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

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ABSTRACT

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

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

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   aqueaous 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 pesticidesconsisted 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 (λ-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 metamitron. 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

the catchment land use (Rasmussen et al., 2013). One stream is located in the Skensved catchment (Fig.

1C), where agriculture represents ca. 99% of the total land use (McKnight et al., 2012).

Although the Hove and Skensved catchments differ in catchment size (and thus number of

tributaries) and degree of physical in-stream (habitat) degradation, they are similar in that the dominant

arable crop types are wheat, barley and canola (visual observations 2010-2012). There is no substantial

input from waste water treatment plants in the investigated first-order streams, but storm water run-off

from the scattered settlements may contribute to stream water quality (McKnight et al., 2012).

Discharge from contaminated sites, and in particular abandoned landfill sites, can include pesticides at

specific locations (Milosevic et al., 2012). All catchments are characterized by low elevations,

clayey/loamy soils, a temperate climate, and an average regional precipitation of 600 mm yr^{-1} (Hansen

et al., 2011). Tile drains, triggered by storm-flow events and wet seasons, are present in agricultural

fields in both catchments.
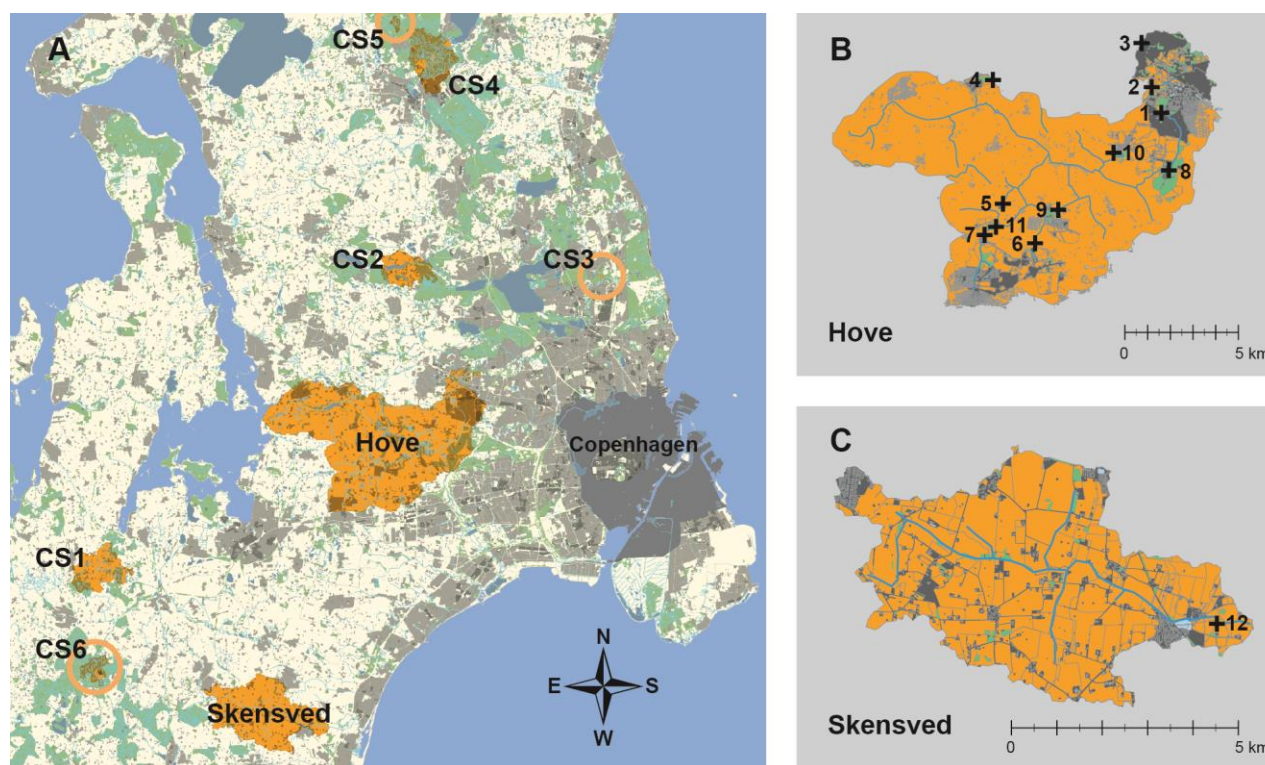
Fig. 1: Location of the study catchments (A) on Sjaelland, Denmark, including their relation to the six least disturbed
control catchments (labelled CS1-6); the (B) Hove and (C) Skensved catchments together with their respective stream
networks. In Fig. (a), green and gray areas indicate natural and urban areas, respectively. In Figs. (B) and (C), green, orange,
light gray and dark gray areas indicate natural areas, cultivated lands, uncultivated lands and residential settlements,
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).

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 mgL^{-1}; University of Hertfordshire (2014)), so will not affect the

171 toxicity calculations (see Section 3.4), and requires an extra analytical protocol.

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 ^{o}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 defendable

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 logTU$\geq$-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; Hyalella azteca*). For the calculation of ∑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∑TU for permitted pesticides and compared them to the average

237 log∑TU for all detected pesticides (*t*-test, α=0.05) for base-flow, storm-flow and the sediment samples.

238 Additionally, we compared the average log∑TU of all detected pesticides in storm-flow samples with

239 the average log∑TU of base-flow samples (*t*-test, α=0.05). The tested data fulfilled the requirements for

240 normality (Shapiro-Wilk) and equal variances (Bartlett's test; α=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.
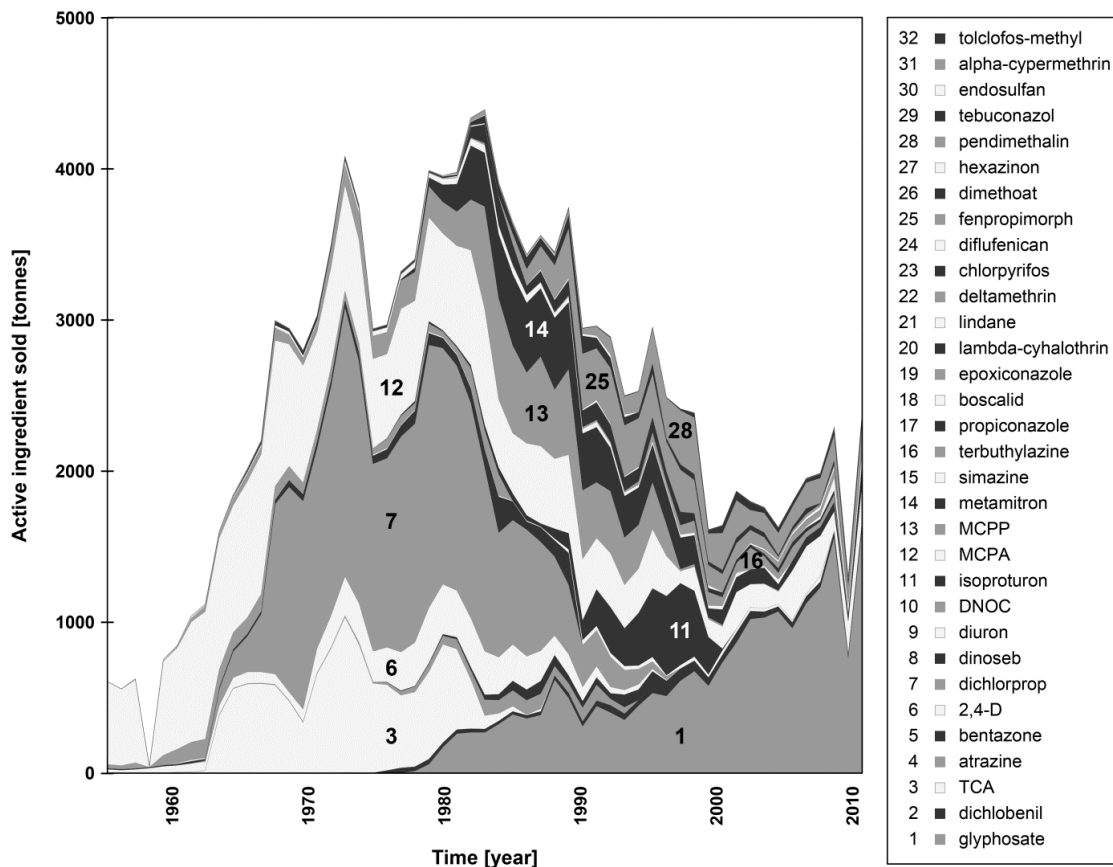
Fig. 2. Sales of detected active ingredients (incl. glyphosate) in Denmark for agricultural use between 1956-2010, shown as stacked shaded areas. Hexachlorobenzene is not present, as it was never authorized for use as a fungicide in Denmark. Adapted from Tuxen et al. (2013).

4.2 Overview of current pesticide findings

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

4.2.1 Dissolved-phase pesticide findings

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

11

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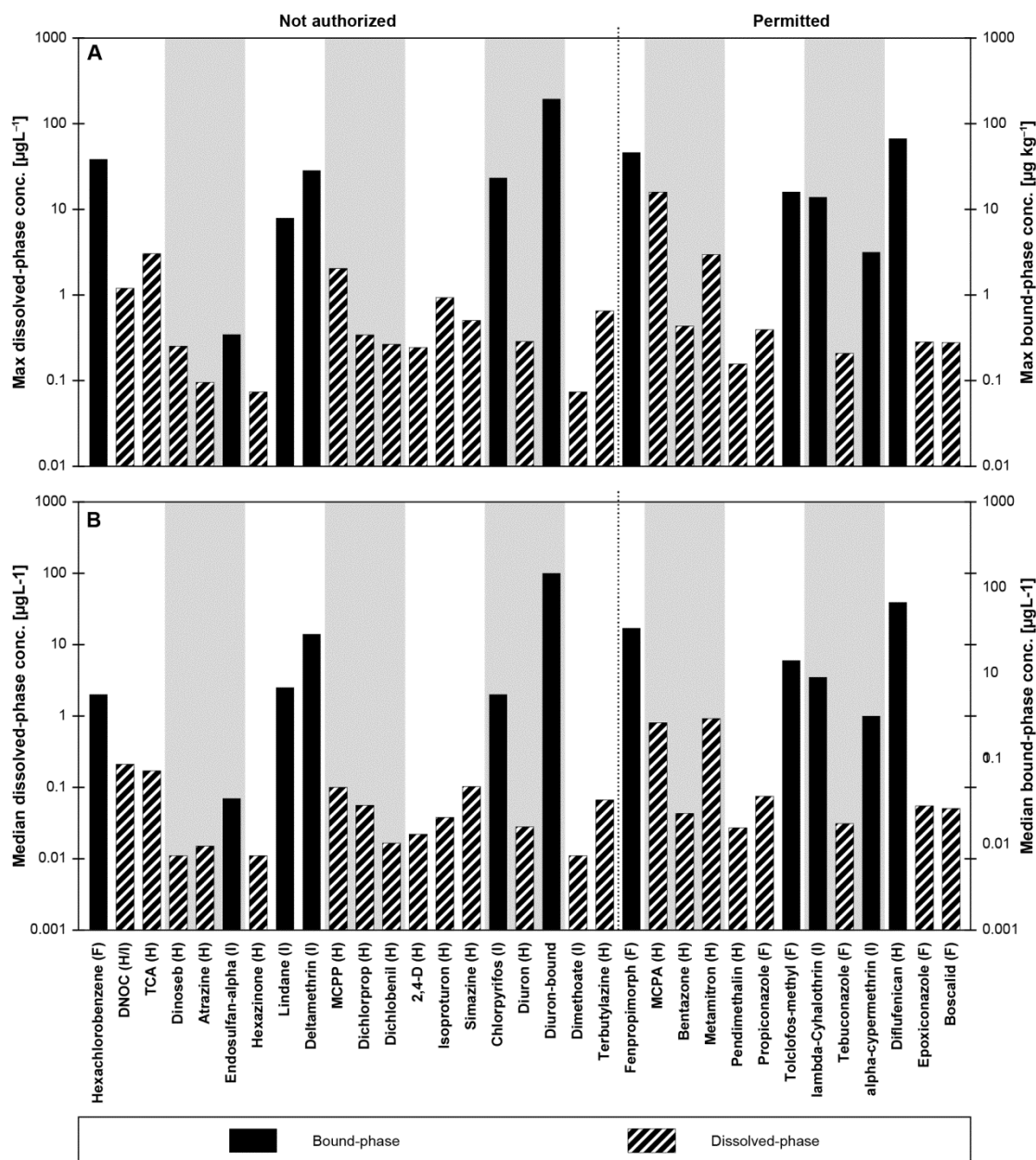
Fig. 3. Overview of (a) maximum and (b) median pesticide concentrations in the water (dissolved phase – striped bars) and associated with sediments (bound-phase – solid bars), for herbicides (H), insecticides (I) and fungicides (F) measured during the field campaign (excluding control sites). Note the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

4.2.2 Pesticides in the sediment-phase

13

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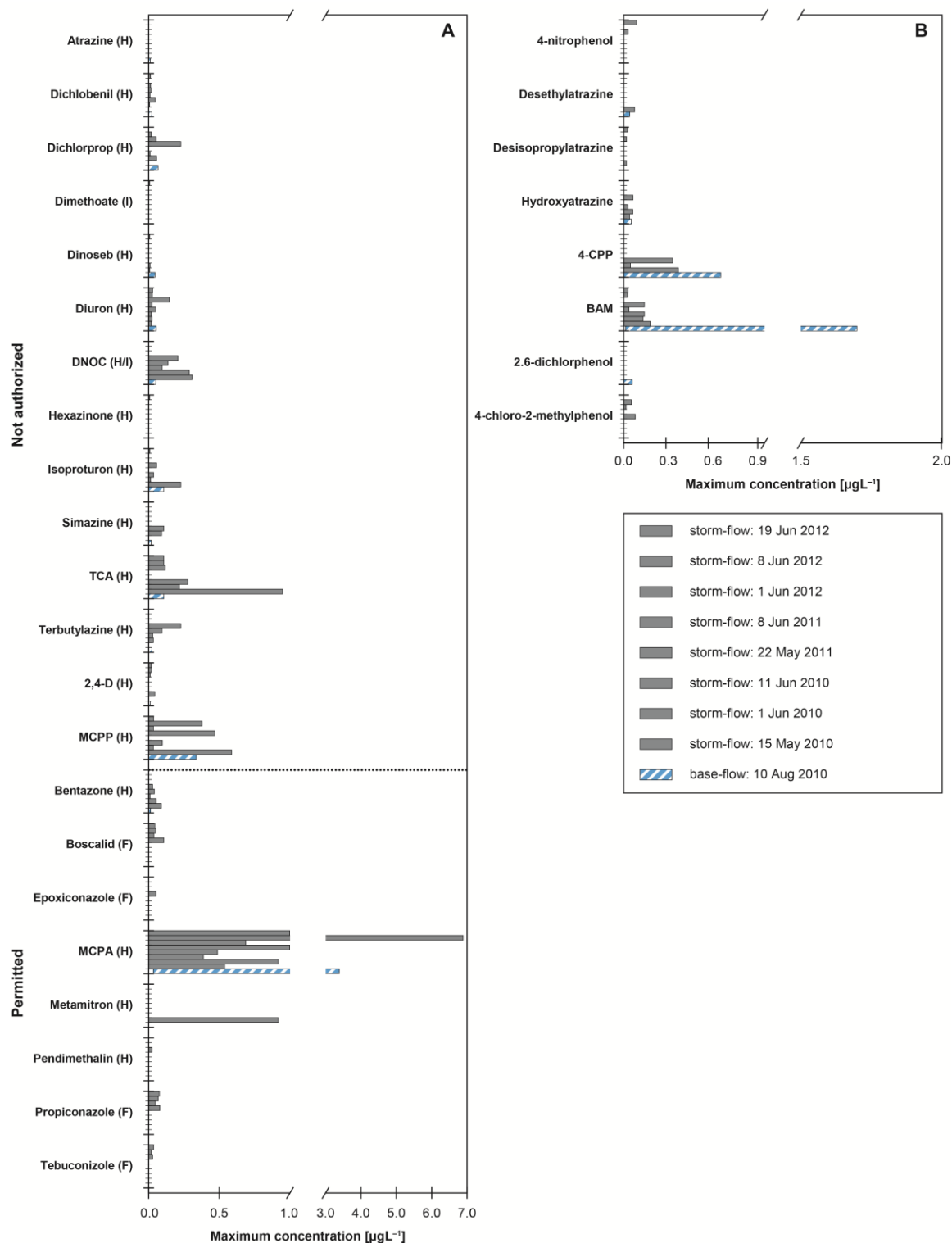
Fig. 4. Overview of dissolved-phase (a) pesticides and (b) metabolites and/or impurities, identified either during the base-flow event (blue-striped bars) or during a storm-flow event (gray-scale bars). Note the dotted black line that marks the transition from not authorized to permitted status according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

353   We can make the strongest case for BAM, dichlobenil, mecoprop (MCPP). 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 MCPP 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 MCPP, these values were close to the value for base-flow.

365   In the case of MCPP, this could indicate that, although we have classified it as not authorized, MCPP-P

366   is still in "restricted" use, although the amounts are low (below 4 metric tonnes, SI Fig. S1, SI Table

367   S1). MCPP 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   (MCPP, 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 μgL^{-1} and 3.4 μgL^{-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 persistant) (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^{-1}]			Median conc. detected per compound [μgL^{-1}]	
	Storm-flow	Base-flow	Groundwater	Storm-flow	Groundwater
DNOC (H)	**0.31**	0.054	0.10	0.21	0.01
TCA (H)	**0.95**	0.11	0.01	0.17	0.01
Dinoseb (H)	0.013	0.048	**0.10**	0.011	0.01
4-nitrophenol	**0.096**	n.d.	n.d.	0.066	n.d.
Atrazine (H)	n.d.	0.015	**0.10**	n.d.	0.01
Desethylatrazine	0.081	0.046	**0.10**	0.081	0.01
Desisoproylatrazine	0.032	n.d.	**0.10**	0.023	0.01

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

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

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

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

Several of the legacy herbicides for which there is weaker evidence for the groundwater pathway (Section 4.3.1) could come in part from the atmosphere, but could also then partly have leached into groundwater and from there entered the stream water. In particular, atrazine, its metabolites, 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).
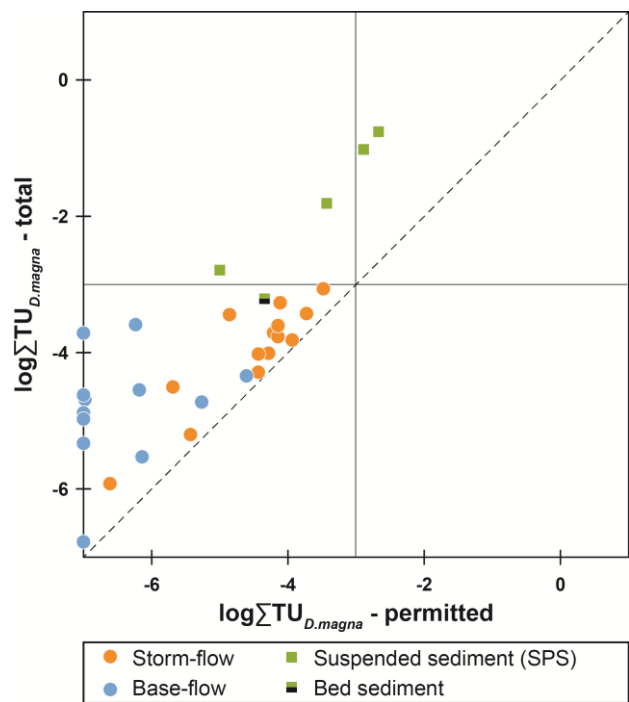
Fig. 5. Log$\sum$TU$_{D.magna}$ for all pesticides detected, compared with the log$\sum$TU$_{D.magna}$ considering only the currently permitted pesticides in Denmark for all pathways analyzed. Solid lines indicate the threshold for acute observed effects on macroinvertebrate community structure in the field (logTU$\geq$-3.0) (Schaefer et al., 2012); dotted line shows 1:1 ratio.

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.

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 (logTU$\geq$-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∑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

5. Implications of pesticide findings in streams

5.1 Historical versus contemporary use

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

Pesticide concentrations in surface water bodies are consistent with trends in current agricultural use (Thurman et al., 1992; Vecchia et al., 2009), particularly for run-off associated with rain events, when only contemporary pesticides are included in the sampling campaign. However, studies of 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 25th (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**

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

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