



## Sources, occurrence and predicted aquatic impact of legacy and contemporary pesticides in streams

McKnight, Ursula S.; Rasmussen, Jes J.; Kronvang, Brian; Binning, Philip John; Bjerg, Poul Løgstrup

*Published in:*  
Environmental Pollution

*Link to article, DOI:*  
[10.1016/j.envpol.2015.02.015](https://doi.org/10.1016/j.envpol.2015.02.015)

*Publication date:*  
2015

*Document Version*  
Peer reviewed version

[Link back to DTU Orbit](#)

*Citation (APA):*  
McKnight, U. S., Rasmussen, J. J., Kronvang, B., Binning, P. J., & Bjerg, P. L. (2015). Sources, occurrence and predicted aquatic impact of legacy and contemporary pesticides in streams. *Environmental Pollution*, 200, 64-76. <https://doi.org/10.1016/j.envpol.2015.02.015>

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Sources, occurrence and predicted aquatic impact of legacy and contemporary**  
2 **pesticides in streams**

3  
4 **Ursula S. McKnight<sup>a,\*</sup>, Jes J. Rasmussen<sup>b</sup>, Brian Kronvang<sup>b</sup>, Philip J. Binning<sup>a</sup>, Poul L. Bjerg<sup>a</sup>**

5 *<sup>a</sup> Department of Environmental Engineering, Technical University of Denmark, Miljoevej Building 113, 2800 Kgs. Lyngby,*  
6 *Denmark*

7 *<sup>b</sup> Department of Bioscience, Aarhus University, Vejlsovej 25, 8600 Silkeborg, Denmark*

8 \*Corresponding author: Tel.: +45 4525 1412; Fax: +45 4593 2850; E-mail: [usmk@env.dtu.dk](mailto:usmk@env.dtu.dk); Co-authors email:  
9 [jr@dmu.dk](mailto:jr@dmu.dk); [bkr@dmu.dk](mailto:bkr@dmu.dk); [pjbi@env.dtu.dk](mailto:pjbi@env.dtu.dk); [plbj@env.dtu.dk](mailto:plbj@env.dtu.dk)

10  
11 **ABSTRACT**

12 We couple current findings of pesticides in surface and groundwater to the history of pesticide usage,  
13 focusing on the potential contribution of legacy pesticides to the predicted ecotoxicological impact on  
14 benthic macroinvertebrates in headwater streams. Results suggest that groundwater, in addition to  
15 precipitation and surface runoff, is an important source of pesticides (particularly legacy herbicides)  
16 entering surface water. In addition to current-use active ingredients, legacy pesticides, metabolites and  
17 impurities are important for explaining the estimated total toxicity attributable to pesticides. Sediment-  
18 bound insecticides were identified as the primary source for predicted ecotoxicity. Our results support  
19 recent studies indicating that highly sorbing chemicals contribute and even drive impacts on aquatic  
20 ecosystems. They further indicate that groundwater contaminated by legacy and contemporary  
21 pesticides may impact adjoining streams. Stream observations of soluble and sediment-bound  
22 pesticides are valuable for understanding the long-term fate of pesticides in aquifers, and should be  
23 included in stream monitoring programs.

24 **Capsule:** Legacy pesticides, particularly sediment-bound insecticides were identified as the primary  
25 source for predicted ecotoxicity impacting benthic macroinvertebrates in headwater streams.

26 **Keywords:** legacy pesticides; groundwater-surface water interaction; sediment-bound residues; benthic  
27 macroinvertebrates; ecotoxicity; aquatic systems

## 28 **1. Introduction**

29 More than 85,000 chemicals are in production and use worldwide, with more than 2,200 produced  
30 in amounts exceeding 450 tons per year. Pesticides are second only to fertilizers in the amount of  
31 chemicals applied and extent of use in the environment (Stokstad and Grullon, 2013), with an estimated  
32 1 to 2.5 million tons of active ingredients used each year, predominantly in agriculture (Fenner et al.,  
33 2013). Nearly 20,000 pesticide products have entered the market since registration began in 1947  
34 (Lyandres, 2012) and more than 1,000 are sold annually in Denmark (Danish EPA, 2011).

35 The term pesticide covers a wide range of compounds that are typically broadly categorized on the  
36 basis of their pesticidal actions, including herbicides, fungicides, insecticides, nematicides, plant  
37 growth regulators, and others, or they can be classified on the basis of their general chemical nature  
38 (e.g. organochlorines; organophosphates) or mode of action (Arias-Estévez et al., 2008; Nollet and  
39 Rathore, 2010). Despite their recognized importance, especially for agricultural production and  
40 pathogen control, pesticides are now considered among the most harmful types of compounds  
41 impacting surface waters (Schwarzenbach et al., 2006). In particular, insecticides have been thought to  
42 be one of the principal stressors affecting stream macroinvertebrates, as well as fundamental ecosystem  
43 functions such as leaf litter decomposition (Peters et al., 2013; Schaefer et al., 2012).

44 The environmental consequences associated with land use intensification are considered one of the  
45 main drivers for increased biodiversity loss and impairment of ecosystem functions (Beketov et al.,  
46 2013), and ways to balance the need to feed the world without disastrous effects on soil, water and  
47 habitat are still being sought after. Pesticides are now widely recognized as prevalent (Konstantinou et  
48 al., 2006), including many of their metabolites (Barth et al., 2007) and production impurities (Holt et  
49 al., 2010; Reitzel et al., 2004) – which are typically not considered in ecotoxicological studies – and  
50 consistently detected over time (e.g. corn herbicides in Vecchia et al., 2009). The occurrence of  
51 pesticides is well-documented for specific environmental compartments, such as in soils (Olsen et al.,  
52 2011) and streambed sediment (Kronvang et al., 2003), groundwater and surface water (Gilliom, 2007;  
53 Roy and Bickerton, 2012), or rainwater (i.e. atmosphere) (Vogel et al., 2008).

54 More recently, studies have begun to focus on the transport and ultimate fate of these compounds as  
55 they move through the different media, for example from agricultural application to soils (cropland)  
56 into the atmosphere (Asman et al., 2005) or into groundwater (Arias-Estévez et al., 2008), from  
57 groundwater to drinking water supply wells (Levi et al., 2014) or groundwater-dependent ecosystems  
58 (Ejrnaes et al., 2014), and then considering the ecotoxicological impacts once these compounds are  
59 present in surface water (Schaefer et al., 2013). Although studies exist that focus on linking pesticide  
60 transport from groundwater to surface water (e.g. Squillace et al., 1993; Kolpin et al., 2001), studies  
61 which endeavor to disentangle the contributions and importance of this entry pathway for aquatic  
62 ecosystems relative to the contribution of other routes, such as pesticide runoff from agricultural land,  
63 are still needed.

64 Moreover, it has become clear that a proper ecotoxicological understanding of the history of low-  
65 dose contamination, impacting stream environments over successive generations, is lacking (Artigas et  
66 al., 2012). This is due in part to the fact that pesticides are specifically designed for topsoil conditions  
67 and subsequently tested under controlled settings that do not accurately reflect the long history of  
68 pesticide exposure. This has contributed to the established viewpoint that contamination of surface  
69 waters by pesticide residues is transitory (occurring primarily during and immediately after application)  
70 (Capel et al., 2001; Holvoet et al., 2007), thus serving to emphasize contemporary pesticides in the  
71 aqueous phase and their related effects on aquatic communities (Schaefer et al., 2011; Schaefer et al.,  
72 2012) with a focus on run-off from rain events (Wittmer et al., 2010; Wittmer et al., 2011).  
73 Importantly, recognition of sediments as an important delivery pathway for pesticide contamination is  
74 increasing (Kuivila et al., 2012; Warren et al., 2003), but studies are still lacking which include a wider  
75 range of legacy pesticides. In addition, a dearth of both chronic and sediment toxicity values has  
76 complicated a more holistic evaluation. A re-examination of the history and legacy of pesticide usage  
77 together with trends in measured pesticide concentrations in groundwater, surface water and the  
78 sediment-phase is therefore crucial to our understanding how to mitigate their impacts.

79 Our main objective was to couple current findings of pesticides in Danish streams and groundwater  
80 (McKnight et al., 2012; Rasmussen et al., 2013) to the history of pesticide usage, in particular focusing  
81 on whether legacy pesticides contribute to the overall predicted toxicity impacting aquatic benthic  
82 macroinvertebrates. This is driven especially by the hypothesis that an examination of only the recent  
83 pesticide application history (one decade or less) may not be sufficient to guarantee the sustainable

84 management and restoration of impaired aquatic resources. This could have ramifications for our  
85 understanding of pesticide fate, pathways and resulting ecotoxicological effects. More specific  
86 objectives were to: (i) assess pesticide trends using five decades of agricultural application data, as well  
87 as stream concentrations for the pesticides found in 14 Danish headwater streams, (ii) evaluate  
88 observed stream water concentrations collected under different hydrological conditions to identify the  
89 role of the groundwater route for pesticides in streams, and (iii) identify which pesticides  
90 (contemporary; legacy) and phases (dissolved, sediment-bound) contribute to predicted  
91 ecotoxicological potential in streams.

## 92 **2. The legacy of pesticide usage**

93 Although the first recorded use of pesticides dates back to ca. 2500 BC when farmers began using  
94 chemical sulfur to kill pests on crops (Unsworth, 2010), the modern-day development and application  
95 of pesticides was initiated after the Second World War. Earlier pesticides consisted predominantly of  
96 inorganic substances derived from natural sources containing large quantities of metals, e.g. arsenic,  
97 copper, and lead, and were heavily in use up until the 1940s (Nollet and Rathore, 2010; Uneke, 2007).  
98 Their application led to the accumulation of metals in the subsurface that could potentially re-mobilize  
99 depending on the prevailing geochemical and climatic conditions. Early organic pesticides such as  
100 nitrophenols, chlorophenols and petroleum oils were by-products of industrial processes such as coal  
101 gas production, and were used against fungal infections and insect pests (Rathore and Nollet, 2012;  
102 Unsworth, 2010). Disadvantages to the use of these substances included the high rates of application,  
103 non-specificity and resulting high (phyto-)toxicity.

104 In order to combat these disadvantages, the 1940s led to an accelerated growth in the development  
105 of natural (plant-based) pesticides, as well as the introduction of synthetic organic compounds with the  
106 discovery of the effects of organochlorines, such as DDT, and phenoxy acids, e.g. dichlorprop. These  
107 chemicals were effective and inexpensive, with DDT being the most popular due to its broad-spectrum  
108 activity, and were thought to consist of less dangerous substances since they appeared to be less toxic  
109 to mammals and reduced insect-borne diseases such as malaria (Unsworth, 2010). However, many of  
110 these chemicals were later found to vary in aquatic (acute) toxicity from moderately toxic (U.S. EPA  
111 category II (Kegley et al., 2008), e.g. DDT; organophosphates: chlorpyrifos) to extremely toxic (U.S.  
112 EPA category I (Kegley et al., 2008), e.g. organochlorines: lindane; dinitrophenols: dinoseb) depending

113 on the specific properties of the substance, such as biodegradability, tendency to bioaccumulate, and  
114 carcinogenicity (e.g. DDT, lindane). Many of the organochlorines have now been banned or  
115 discontinued because of these traits (DG SANCO, 2014).

116 Organophosphates, triazines (e.g. atrazine), phenoxy acids (MCPA), carbamates (pirimicarb),  
117 triazoles (propiconazole) and pyrethroids ( $\lambda$ -cyhalothrin) have been used in contemporary pest control  
118 since the 1940s. These compounds are often characterized as being more stable, albeit less persistent  
119 than the organochlorines, with a mode of action that is less specific compared to the compounds  
120 developed during the 1970s. Pesticides first introduced in the 1970s have been further adapted to  
121 contain only organic substances without metals, must now pass more stringent controls (transport;  
122 degradation; ecotoxicity), and are typically developed for a specific target (e.g. endocrine disruption,  
123 electron transport inhibition); examples include isoproturon, glyphosate and metamiltron. Their frequent  
124 use in modern agricultural and urban areas is attributed to their exceptional pesticidal activity greatly  
125 reducing application rates (Uneke, 2007). However their increased selectivity has led to increased  
126 problems with resistance, leading to the need for management strategies to combat this negative effect  
127 (Unsworth, 2010).

128 More recently, pesticides are being developed solely by genetic engineering. These pesticides are  
129 hypothesized to be safer than previously developed compounds (Uneke, 2007), but their environmental  
130 impacts are still largely unknown (e.g. as a part of the cocktail of pesticides impacting non-target  
131 organisms). New pesticide chemistry utilizing traditional (eco)toxicological methods, has also allowed  
132 better resistance management (in part through the implementation of integrated pest management  
133 systems), improved selectivity and better environmental and toxicological profiles (Unsworth, 2010).

### 134 **3. Methods**

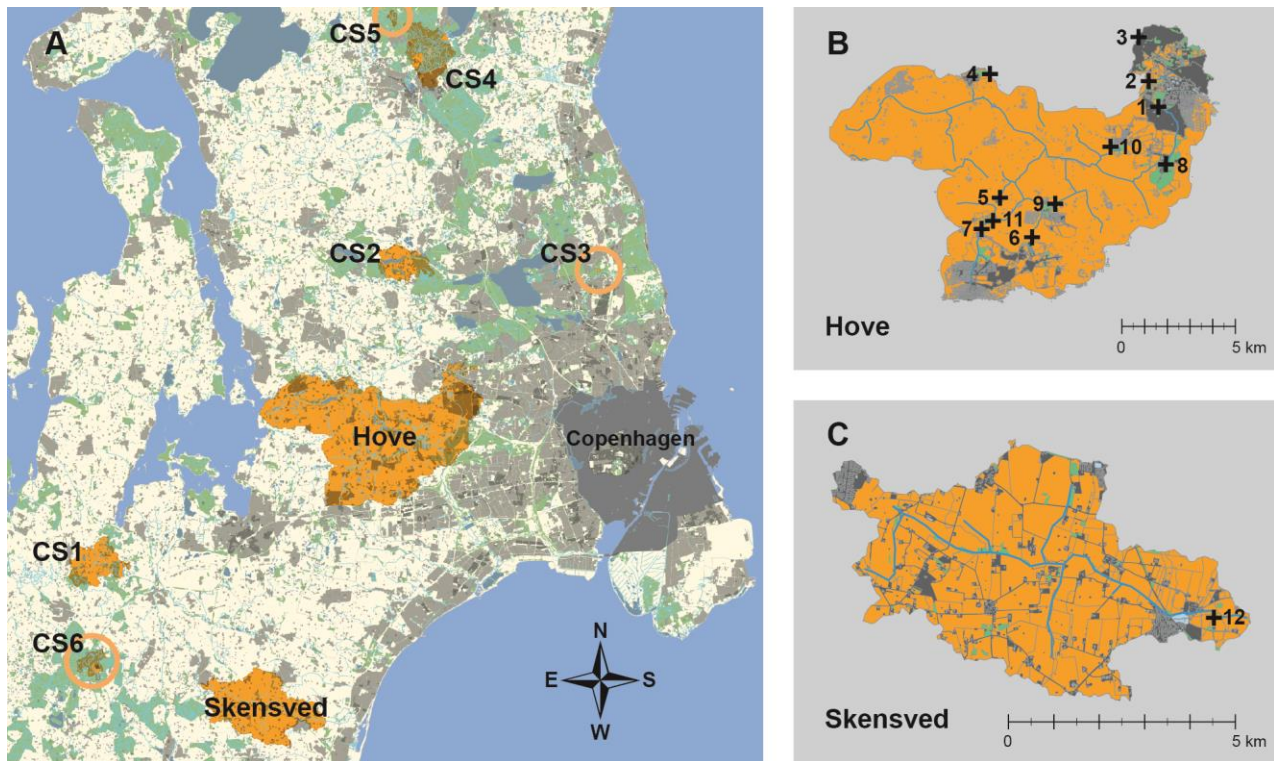
#### 135 *3.1 Study catchments*

136 Fourteen headwater streams in four catchments located on Sjaelland, Denmark were selected (Fig.  
137 1A), and the concentrations of pesticides – including selected metabolites and impurities – were  
138 determined for the dissolved and sediment-bound phases. Two of these streams were chosen as least  
139 disturbed control sites, representing (sub)catchments with >90% forested or natural lands (CS1-2; Fig.  
140 1A). Eleven streams were chosen in the Hove catchment (Fig. 1B), where agriculture represents 80% of

141 the catchment land use (Rasmussen et al., 2013). One stream is located in the Skensved catchment (Fig.  
142 1C), where agriculture represents ca. 99% of the total land use (McKnight et al., 2012).

143 Although the Hove and Skensved catchments differ in catchment size (and thus number of  
144 tributaries) and degree of physical in-stream (habitat) degradation, they are similar in that the dominant  
145 arable crop types are wheat, barley and canola (visual observations 2010-2012). There is no substantial  
146 input from waste water treatment plants in the investigated first-order streams, but storm water run-off  
147 from the scattered settlements may contribute to stream water quality (McKnight et al., 2012).  
148 Discharge from contaminated sites, and in particular abandoned landfill sites, can include pesticides at  
149 specific locations (Milosevic et al., 2012). All catchments are characterized by low elevations,  
150 clayey/loamy soils, a temperate climate, and an average regional precipitation of 600 mm yr<sup>-1</sup> (Hansen  
151 et al., 2011). Tile drains, triggered by storm-flow events and wet seasons, are present in agricultural  
152 fields in both catchments.

153



154

155 **Fig. 1:** Location of the study catchments (A) on Sjælland, Denmark, including their relation to the six least disturbed  
156 control catchments (labelled CS1-6); the (B) Hove and (C) Skensved catchments together with their respective stream  
157 networks. In Fig. (a), green and gray areas indicate natural and urban areas, respectively. In Figs. (B) and (C), green, orange,  
158 light gray and dark gray areas indicate natural areas, cultivated lands, uncultivated lands and residential settlements,  
159 respectively. Black crosses indicate sampling locations within each catchment. Note that streams CS1-2 were actively

160 sampled during this field campaign; all other control catchments was taken from the NOVANA database (Danish EPA,  
161 2011).

### 162 *3.2 Pesticides*

163 The chemicals in the study were generally selected to represent the most relevant pesticides with  
164 respect to legacy (e.g. banned herbicides now commonly found in groundwater) and contemporary-use,  
165 different physicochemical properties (SI Table S1) and toxicity. We included a range of pesticides  
166 determined by evaluating the probable relationships between pesticide, crops and spraying practice (SI  
167 Tables S2-S3), and sales statistics expected to be used in the catchments during 2010-2012. Nine of the  
168 pesticides on the Danish top 15 sales list for 2010 were included (based on metric tonnes active  
169 ingredient sold; SI Table S4). We did not include glyphosate in this study because it has an extremely  
170 low aquatic toxicity ( $LC50_{D,magna} = 40 \text{ mgL}^{-1}$ ; University of Hertfordshire (2014)), so will not affect the  
171 toxicity calculations (see Section 3.4), and requires an extra analytical protocol.

### 172 *3.3 Pesticide sampling and analyses*

173 Sampling was conducted in May-August from 2010-2012 during the main pesticide application  
174 period in Denmark. Dissolved-phase pesticides were sampled using opaque 1-L event-triggered water  
175 samplers as described in Liess and von der Ohe (2005) during May and June to capture surface runoff  
176 and flow through tile drains during heavy precipitation events, resulting in 8 storm-flow event samples.  
177 The bottles were retrieved within 24 h after each precipitation event, not filtered, and stored at 4 °C  
178 until analysis by Eurofins Miljoe A/S Laboratories. One grab-sample was collected at each site in  
179 August 2010 after a period with little to no precipitation and restrictions on the application of pesticides  
180 (close to harvest), representing base-flow conditions. Sediment-bound phases aiming to capture the  
181 most lipophilic pesticides were collected using two methods: suspended sediment (typically fine  
182 particles, e.g. silts; colloidal aggregates) was collected using passive suspended particle samplers (SPS)  
183 placed in 4 selected streams during May-June 2011 (Laubel et al., 2001). The top 2-5 cm of  
184 (stream)bed sediment (BS) (typically coarse particles, e.g. sands; gravel) was collected manually at one  
185 site in August 2012 using Kayak corers following the method described in Kronvang et al. (2003). The  
186 sample consisted of ca. 25 sub-samples collected in depositional areas to meet minimum analytical  
187 requirements for sample mass. Further details of the study catchments, sampling methods and pesticide  
188 screening are provided elsewhere (McKnight et al., 2012; Rasmussen et al., 2013). Analysis of water



189 samples for non-polar compounds were conducted using liquid-liquid extraction followed by  
190 quantification on GC-MS. Polar compounds were solid-phase extracted and quantified by liquid  
191 chromatography tandem mass spectrometry (LC-MS/MS), as described in Jansson and Krueger (2010).  
192 An overview of analytes in the different phases and sampling campaigns is presented in SI Table S5.

### 193 3.4 Toxicity calculation

194 The predicted toxicity for dissolved-phase pesticides was estimated using the toxic unit (TU)  
195 approach (Tomlin, 2001) with *Daphnia magna* as the benchmark organism (OECD, 2010):  
196  $TU=C_i/LC50_i$ , where  $C_i$  is the measured concentration of pesticide  $i$ ,  $LC50_i$  the corresponding acute  
197 48h LC50 value for *D. magna* exposed to pesticide  $i$  (see SI Table S6 for ecotoxicity data). In the  
198 absence of ecotoxicity data for metabolites/impurities, the LC50 values were assigned the same value  
199 as the parent compounds.

200 We calculated the sum of all TUs ( $\sum TU$ ) in order to produce a conservative estimate for the  
201 toxicity, in line with the principle of screening-level risk assessments. For this reason, if a single  
202 substance had multiple test values, the lowest value reported was used.  $\sum TU$  is based on the  
203 assumption of toxic additivity, neglecting potential synergistic and antagonistic effects between  
204 chemicals. Although studies have shown that this approach can result in an overestimation of mixture  
205 toxicity, it is generally within a factor of two or three of the observed toxicity and is as such defensible  
206 as a precautionary default assumption (Faust et al., 2003; Belden et al., 2007). Moreover, Schaefer et  
207 al. (2013) showed that  $\sum TU$  generally correlated as well or better than other toxicity predictions (i.e.  
208 independent action) to an ecological indicator of pesticide pollution (SPEAR) suggesting that the  $\sum TU$   
209 is a reasonable predictor for actual toxic pressure to aquatic biota. Liess and von der Ohe (2005) and  
210 Schaefer et al. (2012) have suggested  $\log TU \geq -3.0$  as a threshold value for acute observed effects on the  
211 aquatic macroinvertebrate community structure in the field. However, their studies were based on  
212  $TU_{\max}$ ; thus it should be noted that our use of  $\sum TU$  is probably slightly more conservative. Note that  
213 samples without pesticides measured at or above the detection limit were assigned a very low TU-value  
214 (-8.0).

215 The predicted toxicity of the sediment-bound pesticides was estimated using measured bulk  
216 sediment concentrations of contaminants, which were converted into aqueous concentrations according  
217 to the equilibrium-partitioning approach for non-ionic organic chemicals (see SI Table S1). The TU

218 could then be determined in accordance with the dissolved-phase approach. This approach is in line  
219 with previous studies focusing on the partitioning, bioavailability and toxicity of pyrethroid  
220 insecticides, suggesting that equilibrium partitioning theory could be used to reasonably predict  
221 sediment toxicity (Maund et al., 2002). It should be noted that this method entails considerable  
222 uncertainty, since the compounds may not be in equilibrium in stream sediment and it is difficult to  
223 measure  $K_{oc}$  for highly hydrophobic compounds. Direct calculation for predicted sediment toxicity was  
224 not possible due to a lack of data (e.g. using chronic 28d NOEC sediment toxicity values) for many of  
225 the detected compounds.

226 We calculated TU for the sediment samples using *Chironomus riparius* as the benchmark organism.  
227 We assembled data for three exposure scenarios: acute 96h LC50, chronic 28d NOEC (spiked water)  
228 and chronic 28d NOEC (spiked sediment) (SI Table S6). Where no data was available, we  
229 supplemented with data for other test organisms (*Chironomus tentans*; *Chironomus dilutus* (previously  
230 called *C. tentans*); *Gammarus lacustris*; *Hyaella azteca*). For the calculation of  $\sum TU$  for sediment  
231 samples, we prioritized data in the following order: 1) chronic 28d NOEC for *Chironomus* sp., 2) 96h  
232 acute LC50 for *Chironomus* sp. and 3) chronic exposure tests for alternative species. Moreover, we  
233 assembled data for 21-d NOEC for *D. magna* for all compounds detected in a sediment sample. For  
234 some compounds, no values could be found (SI Table S7), and our toxicity estimates are therefore  
235 likely to underestimate the potential toxicity of the samples.

236 We calculated the average  $\log \sum TU$  for permitted pesticides and compared them to the average  
237  $\log \sum TU$  for all detected pesticides (*t*-test,  $\alpha=0.05$ ) for base-flow, storm-flow and the sediment samples.  
238 Additionally, we compared the average  $\log \sum TU$  of all detected pesticides in storm-flow samples with  
239 the average  $\log \sum TU$  of base-flow samples (*t*-test,  $\alpha=0.05$ ). The tested data fulfilled the requirements for  
240 normality (Shapiro-Wilk) and equal variances (Bartlett's test;  $\alpha=0.05$ ); the two sampled control sites  
241 were not included in any of the statistical analyses.

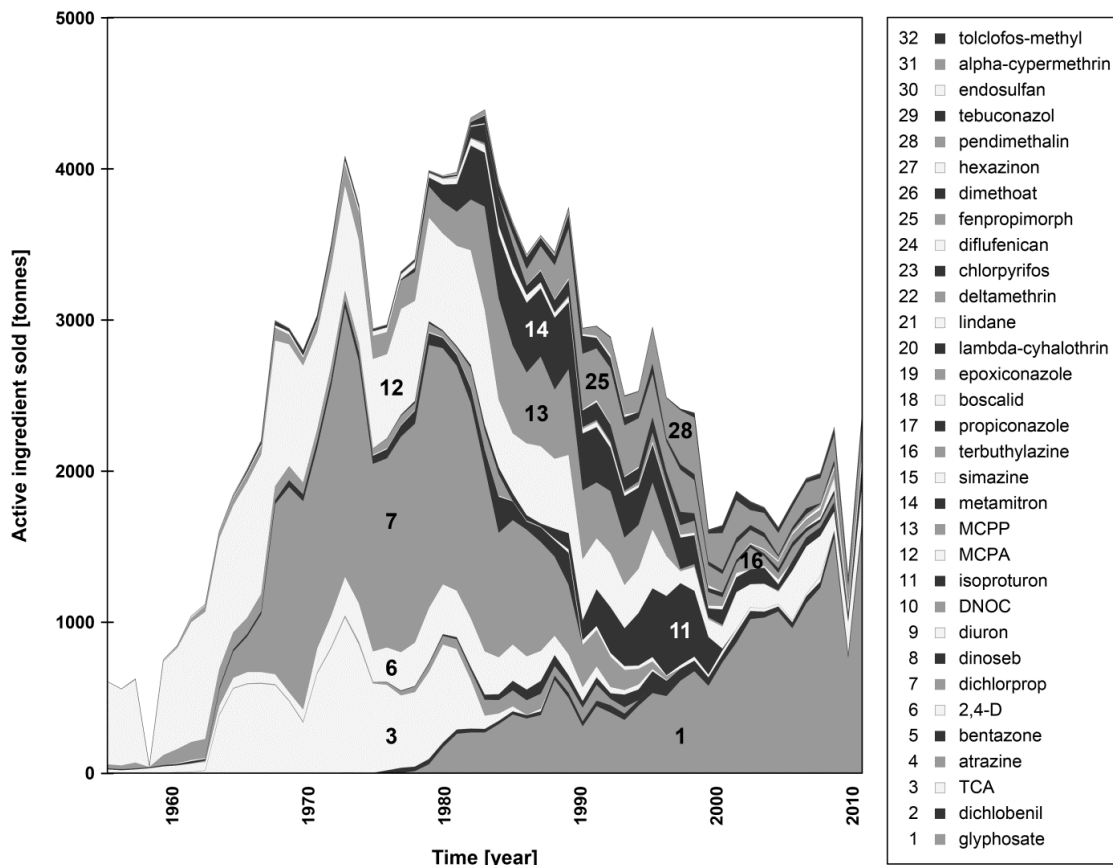
## 242 **4. Results and discussion**

### 243 *4.1 Historical use of pesticides in Denmark*

244 The historical agricultural use of pesticides in Denmark is presented by an overview of sales data for  
245 the active ingredients sold from 1956-2010 (Fig. 2). Specifically, we give an overview for the 32  
246 pesticides detected in this study, with the addition of glyphosate. It can be seen that sales peaked just

247 under 4,400 metric tonnes in 1983. In comparison, the total amount of pesticides sold during this period  
248 peaked at ca. 9,200 tonnes in 1983 of which ca. 5,000 tonnes were herbicides (SI Figure S1). The  
249 pesticides detected in this study thus comprise ca. 45% of the total pesticides sold. The specific  
250 pesticides used have changed greatly over time. Herbicides such as trichloroacetic acid (TCA),  
251 dichlorprop and MCPA dominated the sales from 1960-1970, to be substituted by compounds such as  
252 glyphosate and isoproturon in the early 1980s and 1990s. Further details on the historical use trends in  
253 Denmark for detected compounds are given in SI Table S2.

254 Comparing trends for agricultural land use in Denmark over three decades revealed only a slight  
255 decline in the total area cultivated (from ca. 2.5 M-ha to 2.2 M-ha); thus only small changes were seen  
256 in the total treatment frequency (SI Table S3) and the total number of pesticides sold (e.g. 1,168 in  
257 1990; 1,153 in 2010). However, for some crop types major changes are evident, which could also  
258 reflect the changing use patterns of pesticides, and changes in agricultural practice (Hansen et al.,  
259 2011). For example, for one of the dominant crop types in Denmark (winter cereals) and for corn, the  
260 treatment frequency has been cut by over 60%; whereas for potatoes, it has increased by ca. 35%.  
261 Herbicides, in all cases, are by far the most widely applied pesticides, followed by fungicides and  
262 insecticides.



263

264 **Fig. 2.** Sales of detected active ingredients (incl. glyphosate) in Denmark for agricultural use between 1956-2010, shown as  
 265 stacked shaded areas. Hexachlorobenzene is not present, as it was never authorized for use as a fungicide in Denmark.  
 266 Adapted from Tuxen et al. (2013).

#### 267 4.2 Overview of current pesticide findings

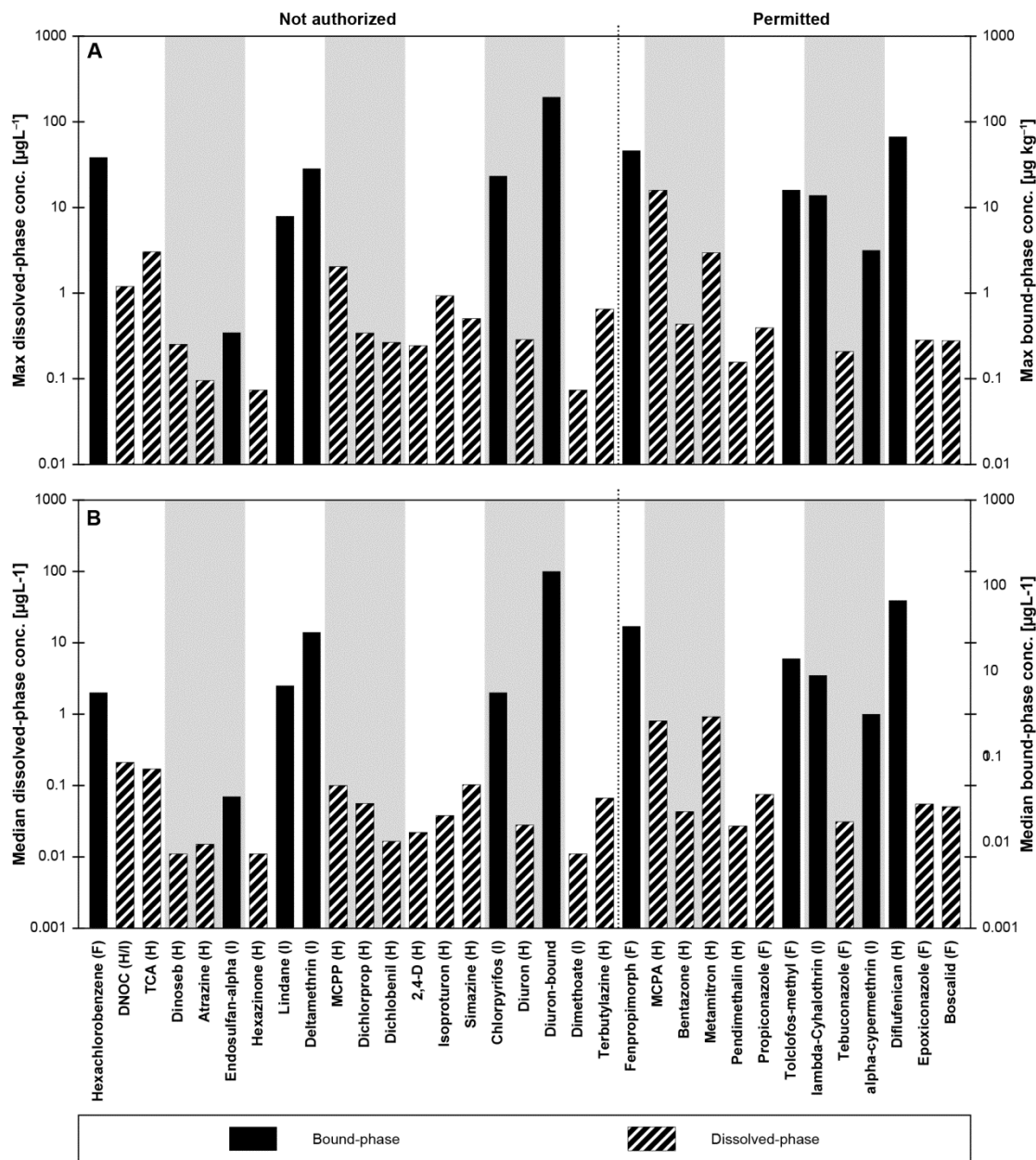
268 Figure 3 presents the findings for all stream site locations sampled in 2010-2012 for both the  
 269 dissolved and sediment samples. In total, 32 pesticides were detected at least once and these findings  
 270 are composed of 18 herbicides, 7 fungicides and 7 insecticides. In addition to hexachlorobenzene,  
 271 another 9 compounds were detected comprising metabolites, intermediates, potential impurities or  
 272 isomers of a pesticide (see also SI Table S8).

##### 273 4.2.1 Dissolved-phase pesticide findings

274 The number of compounds found per stream site ranged from 5 to 24 for storm-flow samples, and  
 275 from 1 to 10 for base-flow samples (SI Table S9). SI Table S9 furthermore gives an overview of which  
 276 compounds were most widespread (found in multiple streams) and in which phase, as well as which  
 277 stream locations were the most impacted (most compounds present per site). DNOC (last sold in 1987),

278 TCA (1988), simazine (2004), 2,6-dichlorobenzamide (BAM, metabolite) and MCPA (4th most sold  
279 pesticide in 2010; SI Table S4) were by far the most prevalent compounds detected in the water  
280 samples. Metamitron additionally belongs to this group as it was detected at 11 of the 12 agriculturally-  
281 impacted sites, although it was detected only during one storm-flow event in 2010 (see Fig. 4).

282 Notably, 4 to 5 compounds were also detected in the control (least disturbed) sites during storm-  
283 flow events, including BAM. BAM is a metabolite of dichlobenil, which is typically associated with  
284 urban applications and not with agricultural use (Clausen et al., 2007) and is one of the most common  
285 contaminants in Danish aquifers (Malaguerra et al., 2012). This finding was expected considering that  
286 scattered settlements are typical of the Danish rural landscape (see Fig. 1).



287

288 **Fig. 3.** Overview of (a) maximum and (b) median pesticide concentrations in the water (dissolved phase – striped bars) and  
 289 associated with sediments (bound-phase – solid bars), for herbicides (H), insecticides (I) and fungicides (F) measured  
 290 during the field campaign (excluding control sites). Note the dotted black line that marks the transition from not authorized  
 291 to permitted status according to Danish legislation, although some may still be permitted within the European Union. We  
 292 consider some pesticides as not being authorized when the yearly registered sales are below 4 metric tonnes (i.e. 2,4-D,  
 293 MCPP), indicating these chemicals are being phased out. SI Table S8 provides an overview of the maximum concentrations  
 294 and features of compounds detected in this study. SI Table S9 presents an overview of the extent of detected compounds in  
 295 streams in the study area. Note that endosulfan-alpha was found above the detection limit in one bed sediment sample, but  
 296 below the quantification limit: its inclusion here is to document its presence, i.e. trace concentration.

297 *4.2.2 Pesticides in the sediment-phase*

298 Eleven different chemicals in total were detected in the sediment samples (Fig. 3), where  
299 hexachlorobenzene, chlorpyrifos and diflufenican were the most prevalent (SI Table S9). Six of these  
300 chemicals are no longer authorized for use in Denmark. Although four chemicals were detected in the  
301 bed sediment, only diuron was measured above the limit for quantification (SI Table S8). The total  
302 number of compounds ranged from 4 to 6 per site. For site 7-V1 (SI Table S9), where both sediment  
303 sampling methods were carried out, chlorpyrifos was additionally detected in the suspended sediment,  
304 which was not present in the streambed sample. The herbicide diuron was the only pesticide found in  
305 all the phases considered in this study (SI Table S5). Notably, many of the (lipophilic) fungicides (e.g.  
306 fenpropimorph) and insecticides (e.g. chlorpyrifos) detected in this study were only found in the  
307 sediment-phase. Findings were similar to the dissolved-phase, showing a mix of banned and  
308 contemporary pesticides.

309 The presence of hexachlorobenzene, never authorized for use in Denmark, could be due to the  
310 importation of products which contain it, such as biocide-treated woods, PVC, fireworks or synthetic  
311 rubber (Paludan et al., 2004). It could also be present since it is a known by- and combustion-product in  
312 the production process for chlorinated solvents and organochlorine pesticides (Gilbert, 2012; Lützhøft  
313 et al., 2012). Chlorpyrifos, banned in 2006 in Denmark but still permitted within the EU (SI Table S2),  
314 is a broad-spectrum chlorinated organophosphate insecticide and biocide (e.g. incorporated in paint as a  
315 means of vector control) (Mackay et al., 2014).

#### 316 *4.2.3 Priority pollutants*

317 Eight of the nine pesticides included on the European 33 priority pollutant list were detected,  
318 including atrazine, chlorpyrifos, diuron, endosulfan-alpha, hexachlorobenzene, isoproturon, lindane  
319 and simazine. Most of the maximum concentrations measured in the study streams fall close to the  
320 reported median value for European streams (SI Table S8). The maximum detected aqueous  
321 concentration for diuron and isoproturon was above or close to the EU aquatic PNEC, respectively  
322 (Klein et al., 1999). The fungicide/impurity hexachlorobenzene and the insecticides lindane and  
323 endosulfan-alpha (isomer of endosulfan) were detected in the sediment at concentrations well-above  
324 the EU sediment PNEC (Klein et al., 1999). Furthermore, the maximum measured concentration of  
325 hexachlorobenzene was double the existing Danish freshwater guideline value of  $10 \mu\text{gkg}^{-1}$  (SI Table  
326 S2) (Danish EPA, 2010), and it was found at all sampled locations (SI Table S9).

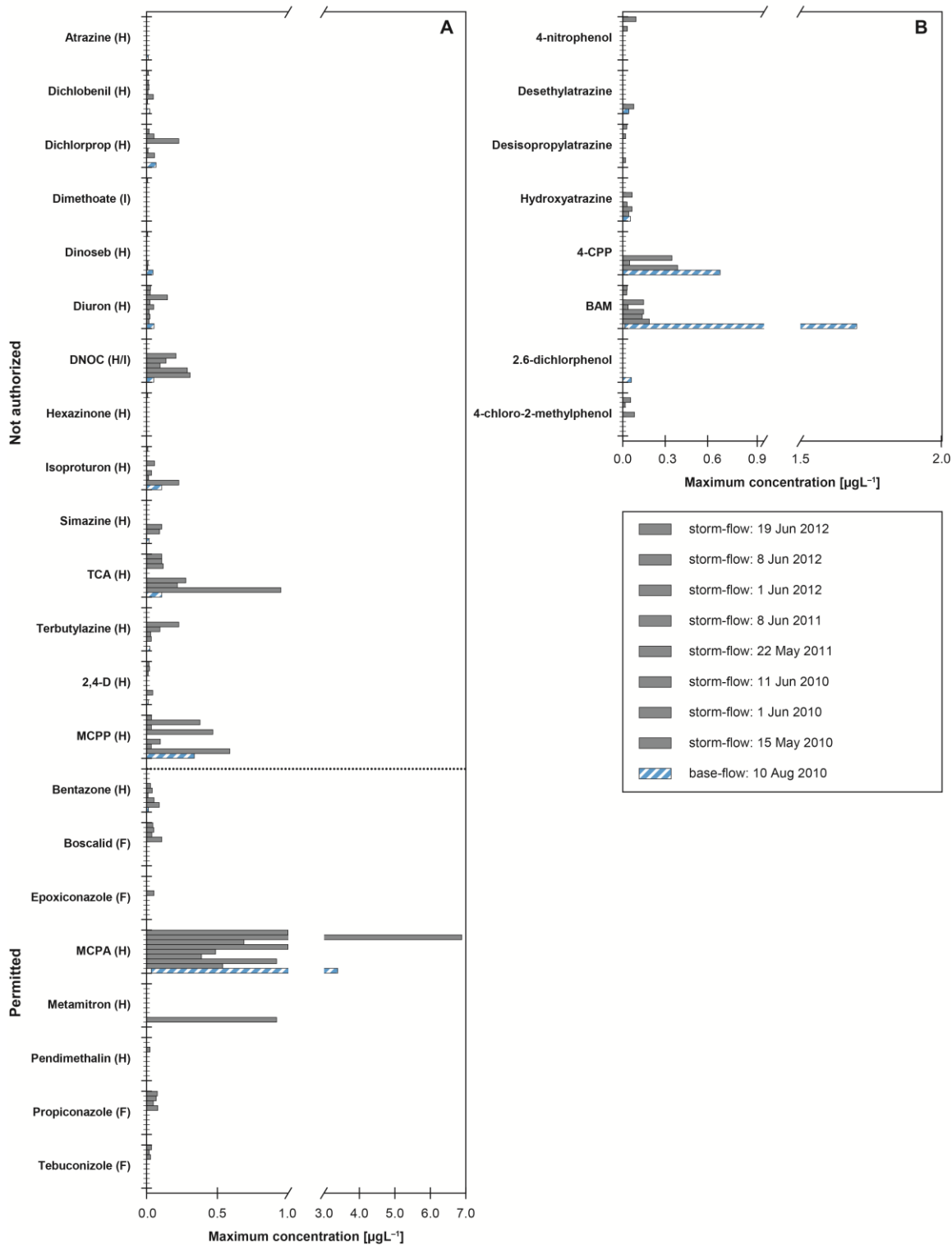
327 *4.3 Complex picture for pesticide entry to surface water*

328 Pesticide application methods vary widely and can strongly affect how much of the applied  
329 chemical reaches the target site (Vogel et al., 2008), where potentially only 0.1% of the pesticides  
330 applied to crops actually reach the target pest (Arias-Estévez et al., 2008). Important factors affecting  
331 the transport of pesticides within a catchment include the meteorological conditions, land use,  
332 physicochemical properties and the conditions of the soil surface (Asman et al., 2005; Holvoet et al.,  
333 2007; Rodrigues et al., 2013). Once released, the fate of pesticides is then determined by environmental  
334 processes such as volatilization (from soil and plant surfaces) and degradation (biotic and abiotic), with  
335 the effect that pesticides will enter different compartments in different quantities and subsequently be  
336 removed at different rates, resulting in widely varying transport distances and residence times. Here we  
337 discuss the contributions and thus importance of different entry pathways for selected compounds,  
338 relying on literature findings to supplement our conclusions for relevant pathways not directly  
339 investigated in this study.

340 *4.3.1 Indications for a groundwater transport route*

341 Almost all pesticides (17 from 22 detected) not authorized for use in Denmark, and metabolites and  
342 impurities, were detected during base-flow conditions when groundwater discharge is expected to be  
343 the most dominant source of inflow to the streams. This is shown in Fig. 4, which separates the storm-  
344 flow and base-flow water samples for pesticides (Fig. 4A) and detected metabolites/impurities (Fig.  
345 4B). Our main criteria for inferring groundwater as a relevant pathway for specific compounds is that  
346 (i) we find it under base-flow conditions in similar concentrations, if not higher, to storm-flow values  
347 (see Table 1), (ii) we find them in groundwater, and (iii) maximum storm-flow and base-flow  
348 concentrations were detected at the same sampling location (i.e. stream), as is discussed further below.





349

350

351

352

**Fig. 4.** Overview of dissolved-phase (a) pesticides and (b) metabolites and/or impurities, identified either during the base-flow event (blue-striped bars) or during a storm-flow event (gray-scale bars). Note the dotted black line that marks the transition from not authorized to permitted status according to Danish legislation.

353 We can make the strongest case for BAM, dichlobenil, mecoprop (MCP)P. 2(4-  
354 chlorophenoxy)propionic acid (4-CPP), and MCPA. For BAM, the highest detected concentration was  
355 in the groundwater, with comparable concentrations in the base-flow (Table 1). BAM is highly soluble  
356 and non-volatile (University of Hertfordshire, 2014), in contrast to its parent compound dichlobenil (SI  
357 Table S1), so that its presence in groundwater is expected. Dichlobenil was found in lower  
358 concentrations in groundwater (Table 1), compared to its metabolite BAM (by one order of magnitude,  
359 Table 1), and was detected in higher but similar concentrations during storm-flow events compared to  
360 base-flow. This could be due to the fact that dichlobenil is typically found in the upper few meters of  
361 soil (Clausen et al., 2007) from where it could be released during heavy rain events.

362 The detected concentrations for MCP)P in storm- and base-flow were comparable to concentrations  
363 detected in groundwater wells for the Hove catchment (Table 1, see also SI Table S10). Although the  
364 storm-flow concentrations were highest for MCP)P, these values were close to the value for base-flow.  
365 In the case of MCP)P, this could indicate that, although we have classified it as not authorized, MCP)P-P  
366 is still in “restricted” use, although the amounts are low (below 4 metric tonnes, SI Fig. S1, SI Table  
367 S1). MCP)P is highly soluble and nonvolatile, further supporting this pathway as being relevant in  
368 explaining its presence in the investigated streams.

369 A similar case can be made for 4-CPP (metabolite/impurity of dichlorprop), although it was only  
370 detected in one groundwater well in the survey (SI Table S10). However, it was found in a later study  
371 in at least one monitoring or drinking water well close to a stream in the Hove catchment (Levi et al.,  
372 2014). In addition, a parallel study conducted at one of the Hove stream locations (8-Ri) confirmed the  
373 existence of groundwater discharge zones entering the stream. Specifically, phenoxy acid herbicides  
374 (MCP)P, dichlorprop, 4-CPP) were detected in groundwater impacted by landfill leachate which enters  
375 the stream particularly during dry periods, i.e. when stream concentrations reached groundwater  
376 concentration levels (Milosevic et al., 2012).

377 MCPA, representing decades of both contemporary and historical use, was found at the highest  
378 concentration of all detected pesticides in water samples. Interestingly, base-flow concentrations for the  
379 restricted-use herbicide MCPA were only ca. 50% lower than the storm-flow concentrations at the  
380 same locations (e.g. maximum detected:  $6.9 \mu\text{gL}^{-1}$  and  $3.4 \mu\text{gL}^{-1}$ , respectively, for 7-V1), indicating the  
381 high importance of groundwater inflow for this compound in addition to the inflow via surface run-off  
382 and tile drains during storm-flow events. This pattern was also seen for three of the other compounds,

383 i.e. maximum storm-flow and base-flow concentrations were found at the same location for 4-CPP (8-  
 384 Ri), BAM (5-In) and dichlobenil (7-V1), suggesting that groundwater is an important contributor for  
 385 these compounds based on our criteria.

386 A lesser case can still be made, however, for bentazone, as well as for atrazine, 2,4-D and its  
 387 metabolite 2,6-dichlorophenol and isoproturon (but see also Section 4.3.2). Bentazone and 2,4-D are  
 388 highly soluble, compared with isoproturon (moderately soluble) and atrazine (low solubility); all of  
 389 these compounds are non-volatile with a low persistence in soils (DT50s) except for atrazine  
 390 (moderately persistent) (SI Table S1). For bentazone, although the highest concentration was detected  
 391 in a storm-flow event, concentrations were in the same order of magnitude for both base-flow and  
 392 groundwater (Table 1), which indicates that groundwater is most likely contributing to the presence of  
 393 bentazone in streams. For 2,4-D, the highest concentration detected was in groundwater (Table 1) with  
 394 similar concentrations detected in base-flow and storm-flow (same order of magnitude). The case for  
 395 isoproturon is similar to 2,4-D, with similar concentrations detected in base-flow and storm-flow (same  
 396 order of magnitude), which could indicate that groundwater is contributing to this compound's  
 397 presence in the investigated streams. In the case of atrazine, its highest concentration was detected in  
 398 groundwater followed by base-flow; it was not detected in storm-flow events, which could be due to  
 399 the fact that its use has been discontinued since 1994 in Denmark.

400 **Table 1:** Overview for maximum and median pesticide concentrations, including metabolites/impurities (right-justified),  
 401 detected in the storm-flow, base-flow and groundwater (Hove catchment only). Note that median equals maximum for base-  
 402 flow, since this was only sampled once. The maximum concentration detected per pathway is highlighted in bold.

Compound	Maximum conc. detected per compound [µgL <sup>-1</sup> ]			Median conc. detected per compound [µgL <sup>-1</sup> ]	
	Storm-flow	Base-flow	Groundwater	Storm-flow	Groundwater
DNOC (H)	<b>0.31</b>	0.054	0.10	0.21	0.01
TCA (H)	<b>0.95</b>	0.11	0.01	0.17	0.01
Dinoseb (H)	0.013	0.048	<b>0.10</b>	0.011	0.01
4-nitrophenol	<b>0.096</b>	n.d.	n.d.	0.066	n.d.
Atrazine (H)	n.d.	0.015	<b>0.10</b>	n.d.	0.01
Desethylatrazine	0.081	0.046	<b>0.10</b>	0.081	0.01
Desisoproylatrazine	0.032	n.d.	<b>0.10</b>	0.023	0.01

Hydroxyatrazine	0.069	0.057	<b>0.10</b>	0.057	0.01
Hexazinone (H)	<b>0.011</b>	n.d.	n.d.	0.011	n.d.
MCPP (H)	<b>0.59</b>	0.34	0.12	0.10	0.01
Dichlorprop (H)	<b>0.23</b>	0.069	0.06	0.056	0.018
4-CPP	0.39	<b>0.69</b>	0.011	0.35	only 1 finding above DL
Dichlobenil (H)	0.051	0.025	<b>0.02</b>	0.017	0.01
BAM	0.19	1.7	<b>2.1</b>	0.14	0.036
2,4-D (H)	0.046	0.014	<b>0.1</b>	0.022	0.01
2,6-dichlorophenol	n.d.	<b>0.064</b>	n.d.	n.d.	n.d.
Isoproturon (H)	<b>0.23</b>	0.11	0.029	0.038	0.029
Simazine (H)	<b>0.11</b>	0.02	0.04	0.1	0.02
Diuron (H)	<b>0.15</b>	0.056	0.026	0.028	0.01
Dimethoate (I)	<b>0.011</b>	n.d.	n.d.	0.011	n.d.
Terbutylazine (H)	<b>0.23</b>	0.026	0.015	0.067	0.01
MCPA (H)	<b>6.9</b>	3.4	0.012	0.81	0.012
4-chlor-2-methylphenol	0.085	n.d.	<b>0.10</b>	0.058	0.05
Bentazone (H)	<b>0.092</b>	0.016	0.023	0.043	0.018
Metamitron (H)	<b>0.92</b>	n.d.	0.02	0.33	0.01
Pendimethaline (H)	<b>0.027</b>	n.d.	n.d.	0.027	n.d.
Propiconazole (F)	<b>0.082</b>	n.d.	n.d.	0.075	n.d.
Tebuconazole (F)	<b>0.038</b>	n.d.	n.d.	0.031	n.d.
Epoxiconazole (F)	<b>0.055</b>	n.d.	n.d.	0.055	n.d.
Boscalid (F)	<b>0.11</b>	n.d.	n.d.	0.051	n.d.

403

404 *4.3.2 Indications for an atmospheric transport route*

405 TCA and DNOC have been banned in Denmark since 1988 and 1987, respectively (SI Table S2),  
406 and they are not found above the detection limit in groundwater in the Hove catchment (SI Table S10).

407 Moreover, maximum storm-flow and base-flow concentrations were found at different locations, e.g.  
408 TCA (3-Kr/storm-flow; 11-V2/base-flow), DNOC (3-Kr/storm-flow; 9-Bi/base-flow) or met amitron  
409 (3-Kr/storm-flow; not detected in base-flow), so the groundwater pathway is considered less likely.  
410 TCA is highly water soluble (SI Table S1) and has been linked to both wet and dry deposition from the  
411 atmosphere, although sources and fate for this compound are still not completely understood (Cape et  
412 al., 2006). Asman et al. (2005) found in a Danish study that the overall wet deposition of nitrophenols  
413 (e.g. DNOC, 4-nitrophenol) were much higher than that for pesticides. Nitrophenols comprise a group  
414 of compounds that are emitted by cars, but can also be formed in the atmosphere by the photochemical  
415 reaction of nitrogen oxides with hydrocarbons (Asman et al., 2005) indicating that DNOC could also be  
416 transported via the atmospheric pathway.

417 Met amitron was found in 11 of the 12 agricultural streams (i.e. Hove; Skensved) during one storm-  
418 event measurement. Met amitron is a pre- and post-emergence herbicide used for weed control in sugar  
419 beet and strawberry fields (SI Table S2). Strawberry fields are indeed found scattered throughout the  
420 area, but are not among the dominant crop type for these catchments and these crops were not observed  
421 directly bordering the sampling locations. Potentially it is transported via the atmosphere, e.g. via wind  
422 drift from nearby fields. This conjecture is supported by previous studies in Danish catchments on  
423 Sjaelland, which showed statistically significant losses of met amitron due to primary (droplet) and  
424 secondary drift (evaporation) (Carlsen et al., 2006a; Carlsen et al., 2006b).

425 Chlorpyrifos, well-known for its persistence and ability to undergo long-range transport (Mackay et  
426 al., 2014), was detected in all four suspended sediment samples, but not in the streambed sediment.  
427 Although it is still permitted within the EU, it has been banned for use as an insecticide in Denmark  
428 since 2006 (SI Table S2), but may still be found in material protection products, e.g. paint as a biocide.  
429 Nearby scattered settlements (Fig. 1) could therefore act as sources emitting chlorpyrifos via storm-  
430 flow runoff to streams. In addition, the atmosphere is also a likely transport pathway; chlorpyrifos was  
431 among the 10 most frequently detected compounds in rainwater in four agricultural watersheds in the  
432 United States (Vogel et al., 2008).

433 Several of the legacy herbicides for which there is weaker evidence for the groundwater pathway  
434 (Section 4.3.1) could come in part from the atmosphere, but could also then partly have leached into  
435 groundwater and from there entered the stream water. In particular, atrazine, its metabolites,  
436 isoproturon, and 2,4-D were detected in rainwater in the study by Asman et al. (2005). In our study,

437 these compounds were detected in the highest concentrations in either base-flow or groundwater,  
438 lending support to the importance of groundwater as a contributor of these compounds to stream water.

439 However, further work is still needed to properly disentangle the relevance of the different sources  
440 and their contribution to the presence of pesticides in surface water. This includes a closer look at the  
441 influence of biocides applied in urban settings, since it is probable that some compounds which are no  
442 longer permitted for agricultural use may still be allowed in biocides (e.g. diuron, mecoprop, Wittmer  
443 et al., 2010, see also Section 4.2.2). Specifically, a much higher temporal and spatial resolution of all  
444 sampling sites for each of these potential sources (e.g. base-flow, storm-flow, groundwater,  
445 precipitation) is essential, preferably also extending the dataset to include more catchments (e.g. mixed  
446 land use for e.g. presence of biocides) in order to support a more statistical evaluation of the data.

#### 447 *4.4 Contribution of banned pesticides to predicted ecotoxicity*

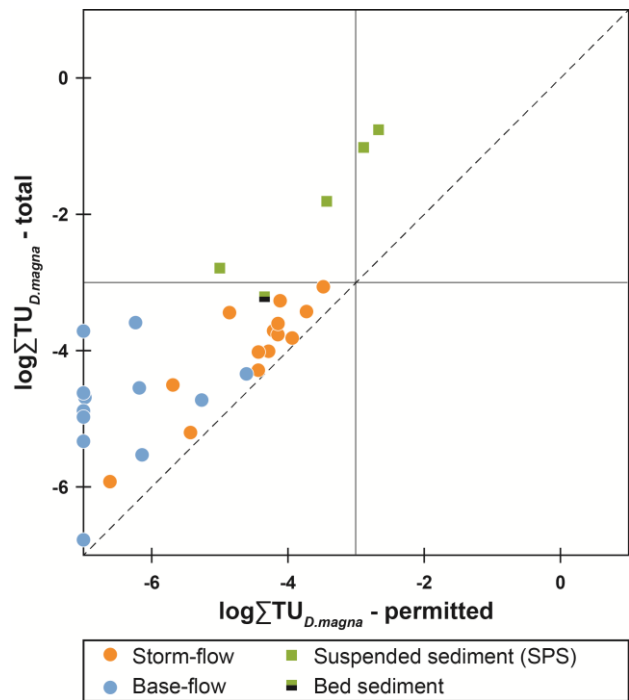
448 The  $\log\sum TU_{D.magna}$  for permitted pesticides is depicted as a function of the  $\log\sum TU_{D.magna}$  for all  
449 compounds for each sample in Fig. 5. For the storm-flow samples, the addition of banned compounds  
450 significantly increased the  $\log\sum TU_{D.magna}$  by up to one order of magnitude ( $P=0.014$ , Table 2). Storm-  
451 flow samples generally exhibited a higher  $\log\sum TU_{D.magna}$  compared to base-flow ( $P<0.001$ ). The higher  
452  $\log\sum TU_{D.magna}$  in storm-flow samples compared to base-flow samples was due in part to the herbicide  
453 metamitron and in part to several fungicides (see SI Table S9). These compounds have low-to-  
454 moderate toxicity to *D. magna*, similar to many of the herbicides detected in the base-flow samples (SI  
455 Table S6). The general increase in  $\log\sum TU_{D.magna}$  of storm-flow samples was therefore due primarily to  
456 the presence of higher concentrations of compounds with similar toxicity (not more toxic compounds).

457 For the base-flow samples, the addition of banned compounds significantly increased the  
458  $\log\sum TU_{D.magna}$  by up to four orders of magnitude ( $P<0.001$ , Table 2). Importantly, contaminated  
459 groundwater inflow constitutes a chronic source of pollution, and given the relatively short time span in  
460 which standard toxicity tests are conducted, the estimated toxicity of these compounds may be  
461 underestimated. Studies using longer exposure durations are needed to fully comprehend the  
462 ecotoxicological potential of groundwater-based pollution in streams. Importantly, these findings show  
463 that non-authorized substances, including metabolites and impurities increase the  $\log\sum TU_{D.magna}$  of  
464 water samples irrespective of the entry pathway.

#### 465 *4.5 Contribution of sediment-bound pesticides to predicted ecotoxicity*

466 Using TU based on 48h acute toxicity tests with *D. magna*, all suspended sediment samples (SPS)  
467 were characterized by a  $\log\sum TU_{D.magna} \geq -3.0$  (converted to water concentrations) when all compounds  
468 were included (Fig. 5). In contrast, only two of the four SPS samples exceeded a  $\log\sum TU_{D.magna}$  of -3  
469 when considering only permitted compounds. Macroinvertebrate community changes have been  
470 observed at  $\log TU_{D.magna}$  of -3 (Liess & von der Ohe, 2005; Schaefer et al., 2012), and this low  
471 threshold for observed effects has been proposed to be due in part to concomitantly acting  
472 environmental stressors and indirect pesticide effects facilitated through community interactions (Liess  
473 & von der Ohe, 2005). Our results suggest that one possible explanatory factor governing this low  
474 threshold for observed community effects in the field could be due to chronic exposure of legacy  
475 pesticides especially via sediments.

476 For the bed sediment sample (Site 7-V1, Table 2), the predicted toxicity value is similar to the more  
477 toxic storm-flow samples, remaining just below the threshold regardless of the inclusion of e.g. legacy  
478 pesticides. Higher  $\log\sum TU_{D.magna}$  in suspended sediment compared to the bed sediment sample is  
479 probably due to the fact that the bed sediment sample was comprised of the top 2-5 cm in depositional  
480 zones. Given that particle-associated pesticides are likely to be primarily associated with the upper few  
481 millimeters of newly deposited sediment, the volume of deeper sediment may dilute the pesticide  
482 concentrations compared to SPS sampling (Liess et al., 1996). The  $\log\sum TU_{D.magna}$  for all compounds  
483 detected in the sediment-phase (both suspended and bed sediment) was higher, but not statistically  
484 different ( $P=0.055$ ), than the  $\log\sum TU_{D.magna}$  when only contemporary pesticides are included. The lack  
485 of a statistically significant difference was most likely due to the small sample size. The  $\log\sum TU$  for  
486 suspended and bed sediment samples were generally higher compared to the  $\log\sum TU$  of water samples,  
487 and the influence of banned pesticides on  $\log\sum TU$  appeared to increase (SI Fig. S2).



488

489

490

491

**Fig. 5.**  $\log \sum TU_{D.magna}$  for all pesticides detected, compared with the  $\log \sum TU_{D.magna}$  considering only the currently permitted pesticides in Denmark for all pathways analyzed. Solid lines indicate the threshold for acute observed effects on macroinvertebrate community structure in the field ( $\log TU \geq -3.0$ ) (Schaefer et al., 2012); dotted line shows 1:1 ratio.

492

493

494

495

496

497

498

499

500

501

502

Our findings, supported also by other published studies (Kuivila et al., 2012; Warren et al., 2003), indicate that sediment-bound pesticide residues could be important for both acute and chronic exposures of the biota since they consistently harbored high toxic potential. However, both the suspended and bed sediment samples were analyzed for a broader spectrum of insecticides that was not included in the aqueous samples. Aqueous samples could therefore have a higher toxic potential to *D. magna* than our results indicate. We furthermore suggest that future research should address chronic exposure of pesticides that have been adsorbed to particles for several months or even years to evaluate whether the predicted toxicity of the sediment-associated pesticides, in particular for the legacy pesticides, detected in agricultural streams is valid. Moreover, we emphasize the need to further develop our knowledge of the temporal dynamics in particular of legacy pesticide concentrations in stream sediments.

503

504

505

506

507

**Table 2:**  $\log \sum TU_{D.magna}$  for all 14 streams based on water samples collected during base-flow, storm flow or in the sediment-phase (SPSs: n=4; kayak coring: n=1, bed sediment). Gray shaded cells represent samples where the calculated toxicity is very close to (n=1) or exceeds (n=7) the threshold ( $\log TU \geq -3.0$ ) for observed effects on macroinvertebrate community structure. N/A=not analyzed, indicating streams where pathways were not sampled for a particular phase (i.e. base-flow; storm-flow; suspended sediment; bed sediment).



Sampling pathways	$\log \sum TU_{D.magna}$													
	1-Ra	2-Ba	3-Kr	4-El	5-In	6-Se	7-V1	8-Ri	9-Bi	10-O	11-V2	12-Sk	13-R	14-Fa
Base-flow: permitted	-6.14	-6.98	-8	-8	-8	-8	-4.61	-6.18	-8	-8	-5.27	-6.24	N/A	N/A
Base-flow: total	-5.53	-4.69	-3.72	-6.78	-4.89	-4.62	-4.34	-4.55	-5.33	-4.98	-4.73	-3.59	N/A	N/A
Storm-flow: permitted	-5.69	-4.86	-3.73	-4.43	-4.21	-4.11	-3.48	-4.28	-4.43	-3.94	-4.15	-4.14	-6.61	-5.43
Storm-flow: total	-4.51	-3.44	-3.43	-4.29	-3.71	-3.27	-3.07	-4.01	-4.02	-3.82	-3.77	-3.61	-5.93	-5.20
Sediment (SPS): permitted	N/A	N/A	-2.67	N/A	N/A	-5.00	-3.43	N/A	N/A	N/A	N/A	-2.89	N/A	N/A
Sediment (SPS): total	N/A	N/A	-0.76	N/A	N/A	-2.79	-1.81	N/A	N/A	N/A	N/A	-1.02	N/A	N/A
Bed sediment: permitted	N/A	N/A	N/A	N/A	N/A	N/A	-4.35	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bed sediment: total	N/A	N/A	N/A	N/A	N/A	N/A	-3.21	N/A	N/A	N/A	N/A	N/A	N/A	N/A

508

## 509 5. Implications of pesticide findings in streams

### 510 5.1 Historical versus contemporary use

511 Our study investigates both the occurrence and pathways for pesticides in stream water. The results  
512 provide a snapshot of the pesticides found in Danish streams, recording only presence/absence of a  
513 small percentage of the total number of chemicals that have been, or currently are on the market. These  
514 findings contribute to a more holistic understanding of stressors potentially impacting freshwater  
515 ecosystems, revealing the presence of legacy pesticides in different media to which aquatic organisms  
516 are exposed.

517 Pesticide concentrations in surface water bodies are consistent with trends in current agricultural  
518 use (Thurman et al., 1992; Vecchia et al., 2009), particularly for run-off associated with rain events,  
519 when only contemporary pesticides are included in the sampling campaign. However, studies of  
520 pesticides in groundwater present similar conclusions to our work, with concentrations being related to

521 the time when these compounds were first introduced (Tesoriero et al., 2007). Here we have clearly  
522 documented the presence of pesticides that have, in some cases, been removed from the Danish and/or  
523 European market for over several decades. Notably, predicted toxicity increased by up to four orders of  
524 magnitude when detected legacy pesticides, and their metabolites and impurities, were included in the  
525 calculation.

526 A closer look at the top 15 pesticides sold over the last decade in Denmark indicates that these  
527 accounted for ca. 63% of all pesticides sold in Denmark in 2000 (or ca. 75% of all herbicides), rising to  
528 ca. 90% in 2010 (ca. 93% of all herbicides; SI Table S4). Seven out of the 9 analyzed pesticides from  
529 this group were detected in this study. Notably, there were no insecticides among these 7 compounds;  
530 in fact, boric acid was the top insecticide sold (in 2010, but since banned in the EU), placing 21st  
531 overall, with cypermethrin – detected in this study – placing 25<sup>th</sup> (Tuxen et al., 2013). This information  
532 is relevant to our findings, where both historical and contemporary insecticides were only rarely  
533 detected in the dissolved-phase, but consistently in the sediment-phase, which also exhibits the highest  
534 predicted toxicity. Herbicides dominate the total sales, are more mobile and thus more likely to be  
535 found in groundwater (SI Table S10). The higher concentrations of herbicides observed in our samples  
536 are likely related to the higher use of herbicides in agriculture, noting that we did not analyze for or  
537 detect many insecticides or fungicides in the dissolved-phase (SI Table S5). This is due to the fact that  
538 insecticides and fungicides found around detection limits in the solid phase will not allow  
539 quantification in the water phase due to their highly lipophilic nature. Groundwater is thus likely to  
540 continue to be an important pathway in particular for herbicides in surface water for decades to come.

#### 541 *5.2 Considerations for determining toxicity*

542 Although our study presents only predicted chemical toxicity findings, which alone can only  
543 support the possibility that chemical stressors may have contributed to impacts on aquatic ecosystems,  
544 we speculate that the role of chronic exposure of pesticides from groundwater and sediments are  
545 currently underestimated (Bundschuh et al., 2014; Peters et al., 2013). We were surprised to discover a  
546 huge paucity of data for chronic sediment toxicity values for benthic macroinvertebrates. Although  
547 there are reasons for this, i.e. non-standardized methods, this is important considering the number of  
548 compounds detected in the sediment-phase in our study. Indeed, current risk assessment methods –  
549 based on no-observed-adverse-effect-levels for single chemicals – do not provide sufficient information

550 about the effects of chronic exposure – over macroinvertebrate generations (Artigas et al., 2012) – to  
551 low-concentration mixtures punctuated by seasonal high-concentration pulses (Gilliom et al., 1999).  
552 They also do not consider the adjuvants pesticides are sold in – i.e. only active ingredients are tested for  
553 ecotoxicity, as the adjuvant is thought to be inert (Benachour and Seralini, 2009) – representing  
554 potential important gaps in our knowledge of the impacts of pesticides on aquatic ecosystems.

### 555 *5.3 Considerations for monitoring programs for streams and groundwater*

556 Our findings indicate that monitoring programs comprised only of current trends in agricultural  
557 pesticide use (one decade or less), will not be representative of what actually exists in streams and  
558 groundwater. The principle of ‘you find what you look for’ is certainly valid here, as assumptions about  
559 the fate and transport of pesticides, including dominant sources and entry pathways, must clearly be  
560 reevaluated. We suggest that legacy pesticides and their metabolites/impurities should be included in  
561 monitoring programs if the ‘true’ toxic effect of pesticides in streams is to be assessed.

562 The importance of the groundwater pathway for headwater streams indicates that stream sampling  
563 during base-flow conditions can provide valuable information about the long-term fate of pesticides in  
564 groundwater. Both aqueous and sediment phases should be sampled to assess the health of streams and  
565 rivers. Ideally, integrative approaches should be developed with monitoring strategies simultaneously  
566 involving chemical analyses, ecotoxicological tools and the study of population/community responses  
567 (Connon et al., 2012) in order to obtain a more holistic picture.

## 568 **6. Conclusions**

569 This study aimed to link the history of pesticide usage to current findings for legacy and  
570 contemporary pesticides in surface and groundwater. We investigated, in particular, how legacy  
571 pesticides contribute to the ecotoxicological impact on stream water ecosystems. Findings comprised a  
572 range of both banned legacy and contemporary pesticides in 14 Danish headwater streams.  
573 Groundwater has been identified as a significant pathway especially for herbicides entering streams,  
574 and should be assessed together with input from atmospheric sources and run-off from rain events to  
575 get the full picture of stream water quality. Legacy pesticides contribute to predicted aquatic  
576 ecotoxicity, which was increased substantially (up to four orders of magnitude) when these pesticides,  
577 and their metabolites and impurities, were included in the toxicity estimations.

578 Sediment-bound insecticides, such as chlorpyrifos and deltamethrin, were a major source for the  
579 estimated ecotoxicity. Their presence could be due either to long-range transport in the atmosphere or  
580 to storm-flow run-off to streams from e.g. urban applications such as biocides. Our results corroborate  
581 other published studies indicating that sediment-bound pesticide residues could be important for both  
582 acute and chronic exposures of the biota. We suggest, however, that chronic toxicity scenarios are  
583 potentially more representative of the overall conditions found in streams, demonstrating the  
584 importance of the lack of long-term chronic exposure data in the literature. Thus monitoring programs  
585 comprised only of current trends in agricultural pesticide use (i.e. one decade or less), will not be  
586 representative of actual conditions in streams. Legacy pesticides and their metabolites and/or impurities  
587 should therefore be included in stream water monitoring programs in order to provide a “true”  
588 assessment of pesticide impact on streams.

### 589 **Acknowledgements**

590 This work was supported by the Danish Strategic Research Council (RiskPoint project). We thank  
591 all persons involved in the field studies analyzed in this paper. We additionally thank two anonymous  
592 reviewers for their valuable comments and suggestions for improving the clarity of this paper.

### 593 **Supporting information**

594 Figures for sales trends of selected pesticides and for the evaluation of different exposure durations  
595 on the toxicity calculation. Tables of the physicochemical properties of the detected compounds, an  
596 overview of their use and legal status, trends of treated areas/frequencies, top-15 Danish pesticide  
597 usage statistics, overview of analytes and sampling campaigns, acute/chronic toxicity data, the number  
598 of compounds for which additional exposure data exists, an overview of the pesticides detected in this  
599 study including EU norms, an overview of the impacted streams, pesticide detection results in  
600 groundwater extraction wells, and for an extended overview of the predicted toxicity for sediment-  
601 bound pesticides.

602

603 **References**

604

- 605 Arias-Estévez, M.; López-Periago, E.; Martínez-Carballo, E.; Simal-Gándara, J.; Mejuto, J.-C.; García-  
606 Río, L. The mobility and degradation of pesticides in soils and the pollution of groundwater  
607 resources. *Agriculture, Ecosystems & Environment* 2008;123:247-260.
- 608 Artigas, J.; Arts, G.; Babut, M.; Caracciolo, A.B.; Charles, S.; Chaumot, A.; Combourieu, B.; Dahllöf,  
609 I.; Despréaux, D.; Ferrari, B.; Friberg, N.; Garric, J.; Geffard, O.; Gourlay-Francé, C.; Hein, M.;  
610 Hjorth, M.; Krauss, M.; De Lange, H.J.; Lahr, J.; Lehtonen, K.K.; Lettieri, T.; Liess, M.; Lofts,  
611 S.; Mayer, P.; Morin, S.; Paschke, A.; Svendsen, C.; Usseglio-Polatera, P.; van den Brink, N.;  
612 Vindimian, E.; Williams, R. Towards a renewed research agenda in ecotoxicology.  
613 *Environmental Pollution* 2012;160:201-206.
- 614 Asman, W.A.H.; Jorgensen, A.; Bossi, R.; Vejrup, K.V.; Mogensen, B.M.; Glasius, M. Wet deposition  
615 of pesticides and nitrophenols at two sites in Denmark: measurements and contributions from  
616 regional sources. *Chemosphere* 2005;59:1023-1031.
- 617 Barth, J.A.C.; Steidle, D.; Kuntz, D.; Gocht, T.; Mouvet, C.; von Tümpling, W.; Lobe, I.; Langenhoff,  
618 A.; Albrechtsen, H.J.; Janniche, G.S.; Morasch, B.; Hunkeler, D.; Grathwohl, P. Deposition,  
619 persistence and turnover of pollutants: First results from the EU project AquaTerra for selected  
620 river basins and aquifers. *Science of The Total Environment* 2007;376:40-50.
- 621 Beketov, M.; Kefford, B.J.; Schaefer, R.B.; Liess, M. Pesticides reduce regional biodiversity of stream  
622 invertebrates. *Proceedings of the National Academy of Sciences* 2013;110(27):11039-11043.
- 623 Belden, J.B.; Gilliom, R.J.; Martin, J.D.; Ludy, M.J. Relative toxicity and occurrence patterns of  
624 pesticide mixtures in streams draining agricultural watersheds dominated by corn and soybean  
625 production. *Integrated Environmental Assessment and Management* 2007;3:90-100.
- 626 Benachour, N.; Seralini, G.E. Glyphosate Formulations Induce Apoptosis and Necrosis in Human  
627 Umbilical, Embryonic, and Placental Cells. *Chemical Research in Toxicology* 2009;22:97-105.
- 628 Bundschuh, M.; Goedkoop, W.; Kreuger, J. Evaluation of pesticide monitoring strategies in agricultural  
629 streams based on the toxic-unit concept — Experiences from long-term measurements. *Science  
630 of The Total Environment* 2014;484:84-91.
- 631 Cape, J.N.; Forczek, S.T.; Gullner, G.; Mena-Benitez, G.; Schroder, P.; Matucha, M. Progress in  
632 understanding the sources, deposition and above-ground fate of trichloroacetic acid.  
633 *Environmental Science and Pollution Research* 2006;13:276-286.
- 634 Capel, P.D.; Larsen, S.J.; Winterstein, T.A. The behavior of thirty-nine pesticides in surface waters as a  
635 function of scale. *Hydrological Processes* 2001;15:1251-1269.
- 636 Carlsen, S.C.K.; Spliid, N.H.; Svensmark, B. Drift of 10 herbicides after tractor spray application. 1.  
637 Secondary drift (evaporation). *Chemosphere* 2006a;64:787-794.
- 638 Carlsen, S.C.K.; Spliid, N.H.; Svensmark, B. Drift of 10 herbicides after tractor spray application. 2.  
639 Primary drift (droplet drift). *Chemosphere* 2006b;64:778-786.
- 640 Clausen, L.; Arildskov, N.P.; Larsen, F.; Albrechtsen, H.-J. Degradation of the herbicide dichlobenil  
641 and its metabolite BAM in soils and subsurface sediments. *Journal of Contaminant Hydrology*  
642 2007;89:157-173.
- 643 Connon, R.E.; Geist, J.; Werner, I. Effect-Based Tools for Monitoring and Predicting the  
644 Exotoxicological Effects of Chemicals in the Aquatic Environment. *Sensors* 2012;12:12741-  
645 12771.

646 Danish EPA. NOVANA: Det nationale program for overvågning af vandmiljøet og naturen.  
647 Proqrambeskrivelse 1. del. ISBN: 978-87-7279-101-7. Miljøministeriet, Naturstyrelsen:  
648 Copenhagen, DK; 1-22; 2011 [in Danish].

649 Danish EPA. Bekendtgørelse om miljøkvalitetskrav for vandområder og krav til udledning af  
650 forurenende stoffer til vandløb, søer eller havet. Miljøministeriet: Copenhagen, DK; 1-18; 2010  
651 [in Danish].

652 Danish EPA. Bekaempelsesmiddelstatistik 2010, Orientering fra Miljøstyrelsen Nr. 5.  
653 Miljøministeriet, Miljøstyrelsen: Copenhagen, DK; 1-48; 2011 [in Danish].

654 DG SANCO. EU Pesticides Database. [Online] [http://ec.europa.eu/sanco\\_pesticides/public/](http://ec.europa.eu/sanco_pesticides/public/); 2014.

655 Ejrnaes, R.; Baatrup-Pedersen, A.; Riis, T.; Pedersen, M.L.; Hoffmann, C.C.; Kronvang, B., Johansen,  
656 O.M. Herbicider i terrestriske vådområder: Herbiciders forekomst, mængde, spredningsveje og  
657 effecter i moser i udvalgte jyske ådale. Bekæmpelsesmiddelforskning nr. 154; ISBN: 978-87-  
658 93178-09-0. Miljøministeriet, Miljøstyrelsen: Copenhagen, DK; 1-122; 2014. [in Danish].

659 Faust, M., Altenburger, R., Backhaus, T., Blanck, H., Boedeker, W., Gramatica, P., Hamer, V.,  
660 Scholze, M., Vighi, M., Grimme, L.H. Joint algal toxicity of 16 dissimilarly acting chemicals is  
661 predictable by the concept of independent action. *Aquatic Toxicology* 2003;63:43-63.

662 Fenner, K.; Canonica, S.; Wackett, L.P.; Elsner, M. Evaluating Pesticide Degradation in the  
663 Environment: Blind Spots and Emerging Opportunities. *Science* 2013;341:752-758.

664 Gilbert, S.G.A Small Dose of Toxicology: The Health Effects of Common Chemicals. Seattle: Healthy  
665 World Press; 2012.

666 Gilliom, R.J. Pesticides in U.S. streams and groundwater. *Environmental Science & Technology*  
667 2007;41:3407-3413.

668 Gilliom, R.J.; Barbash, J.E.; Kolpin, D.W.; Larson, S.J. Testing water quality for pesticide pollution.  
669 *Environmental Science & Technology* 1999;33:164A-169A.

670 Hansen, B.; Thorling, L.; Dalgaard, T.; Erlandsen, M. Trend Reversal of Nitrate in Danish  
671 Groundwater - a Reflection of Agricultural Practices and Nitrogen Surpluses since 1950.  
672 *Environmental Science & Technology* 2011;45:228-234.

673 Holt, E.; Weber, R.; Stevenson, G.; Gaus, C. Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans  
674 (PCDD/Fs) Impurities in Pesticides: A Neglected Source of Contemporary Relevance.  
675 *Environmental Science & Technology* 2010;44:5409-5415.

676 Holvoet, K.M.A.; Seuntjens, P.; Vanrolleghem, P.A. Monitoring and modeling pesticide fate in surface  
677 waters at the catchment scale. *Ecological Modelling* 2007;209:53-64.

678 Jansson, C.; Kreuger, J. Multiresidue Analysis of 95 Pesticides at Low Nanogram/Liter Levels in  
679 Surface Waters Using Online Preconcentration and High Performance Liquid  
680 Chromatography/Tandem Mass Spectrometry. *Journal of AOAC International* 2010;93:1732-  
681 1747.

682 Kegley, S.E., Hill, B.R., Orme, S., Choi, A.H. PAN pesticide database. North America, San Francisco,  
683 CA: Pesticide Action Network; 2008

684 Klein, W.; Denzer, S.; Herrchen, M.; Lepper, P.; Müller, M.; Sehr, R.; Storm, A.; Volmer, J. Revised  
685 Proposal for a List of Priority Substances in the Context of the Water Framework Directive  
686 (COMMPS Procedure): Final Report 98/788/3040/DEB/E1. Schmallenberg, Germany:  
687 Fraunhofer-Institute, Umweltchemie und Ökotoxikologie; 1999.

688 Kolpin, D.W., Thurman, E.M., Linhart, S.M. Occurrence of cyanazine compounds in groundwater:  
689 Degradates more prevalent than the parent compound. *Environmental Science & Technology*  
690 2001;35:1217-1222.

691 Konstantinou, I.K.; Hela, D.G.; Albanis, T.A. The status of pesticide pollution in surface waters (rivers  
692 and lakes) of Greece. Part I. Review on occurrence and levels. *Environmental Pollution*  
693 2006;141:555-570.

694 Kronvang, B.; Laubel, A.; Larsen, S.E.; Friberg, N. Pesticides and heavy metals in Danish streambed  
695 sediment. *Hydrobiologia* 2003;494:93-101.

696 Kuivila, K.M.; Hladik, M.L.; Ingersoll, C.G.; Kemble, N.E.; Moran, P.W.; Calhoun, D.L.; Nowell,  
697 L.H.; Gilliom, R.J. Occurrence and Potential Sources of Pyrethroid Insecticides in Stream  
698 Sediments from Seven U.S. Metropolitan Areas. *Environmental Science & Technology*  
699 2012;46:4297-4303.

700 Laubel, A.; Kronvang, B.; Fjordback, C. Time-integrated suspended sediment sampling from a small  
701 lowland stream. *Vereinigung für theoretische und angewandte Limnologie* 2001;28:1420-1424.

702 Levi, S.; Hybel, A.M.; Bjerg, P.L.; Albrechtsen, H.J. Stimulation of aerobic degradation of bentazone,  
703 mecoprop and dichlorprop by oxygen addition to aquifer sediment. *Science of The Total*  
704 *Environment* 2014;473-474:667-675.

705 Liess, M.; Schulz, R.; Neumann, M. A method for monitoring pesticides bound to suspended particles  
706 in small streams. *Chemosphere* 1996;32:1963-1969.

707 Liess, M.; von der Ohe, P.C. Analyzing effects of pesticides on invertebrate communities in streams.  
708 *Environmental Toxicology and Chemistry* 2005;24:954-965.

709 Lützhøft, H.C.H.; Donner, E.E.; Wickman, T.T.; Eriksson, E.E.; Banovec, P.P.; Mikkelsen, P.S.;  
710 Ledin, A.A. A source classification framework supporting pollutant source mapping, pollutant  
711 release prediction, transport and load forecasting, and source control planning for urban  
712 environments. *Environ Sci Pollut Res Int* 2012;19:1119-1130.

713 Lyandres, O. Keeping Great Lakes Water Safe: Priorities for Protecting against Emerging Chemical  
714 Pollutants. Ed. Campbell, S., [Online] <http://www.greatlakes.org/document.doc?id=1263>.  
715 Chicago, IL: Alliance for the Great Lakes; 2012.

716 Mackay, D.; Giesy, J.P.; Solomon, K.R. Fate in the environment and long-range atmospheric transport  
717 of the organophosphorus insecticide, chlorpyrifos and its oxon. *Reviews of Environmental*  
718 *Contamination and Toxicology* 2014;231:35-76.

719 Malaguerra, F.; Albrechtsen, H.-J.; Thorling, L.; Binning, P.J. Pesticides in water supply wells in  
720 Zealand, Denmark: A statistical analysis. *Science of The Total Environment* 2012;414:433-444.

721 Maund, S.J.; Hamer, M.J.; Lane, M.C.G.; Farrelly, E.; Rapley, J.H.; Goggin, U.M.; Gentle, W.E.  
722 Partitioning, bioavailability, and toxicity of the pyrethroid insecticide cypermethrin in  
723 sediments. *Environmental Toxicology and Chemistry* 2002;21:9-15.

724 McKnight, U.S.; Rasmussen, J.J.; Kronvang, B.; Bjerg, P.L.; Binning, P.J. Integrated assessment of the  
725 impact of chemical stressors on surface water ecosystems. *Science of The Total Environment*  
726 2012;427-428:319-331.

727 Milosevic, N.; Thomsen, N.I.; Juhler, R.K.; Albrechtsen, H.J.; Bjerg, P.L. Identification of discharge  
728 zones and quantification of contaminant mass discharges into a local stream from a landfill in a  
729 heterogeneous geologic setting. *Journal of Hydrology* 2012;446-447:13-23.

730 Nollet, L.M.L.; Rathore, H.S. Handbook of Pesticides: Methods of Pesticide Residues Analysis. Boca  
731 Raton, FL: CRC Press, Taylor and Francis Group, LLC; 2010.

732 OECD. OECD Guidelines for the Testing of Chemicals: Sediment-Water Chironomid Life-Cycle  
733 Toxicity Test Using Spiked Water or Spiked Sediment. Section 2: Effects on Biotic Systems,  
734 Test No. 233. ISBN: 9789264090910; DOI: 10.1787/9789264090910-en. 1-29; 2010.

735 Olsen, P.; Ernsten, V.; Jacobsen, O.H.; Hansen, N.; de Jonge, L.W.; Kjær, J. Transport modes and  
736 pathways of the strongly sorbing pesticides glyphosate and pendimethalin through structured  
737 drained soils. *Chemosphere* 2011;84:471-479.

738 Paludan, E.; Borling, P.; Poulsen, J. 22 hormonforstyrrende aktivstofferkortlægning over anvendelse i  
739 andre produkter end plantebeskyttelsesmidler. Miljøprojekt Nr. 933 2004; Danish EPA; 2004  
740 [in Danish].

741 Peters, K.; Bundschuh, M.; Schaefer, R.B. Review on the effects of toxicants on freshwater ecosystem  
742 functions. *Environmental Pollution* 2013;180:324-329.

743 Rasmussen, J.J.; McKnight, U.S.; Loinaz, M.C.; Thomsen, N.I.; Olsson, M.E.; Bjerg, P.L.; Binning,  
744 P.J.; Kronvang, B. A catchment scale evaluation of multiple stressor effects in headwater  
745 streams. *Science of The Total Environment* 2013;442:420-431.

746 Rathore, H.S.; Nollet, L.M.L. Pesticides: Evaluation of Environmental Pollution. Boca Raton, FL: CRC  
747 Press, Taylor and Francis Group, LLC; 2012.

748 Reitzel, L.A.; Tuxen, N.; Ledin, A.; Bjerg, P.L. Can degradation products be used as documentation for  
749 natural attenuation of phenoxy acids in groundwater? *Environmental Science & Technology*  
750 2004;38:457-467.

751 Rodrigues, E.T.; Lopes, I.; Pardal, M.A. Occurrence, fate and effects of azoxystrobin in aquatic  
752 ecosystems: A review. *Environment International* 2013;53:18-28.

753 Roy, J.W.; Bickerton, G. Toxic Groundwater Contaminants: An Overlooked Contributor to Urban  
754 Stream Syndrome? *Environmental Science & Technology* 2012;46:729-736.

755 Schaefer, R.B.; Gerner, N.; Kefford, B.J.; Rasmussen, J.J.; Beketov, M.A.; de Zwart, D.; Liess, M.; von  
756 der Ohe, P.C. How to Characterize Chemical Exposure to Predict Ecologic Effects on Aquatic  
757 Communities? *Environmental Science & Technology* 2013;47:7996-8004.

758 Schaefer, R.B.; von der Ohe, P.C.; Kuehne, R.; Schueuermann, G.; Liess, M. Occurrence and Toxicity  
759 of 331 Organic Pollutants in Large Rivers of North Germany over a Decade (1994 to 2004).  
760 *Environmental Science & Technology* 2011;45:6167-6174.

761 Schaefer, R.B.; von der Ohe, P.C.; Rasmussen, J.; Kefford, B.J.; Beketov, M.A.; Schulz, R.; Liess, M.  
762 Thresholds for the Effects of Pesticides on Invertebrate Communities and Leaf Breakdown in  
763 Stream Ecosystems. *Environmental Science & Technology* 2012;46:5134-5142.

764 Schwarzenbach, R.P.; Escher, B.I.; Fenner, K.; Hofstetter, T.B.; Johnson, C.A.; von Gunten, U.;  
765 Wehrli, B. The challenge of micropollutants in aquatic systems. *Science* 2006;313:1072-1079.

766 Squillace, P.J.; Thurman, E.M.; Furlong, E.T. (1993). Groundwater as a nonpoint-source of atrazine  
767 and deethylatrazine in a river during base-flow conditions. *Water Resources Research*  
768 1993;29:1719-1729.

769 Stokstad, E.; Grullon, G. Infographic: Pesticide Planet. A global look at the uses, benefits, and  
770 drawbacks of pesticides. *Science* 2013;341:730-731.

771 Tesoriero, A.J.; Saad, D.A.; Burow, K.R.; Frick, E.A.; Puckett, L.J.; Barbash, J.E. Linking ground-  
772 water age and chemistry data along flow paths: Implications for trends and transformations of  
773 nitrate and pesticides. *Journal of Contaminant Hydrology* 2007;94:139-155.

774 Thurman, E.M.; Goolsby, D.A.; Meyer, M.T.; Mills, M.S.; Pomes, M.L.; Kolpin, D.W. A  
775 reconnaissance study of herbicides and their metabolites in surface-water of the midwestern  
776 United States using immunoassay and gas-chromatography mass-spectrometry. *Environmental*  
777 *Science & Technology* 1992;26:2440-2447.

778 Tomlin, C.D.S. The pesticide manual, a world compendium. Farnham, Surrey, UK: Crop Protection  
779 Publications; 2001.



780 Tuxen, N.; Roost, S.; Kofoed, J.L.L.; Aisopou, A.; Binning, P.J.; Chambon, J.; Bjerg, P.L.; Thorling,  
781 L.; Bruesch, W.; Esbensen, K. Skelnen mellem pesticidkilder. Miljøprojekt nr. 1502;  
782 Miljøministeriet, Miljøstyrelsen: Copenhagen, DK; 2013 [in Danish].

783 Uneke, C.J. Integrated Pest Management for Developing Countries: A Systemic Overview. New York:  
784 Nova Science Publishers, Inc.; 2007.

785 University of Hertfordshire. The Pesticides Properties DataBase (PPDB). 2014. [Online]  
786 <http://sitem.herts.ac.uk/aeru/iupac/>. International Union of Pure and Applied Chemistry,  
787 Agriculture & Environment Research Unit, School of Life Sciences, University of  
788 Hertfordshire: Hatfield, Herts., UK.

789 Unsworth, J. History of Pesticide Use. 2010. IUPAC International Union of Pure and Applied  
790 Chemistry; June 24, 2014, [Online]  
791 [http://agrochemicals.iupac.org/index.php?option=com\\_sobi2&sobi2Task=sobi2Details&catid=](http://agrochemicals.iupac.org/index.php?option=com_sobi2&sobi2Task=sobi2Details&catid=3&sobi2Id=31)  
792 [3&sobi2Id=31](http://agrochemicals.iupac.org/index.php?option=com_sobi2&sobi2Task=sobi2Details&catid=3&sobi2Id=31).

793 Vecchia, A.V.; Gilliom, R.J.; Sullivan, D.J.; Lorenz, D.L.; Martin, J.D. Trends in Concentrations and  
794 Use of Agricultural Herbicides for Corn Belt Rivers, 1996-2006. *Environmental Science &*  
795 *Technology* 2009;43:9096-9102.

796 Vogel, J.R.; Majewski, M.S.; Capel, P.D. Pesticides in rain in four agricultural watersheds in the  
797 United States. *Journal of Environmental Quality* 2008;37:1101-1115.

798 Warren, N.; Allan, I.J.; Carter, J.E.; House, W.A.; Parker, A. Pesticides and other micro-organic  
799 contaminants in freshwater sedimentary environments—a review. *Applied Geochemistry*  
800 2003;18:159-194.

801 Wittmer, I.K.; Bader, H.P.; Scheidegger, R.; Singer, H.; Lück, A.; Hanke, I.; Carlsson, C.; Stamm, C.  
802 Significance of urban and agricultural land use for biocide and pesticide dynamics in surface  
803 waters. *Water Research* 2010;44:2850-2862.

804 Wittmer, I.K.; Scheidegger, R.; Bader, H.-P.; Singer, H.; Stamm, C. Loss rates of urban biocides can  
805 exceed those of agricultural pesticides. *Science of The Total Environment* 2011;409:920-932.