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1 **The legacy of pesticide pollution: An overlooked factor in current risk**
2 **assessments of freshwater systems**

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23 ABSTRACT

24 We revealed a history of legacy pesticides in water and sediment samples from 19 small streams
25 across an agricultural landscape. Dominant legacy compounds included organochlorine pesticides,
26 such as DDT and lindane, the organophosphate chlorpyrifos and triazine herbicides such as
27 terbutylazine and simazine which have long been banned in the EU. The highest concentrations of
28 legacy pesticides were found in streams draining catchments with a large proportion of arable
29 farmland suggesting that they originated from past agricultural applications. The sum of toxic units
30 ($\text{SumTU}_{D.magna}$) based on storm water samples from agriculturally impacted streams was
31 significantly higher when legacy pesticides were included compared to when they were omitted.
32 Legacy pesticides did not significantly change the predicted toxicity of water samples to algae or
33 fish. However, pesticide concentrations in bed sediment and suspended sediment samples exceeded
34 safety thresholds in 50 % of the samples and the average contribution of legacy pesticides to the
35 $\text{SumTU}_{C.riparius}$ was > 90%. Our results suggest that legacy pesticides can be highly significant
36 contributors to the current toxic exposure of stream biota, especially macroinvertebrate
37 communities, and that those communities were primarily exposed to legacy pesticides via the
38 sediment. Additionally, our results suggest that neglecting legacy pesticides in the risk assessment
39 of pesticides in streams may severely underestimate the risk of ecological effects.

40

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44 KEYWORDS: Legacy pesticides, Environmental Risk Assessment, Mixture Toxicity, Pesticide
45 Monitoring, Streams

46 INTRODUCTION

47 Publication frequency of articles characterising the contamination dynamics of freshwater systems
48 in space and time has increased over the past decade in recognition of the need to increase realism
49 of current exposure and risk assessments to support an informed management of these systems.
50 Pesticides in particular have received increasing attention given their suggested important role in
51 the global loss of freshwater biodiversity and ecosystem functioning (Beketov et al. 2013; Malaj et
52 al. 2014; Rasmussen et al. 2012; Schäfer et al. 2012). In this article, we subdivide pesticides into
53 those still registered for agricultural use in the European Union and in Denmark (referred to as
54 contemporary pesticides) and those that have been discontinued or banned for usage in conventional
55 agriculture (referred to as legacy pesticides).

56

57 Pesticides applied to agricultural fields may reach surface water through a series of different
58 pathways with surface runoff and tile drains being widely accepted as the most important routes for
59 contemporary pesticides (Schulz 2004). These transport routes are primarily initiated during heavy
60 precipitation events and lead to transient peak concentrations often exceeding current ecological
61 quality criteria (Bundschuh et al. 2014; Liess and von der Ohe 2005; Schulz 2004). In contrast,
62 legacy pesticides may enter surface water continuously via groundwater inflow (Barth et al. 2007;
63 Gilliom 2007; McKnight et al. 2015), atmospheric deposition (Konstantinou et al. 2006; Weber et
64 al. 2010) or through continuous leaching from agricultural soils and landfills (Aliyeva et al. 2013).
65 Consequently, legacy pesticides may generate a relatively constant exposure regime in surface
66 waters. The yearly flux of legacy pesticides to freshwater ecosystems may comprise up to several
67 percent of the historical yearly applied amounts in a catchment (Barth et al. 2007). Importantly,
68 pesticides and their residues may persist and even accumulate in sediments of freshwater
69 ecosystems (Dai et al. 2014; Kuivila et al. 2012; Nowell et al. 2013).

70

71 Factors controlling the fate of a pesticide in agricultural landscapes include a variety of chemical
72 and environmental properties of the pesticide (e.g. degradation rate, adsorption to organic carbon
73 and water solubility), climatic factors (e.g. temperature and precipitation), soil characteristics,
74 topography and agricultural practices (Leonard 1990; Wauchope 1978). More than 20,000 pesticide
75 products have entered the market since registration became legislatively required in 1947, and it is
76 therefore not surprising that the combined effect of multiple factors influencing the environmental
77 transport and fate of each pesticide generates highly complex exposure profiles of pesticide
78 mixtures in time and space (Konstantinou et al. 2006; Wauchope 1978). However, pesticides that
79 are currently applied in the highest quantities are also those that occur most often in surface waters
80 with the more water soluble and persistent compounds reaching the highest concentrations
81 (Bundschuh et al. 2014; Kreuger and Tornqvist 1998; Li et al. 2013; Moschet et al. 2014).

82 Therefore, current pesticide usage is often used to guide the prioritisation of active ingredients
83 included in monitoring programmes and research activities. Moschet et al. (2014) showed that a
84 stringent focus on EU priority pollutants or a subset of the active ingredients applied in the highest
85 quantities on the national level may seriously underestimate predicted toxic pressures in streams.

86 Whereas Moschet et al. (2014) aimed to document that an extensive pesticide screening (249 active
87 ingredients) translates into significantly higher predicted mixture toxicities compared to screenings
88 restricted to fewer pesticides (≤ 36), the authors did not distinguish between the toxic contribution
89 of contemporary and legacy pesticides. Based on water samples mainly analysed for herbicides and
90 four sediment samples mainly analysed for insecticides, McKnight et al. (2015) suggested that
91 legacy pesticides could still be prominent players driving observed impairments of freshwater
92 invertebrates, and the authors urged for more extensive studies that allow for quantifying the
93 predicted toxicological potency of legacy pesticides in comparison to current use pesticides. To our

94 knowledge, such an extensive study of the potential toxicity of legacy pesticides to aquatic biota
95 relative to that of contemporary pesticides is still lacking despite a substantial body of literature
96 addressing the occurrence, concentrations and predicted toxicities of selected legacy pesticides
97 (Aliyeva et al., 2013; Gilliom, 2007; McKnight et al., 2015; Weber et al., 2010). The novelty
98 element is therefore to quantify the possible toxicity of legacy pesticides as an integral part of
99 current risk assessment. Such an integration has a number of potentially vital implications for the
100 usability of risk assessment, including that i) contemporary regulatory actions are only targeting
101 substances that are still in use; ii) it gives an increased explanatory power in river quality
102 assessment by quantifying the impact of current unknowns, which will additionally reduce the
103 potential underestimation of the role of pesticides as stressors in stream ecosystems, which is
104 currently most likely the case (Beketov et al., 2013; Malaj et al., 2014), and iii) it provides highly
105 needed insight into pesticide exposure profiles in time and space that may be used as improved
106 benchmarks for the interpretations of ecological response parameters.

107

108 This article aims to compare the toxicity of legacy pesticides and their metabolites to those of
109 contemporary pesticides in 19 Danish 1st and 2nd order streams situated in agricultural landscape
110 covering a range of agricultural intensity, local climate and soil types. Water samples were collected
111 during base flow and peak flow for pesticide analyses, and bulk sediment and suspended sediment
112 were sampled to optimize detections of pesticides with low water solubility. In more detail, our
113 objectives were to: i) characterize the occurrence of legacy pesticides in Danish headwater streams,
114 ii) estimate the predicted toxicity of legacy pesticides and their residues using the Toxic Units (TU)
115 approach, iii) evaluate the relative contribution of legacy pesticides and their residues to the
116 summed TU of contemporary pesticides, and iv) evaluate which legacy pesticides are of highest
117 ecotoxicological concern.

118

119 METHODS

120 *Study streams*

121 Nineteen Danish 1st and 2nd order streams (Fig. S1) were sampled for pesticide occurrences. Nine
122 streams with < 50% agricultural land-use in a two-sided buffer extending 2,000 m upstream of the
123 sampling site were selected in addition to 10 streams with expected high impact of pesticides
124 (conventional agriculture > 60% in the two-sided 100 m buffer). Furthermore, all study sites
125 complied with the following selection criteria: i) forest should occupy < 50% of a two-sided 50m
126 buffer extending from the study site and 2,000 m upstream, ii) proportional coverage of silt and
127 mud in stream substrates (indicative of drainage ditches) should be < 50%, and iii) no influence
128 from waste water treatment plants, but scattered settlements may influence the chemical water
129 quality. Detailed information on the study streams and catchments is provided in Table S1). In this
130 article, we refer to the nine streams with expected low agricultural impact as controls and the ten
131 streams with expected high agricultural impact as agricultural streams. All catchments are
132 characterised by loam or sandy loam, low elevation and precipitation ranges from ca. 800-850 mm
133 year⁻¹ for central Jutland and on Funen and 700-750 mm year⁻¹ on Zealand.

134

135 Base flow discharge was calculated as the product of the mean stream width, mean depth and mean
136 water velocity, based on measurements at ten transects along a 100 m stream reach extending
137 upstream from the sampling point (depth and velocity measured at 0, 25, 50 and 75% of the width
138 of each transect). Moreover, yearly mean discharge was estimated as the product of yearly mean
139 discharge coefficients ($L s^{-1} km^{-2}$), calculated for national hydrological monitoring stream sites
140 geographically/geologically selected as representative for the study streams, and catchment area for
141 the study streams (km^2). In a few cases national monitoring sites could not be regarded as truly

142 representative, and yearly mean discharge was designated as > base flow (Table S1). The
143 proportion of conventional agriculture was quantified for the catchments of each study stream and
144 for a two-sided 100 m buffer extending 2,000 m upstream of the sampling site were quantified in
145 ArcGis 10.1 for windows.

146

147 *Pesticide sampling*

148 Sampling was conducted during May – August in 2012 coinciding with the main pesticide
149 application season in this part of Europe. Dissolved phase pesticides were sampled with: i) manual
150 grab samples in August during low flow conditions to optimize detections of pesticides originating
151 from groundwater inflow (one sample per stream) and ii) event-triggered water samplers designed
152 to capture water during storm flow (Liess et al. 1999). Manual collection of water samples during
153 low-flow conditions were consistently preceded by one week without precipitation. Event-triggered
154 water samplers were checked every week during May, June and July and collected if full, resulting
155 in 64 storm flow water samples. The event-triggered water samplers strategically collect water
156 representing a temporal point measurement during the first hours of a heavy rain incident (Liess et
157 al. 1999).

158

159 Sediment associated pesticides were sampled with two different methods. Bed sediment was
160 collected (top 1 cm) in depositional areas using Kajak corers (8 cm in diameter). Each bed sediment
161 sample was comprised of 20-30 subsamples to obtain samples representative for the stream reach.
162 Bed sediment was collected in all streams in mid-August reflecting newly deposited material during
163 the summer period. Suspended sediment was additionally collected since the mobile sediment
164 fraction may provide a stronger estimate for worst case scenarios (Liess et al. 1996). The Suspended
165 Particle Samplers (SPS) used in this study are described in detail elsewhere (Laubel et al. 2001).

166

167 *Chemical analyses*

168 Water samples were screened for 70 pesticides and metabolites comprising 42 contemporary
169 pesticides, 26 legacy pesticides and 2 metabolites (Table S2). The 68 active ingredients included 35
170 herbicides, 16 fungicides and 17 insecticides. Bed sediment and suspended sediment samples were
171 screened for 38 pesticides and residues comprising 16 contemporary pesticides, 18 legacy pesticides
172 and 4 metabolites (Table S3). The 34 active ingredients included in the screening included 12
173 herbicides, 5 fungicides and 17 insecticides.

174

175 Analysis of water samples for the non-polar compounds was done by liquid/liquid extraction
176 followed by gas chromatography mass spectrometry (GC-MS). For the polar and semi-polar
177 compounds online solid-phase extraction followed by liquid chromatography tandem mass
178 spectrometry (LC-MS/MS) was performed as described by Jansson & Kreuger (2010).

179 Wet sediment sample (20 g) was mixed with a drying agent (10 g). A sub-sample of the mixture (9
180 g, corresponding to 6 g sediment) was placed in pre-cleaned (400 °C) glass fibre cartridges and
181 extracted together with the internal standards ethion and terbuthylazin-D5 by a Soxtec Avanti 2050
182 Auto System using dichloromethane and acetone (1:1). The extract was evaporated and diluted in
183 cyclohexane and dichloromethane (1:1) before purification by Gel Permeation Chromatography
184 (GPC), followed by evaporation and dilution in cyclohexane and acetone (9:1). The volume was
185 adjusted to 1 ml. The extract was injected on two separate GC-MS systems, one in negative
186 chemical ionization (NCI) mode (Agilent Technologies GC 7890, MS 5975C) and one in electron
187 impact (EI) mode (Agilent Technologies GC 6890, MS 5973), quantifying against an external
188 standard calibration. In order to enhance the sensitivity of the DDTs, a part of the initial extract was
189 purified with sulphuric acid and with the internal standards added once again before injection. The

190 standards used were obtained from Dr Ehrenstorfer GmbH. Dry-weight measurements of sediment
191 were performed in a dry oven (105 °C) during ca. 16 hours, with analytical results presented as µg
192 per kg of dry weight.

193

194 Values between the limit of detection (LOD) and the limit of quantification (LOQ) were given as trace
195 concentrations. At this level, the uncertainty of the concentration might be higher than stipulated (i.e. above
196 30 %), but the identity of the compound has been confirmed and was therefore considered appropriate to be
197 included in the subsequent data analysis.

198

199 *Data analysis*

200 All pesticide properties including effect concentrations (Tables S1 and S2) were acquired from the
201 Pesticide Properties Database (<http://sitem.herts.ac.uk/aeru/ppdb/en/> accessed 18.08.2014) and from
202 the US EPA Ecotox Database (<http://cfpub.epa.gov/ecotox/> accessed on 25.08.2014). In the cases
203 where more than one effect concentration was available for a pesticide, the lowest value was
204 selected. Legal status of the pesticides in Denmark and the EU was acquired from the Danish
205 Pesticide Database (<http://middeldatabasen.dk/Middelvalg.asp> accessed on 04.09.2014) and the EU
206 Pesticides Database (http://ec.europa.eu/sanco_pesticides/public/?event=homepage accessed on
207 04.09.2014), respectively (Tables S1 and S2).

208

209 For all water samples and sediment samples with pesticide detections above the LOD, the sum of
210 toxic units (SumTU) was calculated to standardise exposure concentrations according to a
211 benchmark organism. For water samples we used 96h growth inhibition tests on the green algae
212 *Pseudokirchneriella subcapitata* to benchmark sample toxicity to primary producers. In the cases
213 where no data existed, we used data for *Scenedesmus subspicatus* as an alternative. Acute 48h
214 mortality tests on *Daphnia magna* were used to benchmark the toxicity to invertebrates and 96h

215 mortality tests on *Oncorhynchus mykiss* were used to benchmark sample toxicity to fish. *Lepomis*
216 *macrochirus* was used as an alternative species in the few cases where no data was available for *O.*
217 *mykiss*.

218 The sum of toxic units (SumTU) is calculated as:

$$219 \text{ SumTU} = \sum_{i=1}^n \frac{C_i}{\text{EC50}_i} \quad (1)$$

220 where C_i is the concentration of pesticide i in the sample, and EC50_i is the concentration of
221 chemical i causing a 50% effect to the benchmark organisms.

222

223 Bed sediment and suspended sediment pesticide concentrations were converted to TU using 96h
224 acute mortality tests for the sediment dwelling non-biting midge *Chironomus riparius*
225 supplemented with 28d chronic exposure tests on emergence success for *C. riparius* in the cases
226 where no 96h acute mortality test data existed. Often, only one of the tests was available for a
227 pesticide, but in the few cases where data for both acute and chronic tests existed, we selected the
228 lowest effect concentration. Effect concentrations in the *C. riparius* tests were based on measured
229 pore water concentrations. In the cases where no sediment test data existed for a pesticide, we used
230 the 48h LC50 for *D. magna* as surrogate measure for sediment toxicity. Plotting the *C. riparius*
231 toxicity data as a function of 48h LC50 for *D. magna* for the pesticide compounds having both sets
232 of toxicity data revealed that the deviation from the 1:1 line rarely exceeded one order of magnitude
233 (Fig. S2).

234

235 Measured sediment-associated pesticide concentrations were converted to pore-water
236 concentrations according to the equilibrium-partitioning approach to comply with the sediment
237 benchmark toxicity tests that are based on dissolved phase pesticides in pore water. Moreover, pore

238 water concentrations are superior predictors of sediment toxicity to invertebrates compared to
239 pesticides adsorbed to sediment particles (Xu et al. 2007).

240 Pore water concentrations from bed sediment and suspended sediment were calculated according to
241 Ditoro et al. (1991) as:

$$242 \quad C_{PW} = \frac{C_s}{K_d} \quad (2)$$

243 where K_d is the partitioning coefficient, C_s is the sediment concentration and C_{PW} the pore water
244 concentration of the pesticide. K_d was calculated as:

$$245 \quad K_d = K_{OC} \times f_{OC} \quad (3)$$

246 where K_{OC} is the dimensionless organic carbon-water partitioning coefficient for the pesticide and
247 f_{OC} is the fraction of total organic carbon measured in the sediment sample. Kronvang et al. (2003)
248 found the fraction of total organic carbon in bed sediments from 27 Danish agricultural streams to
249 range from 5.5 to 16.1% with an average of 8.5%. Hence, the f_{OC} was set to 0.085 in our study. The
250 K_{OC} was calculated as:

$$251 \quad \log K_{OC} = a \times \log K_{OW} + b \quad (4)$$

252 where K_{OW} is the octanol-water partitioning coefficient. The constants a and b were set to 0.72 and
253 0.49, respectively, according to Schwarzenbach and Westall (1981).

254

255 We tested correlations between pesticide concentrations (ppm) among sample types ($n = 19$) using
256 Spearman-Rank analysis. Stream specific (arithmetic) mean concentrations of storm flow samples
257 were used. The number of storm flow samples ranged between two and five among streams (Table
258 S4). Moreover, we tested correlations between sumTU of legacy pesticides and sum TU of
259 contemporary pesticides within base flow, storm flow and sediment samples. For water samples, the
260 correlations were based on data for all benchmark organisms. All data used in the Pearson

261 correlation analyses were log-transformed to obtain normal distribution. The Spearman Rank
262 correlation analyses were conducted in JMP 11.1.1 for Windows.

263

264 We tested if the addition of legacy compounds significantly increased the sumTU of water and
265 sediment samples in control and agricultural streams, respectively, by comparing the sumTU of
266 contemporary pesticides to the sumTU of all pesticides using Mann-Whitney tests in JMP 11.1.1 for
267 Windows.

268

269 RESULTS AND DISCUSSION

270 *Pesticide occurrence and toxicity patterns*

271 We found a significant positive relationship among pesticide concentrations in all combinations of
272 sample types ($P < 0.05$) (Table 1, Fig. S3). The strongest correlations were obtained between
273 suspended sediment and bed sediment samples, between storm flow water and suspended sediment
274 and between storm flow water and bed sediment (Table 1). Thus, streams with high pesticide
275 concentrations in especially storm flow samples also had a high probability of having high pesticide
276 concentrations in sediments and to a lesser extent during base flow. Importantly, SumTU based on
277 contemporary pesticides was additionally a strong indicator for SumTU based on legacy pesticides
278 in base flow samples (daphnia: $r = 0.724$, $P < 0.001$; fish: $r = 0.578$, $P = 0.009$; algae: $r = 0.460$, $P =$
279 0.046), storm flow samples (daphnia: $r = 0.603$, $P < 0.001$; fish: 0.468 , $P < 0.001$; algae: $r = 0.359$,
280 $P = 0.009$), suspended sediment samples (chironomids: $r = 0.563$, $P = 0.012$) and sediment samples
281 (chironomids: $r = 0.696$, $P < 0.001$) (Fig. 1). This indicates that streams which are currently the
282 most impacted by contemporary pesticide pollution, have probably also been so in the past. This is
283 perhaps not surprising as areas with productive conventional agriculture rarely are converted into
284 non-farming activities (Harding et al. 1998).

285

286 *Quantification of pesticide toxicity*

287 In 11 ($\approx 17\%$), 12 ($\approx 18\%$) and 35 ($\approx 55\%$) of the storm water samples, pesticide concentrations
288 exceeded safety thresholds for daphnia (1/100 48h LC50), fish (1/100 96h LC50) and algae (1/10
289 96h EC50), respectively (Panel 2013) (Fig. 2, Table 2). Concentrations of legacy pesticides alone
290 exceeded the safety thresholds for daphnia and fish in six and three of the storm flow water
291 samples, respectively, while none of the samples contained legacy pesticide concentrations
292 exceeding the safety threshold for algae. Note however, that the average SumTU for daphnia, fish
293 and algae in agricultural streams all exceeded the respective safety thresholds (Table 2).
294 Importantly, and confirming the early findings of McKnight et al. (2015), the addition of
295 SumTU_{D.magna} based on legacy pesticides to the SumTU_{D.magna} based on contemporary pesticides
296 significantly increased the SumTU_{D.magna} in storm water samples from agricultural streams (Fig. 2B,
297 $P = 0.039$). None of the base-flow water samples exceeded existing guideline values for
298 invertebrates, fish or algae (Fig. 2A, Table 2).

299

300 Sediment and suspended sediment samples contained pesticide concentrations exceeding safety
301 thresholds in 10 of 20 samples from agricultural streams. In seven of these samples, legacy
302 pesticide concentrations alone exceeded the safety threshold, and the addition of SumTU_{C.riparius} for
303 legacy pesticides to the SumTU_{C.riparius} for contemporary pesticides significantly ($\alpha = 0.1$) increased
304 the SumTU_{C.riparius} in suspended sediments (Fig. 3, $P = 0.038$) as well as in bed sediments (Fig. 3, P
305 $= 0.064$). In fact, the average contribution of legacy pesticides to SumTU_{C.riparius} for bed sediments
306 and suspended sediments was $> 90\%$, and the average SumTU_{C.riparius} > 0.1 (Table 2).

307

308 Our results suggest that legacy pesticides can be highly significant contributors to the contemporary
309 toxic exposure of stream biota, especially macroinvertebrate communities, and that those
310 communities were primarily exposed to legacy pesticides via the sediment. However, Liess and von
311 der Ohe (2005) and Schäfer et al. (2012) showed that stream dwelling macroinvertebrate
312 communities were significantly different in streams containing peak flow concentrations of
313 pesticides at $1/1000$ 48h LC50_{*D.magna*}, and this threshold was exceeded in approximately 50% of the
314 storm water samples in our study (30% for legacy pesticides alone) (data not shown). This clearly
315 suggests that the exposure of stream biota to dissolved phase legacy pesticides as well as legacy
316 pesticides adsorbed to sediment particles are likely both important stressors in these streams.
317 Integrating past land use should therefore improve the prediction of pesticide impacts on
318 macroinvertebrate communities compared to the stringent focus on current use chemicals in the
319 water and sediment phases (Harding et al. 1998). Highly important is the fact that our results,
320 supported by the findings of McKnight et al. (2015), strongly suggest that disregarding legacy
321 pesticides, in particular those adsorbed to sediment particles, in ecotoxicological field studies and
322 pesticide monitoring programs probably leads to significant underestimations of total risk and
323 significant underestimations of the relative importance of pesticides compared to other important
324 anthropogenic stressors (Harding et al. 1998; Matson et al. 1997). However, we recognize that the
325 bioavailability of the highly lipophilic pesticides adsorbed to particles may decrease with increasing
326 age of the pesticide-particle complex (Xu et al. 2008). Hence the predicted sumTU for sediment-
327 dwelling organisms may be overestimated when large proportions of the pesticide-particle
328 complexes have been long-established.

329

330 Predicting the toxicity of pesticide mixtures based on the assumption of toxic additivity
331 (Concentration Addition, CA), as done in the present study, may be problematic when the pesticides

332 in the sample have dissimilar Modes Of Action (MOA) (Belden et al. 2007; Cedergreen et al.
333 2013). However, CA appears to be a slightly conservative and broadly applicable model for
334 pesticide mixtures with similar, dissimilar and unknown MOAs and has a relatively small risk of
335 underestimating the effects (Backhaus and Faust 2012; Nowell et al. 2014). Moreover, the SumTU
336 approach has been shown to strongly correlate with an ecological indicator for pesticide pollution
337 (SPEAR) (Liess and von der Ohe 2005) and provides as strong a correlation to SPEAR as other
338 models that consider different MOAs of sample constituents, e.g. the msPAF (Schäfer et al. 2013).

339

340 *Potential sources of the legacy pesticides*

341 The majority of the legacy pesticides included in this study (e.g. organochlorines and triazines) have
342 the potential to persist for several decades in agricultural soils to which the compounds have been
343 applied in the past (Aliyeva et al. 2013; Manz et al. 2001). In consequence, agricultural soils may
344 still be important sources providing continuous fluxes of legacy pesticides to freshwater ecosystems
345 (Barth et al. 2007; Gilliom 2007). The detection frequency of legacy pesticides was highest in base-
346 flow water samples and sediment samples; although their concentrations increased 2 to 15 fold in
347 water during storm flow (Table 2). This could indicate that a dominant source of legacy pesticides
348 was upper soil layers in the catchments, originating from past agricultural applications, where
349 surface runoff occurs (Manz et al. 2001). Re-suspension of contaminated sediment may have altered
350 the partitioning between particle bound and dissolved phases of pesticides and hence could be an
351 additional important source governing the observed increase in legacy pesticide concentrations
352 during storm flow (Quesada et al. 2014). Additional sources of potential importance may include
353 atmospheric deposition (Weber et al. 2010), point sources such as waste dumps (Aliyeva et al.
354 2013), industrial use and commercial products (Connor et al. 2007), and illegal private use (see

355 McKnight et al. (2015) for a detailed description of potential sources of legacy pesticides in
356 streams).

357

358 Since the dominant source of legacy pesticides is likely agricultural soils, we expect the flux of
359 legacy pesticides to streams to be relatively comparable between summer and winter, i.e. peaks
360 associated with storm events in winter would be less strong than peaks associated with the
361 additional application of contemporary pesticides in the summer. Data from the extensive Swedish
362 pesticide monitoring program documents that legacy pesticides are still found in stream water
363 outside the primary crop growing season of Nordic countries (Nanos et al., 2012). Hence, in
364 contrast to contemporary pesticides, the toxic pressure of legacy pesticides in streams is likely
365 relatively constant across seasons, additionally indicating that the relative toxic contribution of
366 legacy pesticides to the sumTU increases outside the primary crop growing seasons.

367

368 *Identifying compounds of concern*

369 Among the legacy pesticides, the organophosphate chlorpyrifos and organochlorines such as DDT
370 (and degradation products) and lindane were the strongest drivers of high SumTU for daphnia, fish
371 and sediment dwelling invertebrates, whereas diuron and the triazine herbicides (terbutylazine and
372 simazine) were the strongest drivers of high SumTU for algae (Table S5). Chlorpyrifos is still
373 permitted for agricultural purposes in some EU countries but has been banned in Denmark since
374 2008. The remaining pesticides mentioned are forbidden for agricultural purposes in the EU (DDT
375 since 1979, lindane since 2001 (but 1994 in Denmark), simazine since 2005, diuron since 2008 and
376 terbutylazine since 2009).

377

378 Since the legacy pesticides significantly increased the sumTU_{D.magna} in storm flow water and
379 sumTU_{C.riparius} in sediments we further evaluated the relative contribution of specific groups of
380 pesticides to sumTU_{D.magna} and sumTU_{C.riparius} in storm flow water and sediment samples,
381 respectively. The sumTU_{D.magna} in storm flow water was most strongly influenced by contemporary
382 pyrethroid insecticides (62.6%) and the legacy pesticide chlorpyrifos (15.3%) in agricultural
383 streams, whereas the sumTU_{D.magna} was most strongly influenced by legacy and contemporary
384 pyrethroid insecticides (26.3% and 24.3%, respectively) and chlorpyrifos (42%) in control streams
385 (Table 3). The SumTU_{C.riparius} of suspended sediment and bed sediment samples were almost
386 entirely governed by chlorpyrifos in agricultural streams whereas the sumTU_{C.riparius}, especially for
387 bed sediments, was more influenced by organochlorine insecticides in control streams (Table 3).
388 Since the half-life of chlorpyrifos in aquatic sediments is proposed to be 20-180 days (Mackay et al.
389 2014), which is comparable to the half-lives of pyrethroids, our findings could indicate that this
390 active ingredient is illegally used in Denmark. Alternatively, as pointed out by McKnight et al.
391 (2015), chlorpyrifos is well-known for its ability to undergo long-range transport and/or may still be
392 permitted for use in material protection products (e.g. as a biocide).

393

394 *Conclusions*

395 Risk assessment, the identification of pesticides of particular concern and the prioritization of
396 mitigation activities strongly rely on monitoring data from streams, and keeping up with the
397 increasing number of (emerging) active ingredients entering the market remains a serious challenge.
398 However, our results suggest that increasing attention should additionally be directed towards
399 legacy pesticides due to their predicted high impacts on the biota of especially agricultural streams.
400 Neglecting central legacy pesticides in stream monitoring programs may underestimate the
401 predicted toxicity of stream sediments by up to 90%. Future assessment schemes and management

402 strategies should seek to quantify the actual toxicity of sediments containing high concentrations of
403 legacy pesticides, and moreover seek to benchmark ecological entities of streams against more
404 extensive pesticide screening programs, including legacy pesticides, in order to evaluate if the
405 combined measurements of past and current use pesticides increase the explanatory power of
406 correlations between all types of pesticides and their ecological effects. Monitoring programs
407 should continuously re-address the status of legacy pesticides in freshwater systems to register
408 developments in long term exposure profiles. To reduce costs, the frequency and concentration
409 might be related to land-use history which can then be used as a proxy for potential exposure risk.
410 Our understanding of pesticide exposure in streams needs expansion and should progress towards
411 interpreting ecosystem responses in a temporal context where land use history is a key determinant
412 to when and where to sample.

413

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419

420 SUPPORTING INFORMATION

421 Figures providing a schematic overview of the study sites, correlations between pesticide
422 concentrations between sample types and relationships between compound-specific effect
423 concentrations for *D. magna* and *C. riparius*. Tables presenting legal status and analytical detection
424 limits for pesticides included in the field screening, stream and catchment characteristics,
425 supporting statistical information and an overview of compounds responsible for highest sumTU.

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553

554 Fig. 1. SumTU for legacy pesticides as a function of the SumTU for contemporary pesticides for
555 base flow water samples (A), storm flow water samples (B) and sediment samples (C). Sediment
556 was sampled with two methods representing the bed sediment and suspended sediment. The
557 diagonal lines indicate 1:1 relationships. For all water samples, the SumTU was calculated for algae
558 (*R. subcapitata*), fish (*O. mykiss*) and invertebrates (*D. magna*), whereas SumTU calculations for
559 sediment samples were based on *C. riparius*.

560

561 Fig. 2. Average SumTU for base-flow water samples (A) and storm flow water samples (B).
562 SumTU is grouped according to stream category (control, n=9; agricultural, n=10) and according to
563 benchmark organisms (*D. magna*, *O. mykiss* and *R. subcapitata*). Asterisks indicate significant
564 differences in the pairwise tests at the 5% level (**). The boxplots display the median (bold line),
565 first and third quartiles (upper and lower end of box) and the 1.5-fold interquartile range (error
566 bars). Outliers are indicated with open circles.

567

568 Fig. 3. Average SumTU_{*C. riparius*} for bed sediment and suspended sediment samples. SumTU is
569 grouped according to stream category (control, n=9; agricultural, n=10). Asterisks significant
570 differences at the 10% level (*) and 5% level (**). The boxplots display the median (bold line), first
571 and third quartiles (upper and lower end of box) and the 1.5-fold interquartile range (error bars).
572 Outliers are indicated with open circles.

573

574 Table 1. Results from the Spearman Rank analyses comparing the summed pesticide concentrations
 575 (ppm) between all sample types. The correlation coefficients (r, first line) and significance levels (P,
 576 second line) are given.

	Base-flow water	Storm flow water	Suspended sediment	Bed sediment
Base-flow water		0.658 0.002	0.523 0.026	0.694 < 0.001
Storm flow water			0.794 < 0.001	0.782 < 0.001
Suspended sediment				0.984 < 0.001
Bed sediment				

577

578 Table 2. Overview of central parameters for the pesticides monitored during base-flow and storm
 579 flow as well as in bed sediments (BS) and suspended sediments (SS). Parameter values are given \pm
 580 SE for control streams (n=9) and agricultural streams (n=10).

Parameter	Control streams	Agricultural streams
<i>Base-flow water samples</i>		
Average # compounds (all)	3.1 \pm 0.9	8.8 \pm 1.6
Average # compounds (legacy)	2.1 \pm 0.4	4.1 \pm 0.9
Average sum conc. ($\mu\text{g L}^{-1}$) (all)	0.033 \pm 0.014	0.192 \pm 0.099
Average sum conc. ($\mu\text{g L}^{-1}$) (legacy)	0.003 \pm 0.001	0.055 \pm 0.045
Average SumTU _{D.magna} (all)	6.8*10 ⁻⁶ \pm 3.9*10 ⁻⁶	0.0007 \pm 0.0004
Average SumTU _{D.magna} (legacy)	1.3*10 ⁻⁷ \pm 1.7*10 ⁻⁸	0.0006 \pm 0.0003
Average SumTU _{O.mykiss} (all)	1.7*10 ⁻⁵ \pm 8.7*10 ⁻⁶	0.0004 \pm 0.0002
Average SumTU _{O.mykiss} (legacy)	6.3*10 ⁻⁷ \pm 6.8*10 ⁻⁹	0.0002 \pm 6.3*10 ⁻⁵
Average SumTU _{P.subcapitata} (all)	0.006 \pm 0.003	0.036 \pm 0.008
Average SumTU _{P.subcapitata} (legacy)	0.0002 \pm 0.00008	0.002 \pm 0.002
<i>Storm flow water samples</i>		
Average # compounds (all)	7.7 \pm 0.9	21.3 \pm 1.4
Average # compounds (legacy)	3.5 \pm 0.3	6.9 \pm 0.5
Average sum conc. ($\mu\text{g L}^{-1}$) (all)	0.277 \pm 0.088	1.845 \pm 0.339
Average sum conc. ($\mu\text{g L}^{-1}$) (legacy)	0.045 \pm 0.015	0.129 \pm 0.018
Average SumTU _{D.magna} (all)	0.002 \pm 0.001	0.016 \pm 0.007
Average SumTU _{D.magna} (legacy)	0.001 \pm 0.001	0.003 \pm 0.001
Average SumTU _{O.mykiss} (all)	0.004 \pm 0.003	0.011 \pm 0.003
Average SumTU _{O.mykiss} (legacy)	0.001 \pm 0.001	0.001 \pm 0.001
Average SumTU _{P.subcapitata} (all)	0.101 \pm 0.045	0.892 \pm 0.292
Average SumTU _{P.subcapitata} (legacy)	0.004 \pm 0.002	0.012 \pm 0.005
<i>Sediment samples</i>		
Average # compounds (BS, all)	1.3 \pm 0.5	5.9 \pm 1.2
Average # compounds (SS, all)	2.1 \pm 0.5	6.9 \pm 1.1
Average # compounds (BS, legacy)	0.9 \pm 0.4	3.8 \pm 0.9
Average # compounds (SS, legacy)	1.3 \pm 0.4	4.2 \pm 0.8
Average sum conc. ($\mu\text{g kg}^{-1}$ DW) (BS, all)	6.0 \pm 2.5	65.1 \pm 14.2
Average sum conc. ($\mu\text{g kg}^{-1}$ DW) (SS, all)	13.1 \pm 3.6	167.6 \pm 57.0
Average sum conc. ($\mu\text{g kg}^{-1}$ DW) (BS, legacy)	2.5 \pm 1.1	22.7 \pm 7.6
Average sum conc. ($\mu\text{g kg}^{-1}$ DW) (SS, legacy)	6.6 \pm 2.8	48.4 \pm 21.3
Average SumTU _{C.riparius} (BS, all)	0.0003 \pm 0.0001	0.141 \pm 0.083
Average SumTU _{C.riparius} (SS, all)	0.001 \pm 0.001	0.117 \pm 0.090
Average SumTU _{C.riparius} (BS, legacy)	7.8*10 ⁻⁵ \pm 2.6*10 ⁻⁵	0.137 \pm 0.082
Average SumTU _{C.riparius} (SS, legacy)	0.001 \pm 0.001	0.108 \pm 0.090

581

582 Table 3. Relative contribution of selected groups of pesticides to the sumTU based on *D. magna* for
 583 storm flow water samples and *C. riparius* for sediment samples. The values are grouped according
 584 to the stream category (control and agriculture). The median sumTU values for the respective
 585 samples are given.

		Storm flow water		Suspended sediment		Bed sediment	
		Control	Agriculture	Control	Agriculture	Control	Agriculture
median sumTU		<0.001	0.004	<0.001	0.011	<0.001	0.014
Contemporary pesticides	Herbicide	5.9	6.9	70.4	0.9	22.8	2.3
	Fungicide	1.5	9.5	<0.1	<0.1	<0.1	<0.1
	Pyrethroid	24.3	62.6	<0.1	1.5	8.7	5.2
	Other insecticide	<0.1	0.9	<0.1	<0.1	<0.1	<0.1
Legacy pesticides	Herbicide	<0.1	<0.1	4.0	<0.1	<0.1	0.1
	Fungicide	<0.1	<0.1	25.6	<0.1	1.1	<0.1
	Organochlorine	NA	NA	<0.1	0.2	67.4	0.8
	Organophosphate	42.0	15.4	<0.1	97.4	<0.1	91.6
	Pyrethroid	26.3	2.7	<0.1	<0.1	<0.1	<0.1
	Other insecticide	<0.1	2.0	<0.1	<0.1	<0.1	<0.1

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