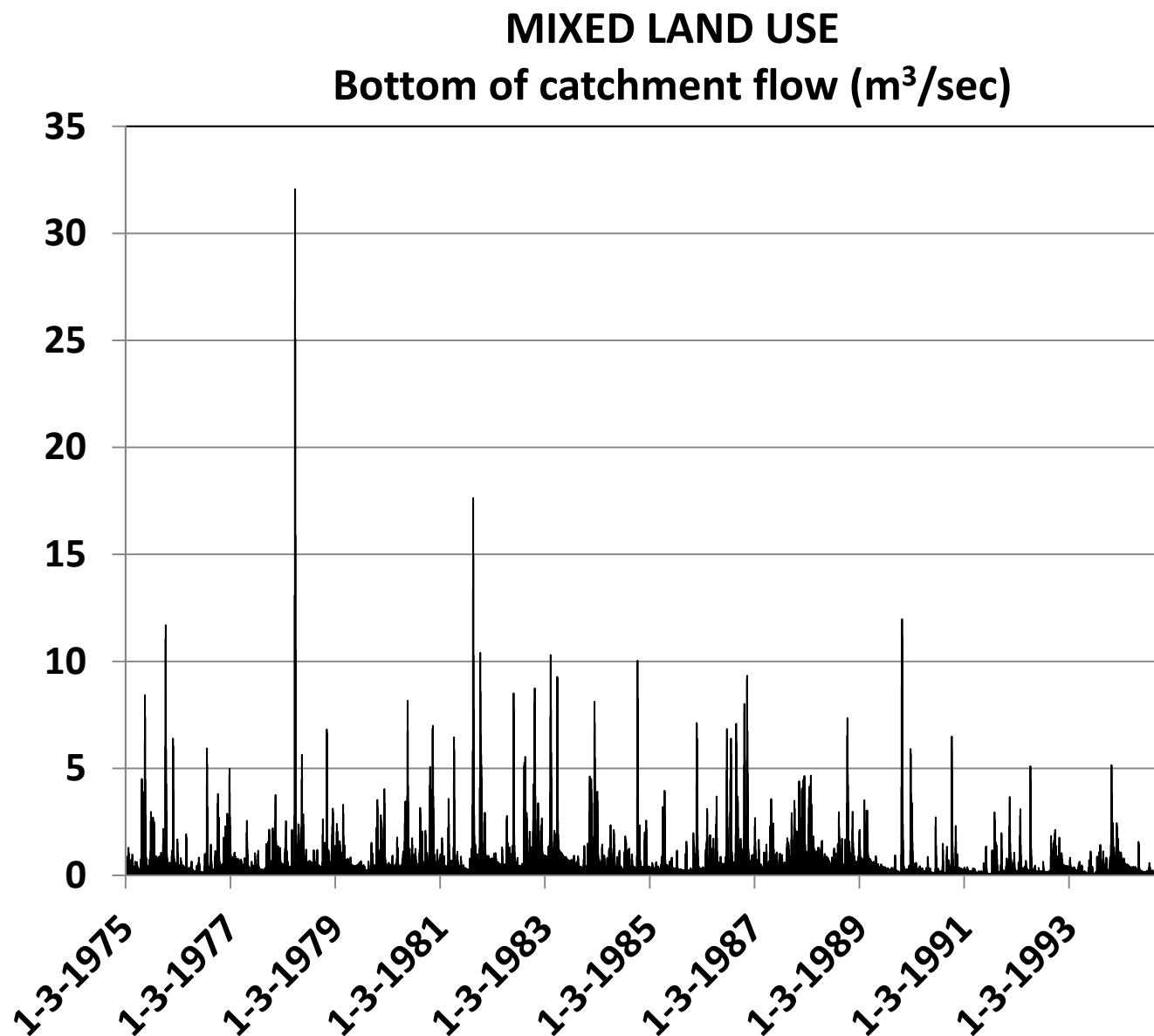
Supporting Information Figure 2 (left)\_Posthuma et al

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### CITY ONLY (DOMESTIC and RUNOFF) Bottom of sub-catchment flow (m<sup>3</sup>/sec)



Supporting Information Figure 2 (middle)\_Posthuma et al

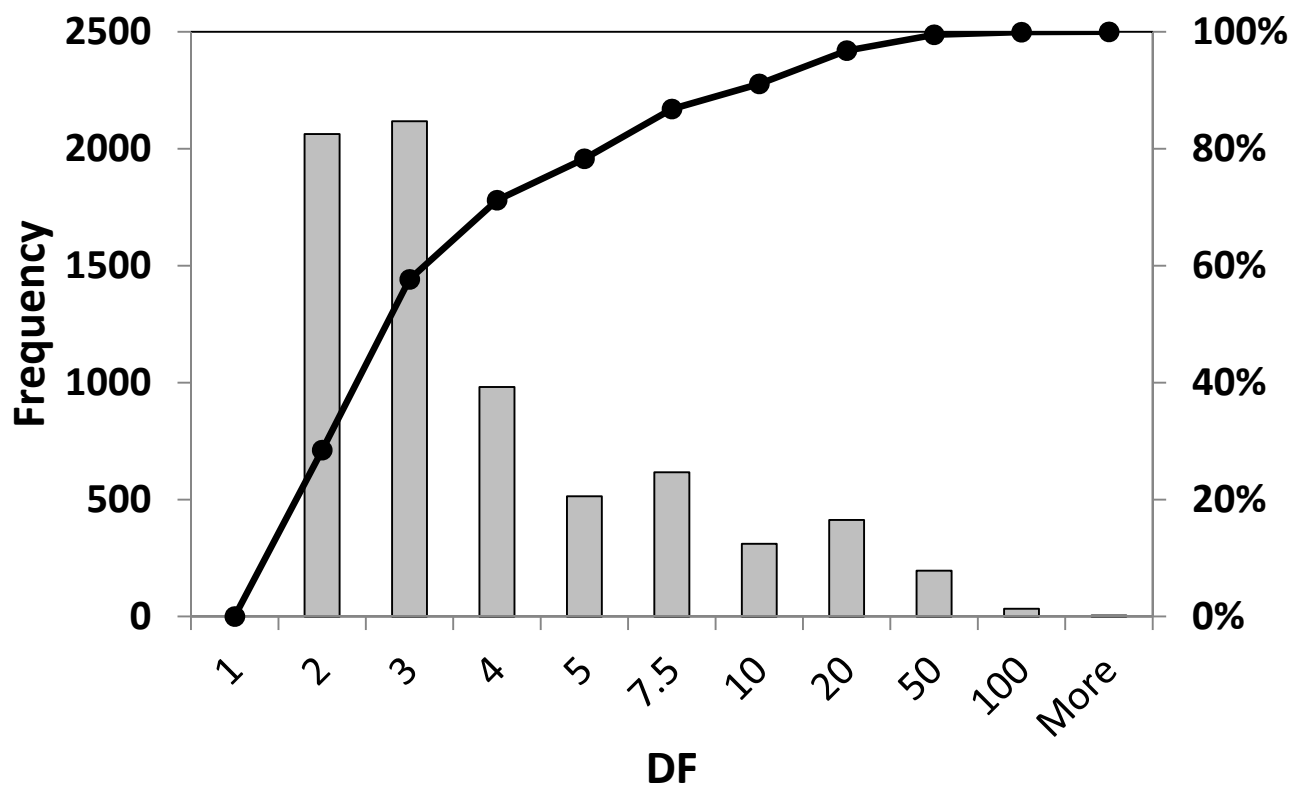


Supporting Information Figure 2 (right)\_Posthuma et al



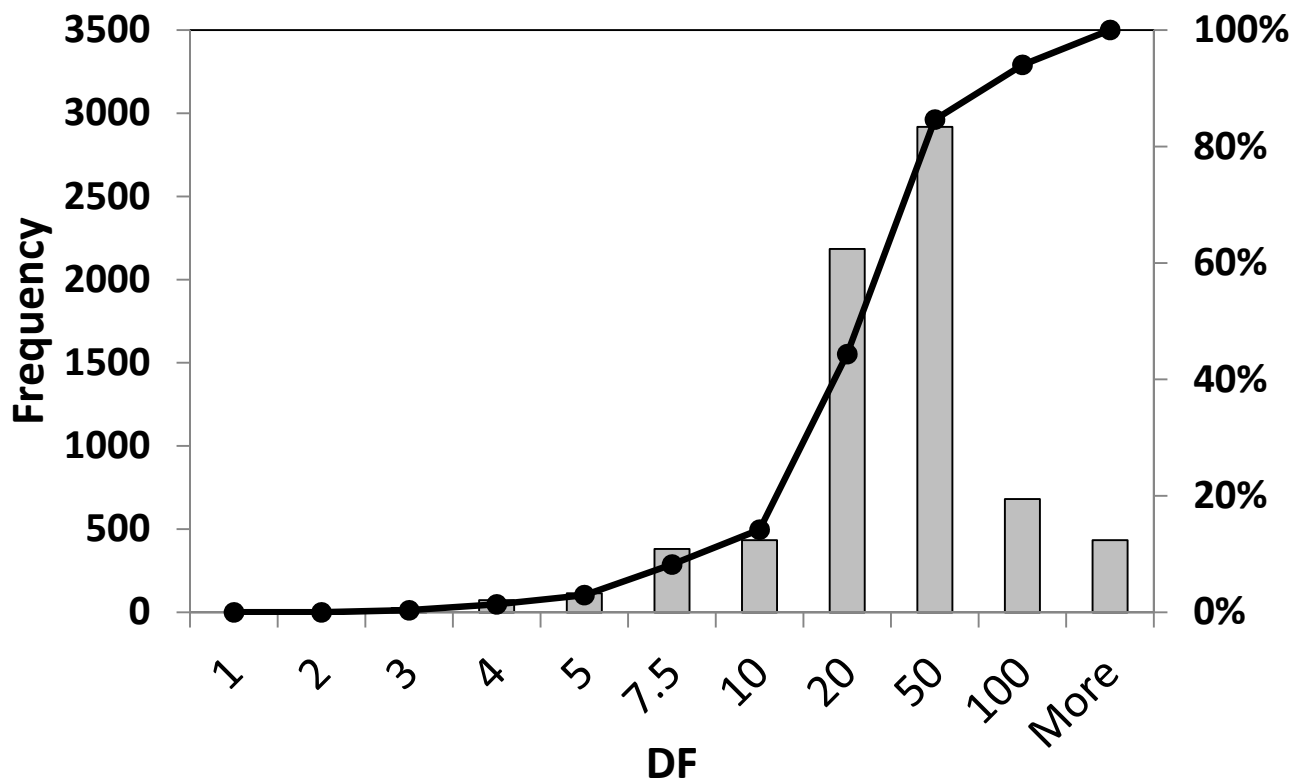
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### Dilution Factor - DOM sub-catchment



Supporting Information Figure 3 (left)\_Posthuma et al

### Dilution Factor - MIXED catchment



Supporting Information Figure 3 (right)\_Posthuma et al

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