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Bioactive chemicals in Zürich streams in context of road runoff and tire wear particles

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Cover photo: Spitalerbach at low and high flows. Daniel Olbrich, Swiss Centre for Applied Ecotoxicology

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Summary

Urban waterways can be heavily contaminated with chemicals originating from vehicles and washing from roads. Toward the aim of assessing (1) the condition of Zurich streams and (2) the contribution of stormwater pollutants, we conducted a pilot study of two streams in Zürich. The streams were targeted with different stormwater infrastructure: one with generally little input, the other receiving heavy stormwater input. In two phases, we measured first near the confluences with the receiving river, and a second time in a follow-up campaign with paired with upstream locations. Both sampling events targeted rain events expected to mobilize roadway pollutants. We measured three chemicals as markers of vehicle pollution: DPG, 6PPD-Q, and HMMM, and opportunistically tracked other common water pollutants (caffeine, diuron, diclofenac). This established which streams were impacted by stormwater during the sampling events. We investigated bioactive chemicals in the streams in a non-targeted approach using bioassays coupled to high performance thin layer chromatography (HPTLC). Estrogenic chemicals, bacterial toxicants, and agonists of the aryl hydrocarbon receptor were evaluated.

The highest concentrations of tire marker chemicals were detected in the downstream location of the stream heavily impacted by stormwater. The same samples were the most active for all three bioassay endpoints. A comparison of upstream to downstream, and between two streams with or without direct stormwater inputs shows that stormwater runoff is a source of bioactive chemicals to Zurich waters. Some of the unknown bioactive chemicals match the HPTLC retention factors of chemicals from ground tire tread. This suggests but does not prove contribution of tire chemicals to the bioassay response. While vehicle emissions may be contributing to the pollution, other sources were indicated by specific chemicals. Diuron was detected, which likely washes from building facades, and the toxicity profiles in the HPTLC bioassays detected bioactivity in samples from upstream locations, which should have a low influence from stormwater.

Overall, the chemical pollution was most concentrated in the stormwater receiving stream. This shows the effectiveness of the infrastructure to reduce contamination in open streams. However, this work also highlights that many emerging and unknown substances are being released to the receiving river. Risk from these chemicals after dilution into the receiving water requires further study.

Content

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1 Introduction

Stormwater runoff from paved roads contains pollutants from traffic that could endanger organisms in receiving waters. Chemicals released during the normal use of vehicles include exhaust emissions from the tailpipe and from the abrasion of brakes and tires. Along with known chemicals like combustion byproducts, metals, and chemical additives, are many unknown substances that are impurities of the intentionally added chemicals or form as transformation products of the additives. A prominent example is the recent discovery of N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine-quinone (6PPD-Q), which forms during the ozonation of an antioxidant tire additive, N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD).(1) 6PPD-Q is lethal to certain salmonid species, notably to coho salmon. While coho are not present in Europe, the story of 6PPD-Q highlights the lack of understanding of what, exactly, is entering the environment from roads. To protect environmental and human health from traffic related pollution, we need to work to fully characterize hazards in the complex chemical mixture that enters the environment through stormwater.

Chemicals known to be associated with tires can be applied as markers of road runoff. Along with 6PPD-Q, 1,3-diphenylguanidine (DPG) and (hexa(methoxymethyl)melamine) HMMM have been used.(2) DPG is an ingredient in tire rubber as a vulcanization agent. HMMM is used as an adhesive between layers of tires such as steel cable and belt, textile sheets, and the rubber layers. HMMM is therefore not directly used in the rubber itself but possibly diffuses to the tire tread during the lifetime of a tire. HMMM and DPG are not only used in tires, but other rubber materials. Therefore, each chemical alone is not a perfect indicator of tire wear, but together the three chemicals can indicate vehicle pollution in road runoff. In addition, stormwater from roads may also contain chemicals originating from nearby constructions, notably biocides used in building facades such as diuron.

Beyond chemicals known to be associated with tires are many more chemicals that have not been identified. Peter et al. detected thousands of chemicals in stormwater runoff and in the leachates of several automotive materials.(3) Of these, only a few could be matched to known substances. Because it is time intensive to identify all of the unknown chemicals, we use in vitro bioassays to help detect and prioritize the potentially hazardous substances. These bioassays detect all chemicals that act on a specific receptor or organism, without full knowledge of their structure/identity. There are additional advantages when coupling bioassays to high performance thin layer chromatography (HPTLC). Through chromatographic separation, potentially interfering (e.g. cytotoxic) chemicals are removed from the chemicals of interest.(4) Multiple bioactive chemicals can be detected per sample, displaying a profile of toxicity for each sample. In previous work, we applied HPTLC-bioassays to evaluate the toxicity profiles of chemicals extracted and leached from ground car tire tread.(5)

This report describes preliminary efforts to use HPTLC-bioassays to evaluate the status of streams in the city of Zürich that are impacted to varying degrees by stormwater runoff. In two streams with very different infrastructure, we aimed to use 6PPD-Q, DPG, and HMMM as markers of road runoff to establish which samples were affected by vehicle pollution. Then, we applied HPTLC bioassays for general bacterial toxicity, estrogenicity, and induction of the aryl hydrocarbon receptor. By looking at chemical and toxicity profiles, we aimed to detect and start to characterize the impact of road runoff in the streams of Zurich.

2 Material and Methods

2.1 Sample collection

2.1.1 Sample locations and timing

Two streams in Schwamendingen were targeted as case study streams as they cover a similar urban stretch but have different characteristics and inputs (Figure 1). Schwamendinger Dorfbach begins in the forest on Züriberg, where it is called Brandbach. After a diversion and a hochwasserentlastung at Bocklerstrasse, Schwamendinger Dorfbach is mostly above ground (open) and receives few direct inputs from road runoff drains. It discharges into the Glatt under highway A1L. Spitalerbach divides at the edge of the forest near Irchel Campus into Strickhofbach and Spitalerbach. Spitalerbach divides at the edge of the forest near Irchel Campus into Strickhofbach and Spitalerbach. Both streams flow through ponds on the campus before rejoining as canalized Hochwasserentlastung Spitalerbach after Zoologischer Teich. Another part of Spitalerbach flows into the Allmendsee. The Spitalerbach canal is the focus of this study, so further mentions of Spitalerbach refer to the canalized stream flowing to the Glatt. The source of the water to Spitalerbach varies depending on the water levels and rain conditions. Therefore, the most reliable upstream location for Spitalerbach is below the Zoological Teich at the Tierspital where diversions from the two forest streams converge. At this point, Spitalerbach is underground (closed) and receives road runoff from the nearby streets. Near the outflow, the water passes through a mineral oil separator (Mineraloelabscheider), which should remove buoyant components of the stream. Finally, it discharges into the Glatt about 100 meters downstream of Schwamendinger Dorfbach.

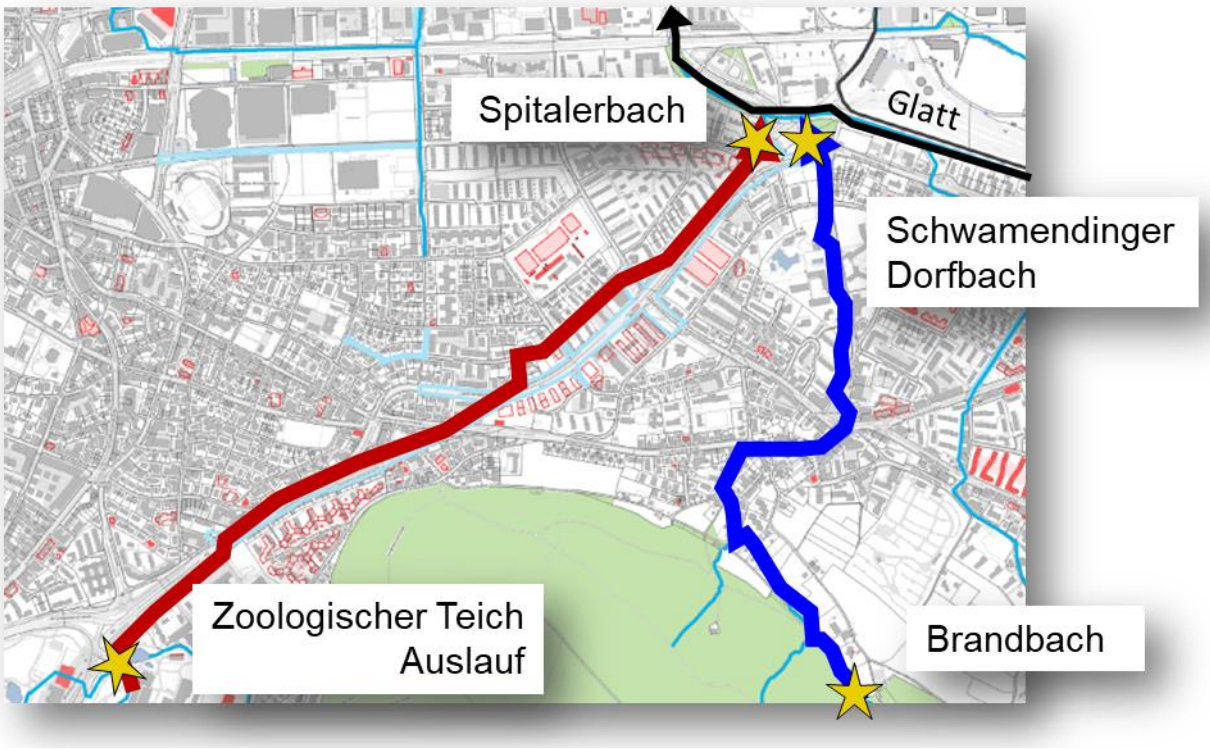

Figure 1. Map of studied area. Two streams of study are traced in red (Spitalerbach) and blue (Brandbach, Schwamendinger Dorfbach). The receiving river (Glatt) is traced in black. Arrows show the direction of flow. Sample locations are labelled with yellow stars.

Sampling was conducted in two phases: a preliminary sampling in November 2024 and slightly extended sampling in May 2025. The samples collected in November 2024 were partly a trial to

determine if a chemical road runoff signature, and any bioactivity, could be detected. Locations at Schwamendinger Dorfbach and Spitalerbach near the confluence with the Glatt were selected. Additional samples were collected from the Glatt next to a separate discharge pipe just downstream of Schwamendinger Dorfbach. This outfall pipe drains stormwater runoff from streets near Schwamendinger Dorfbach. A location on the Glatt upstream of the other locations was also selected.

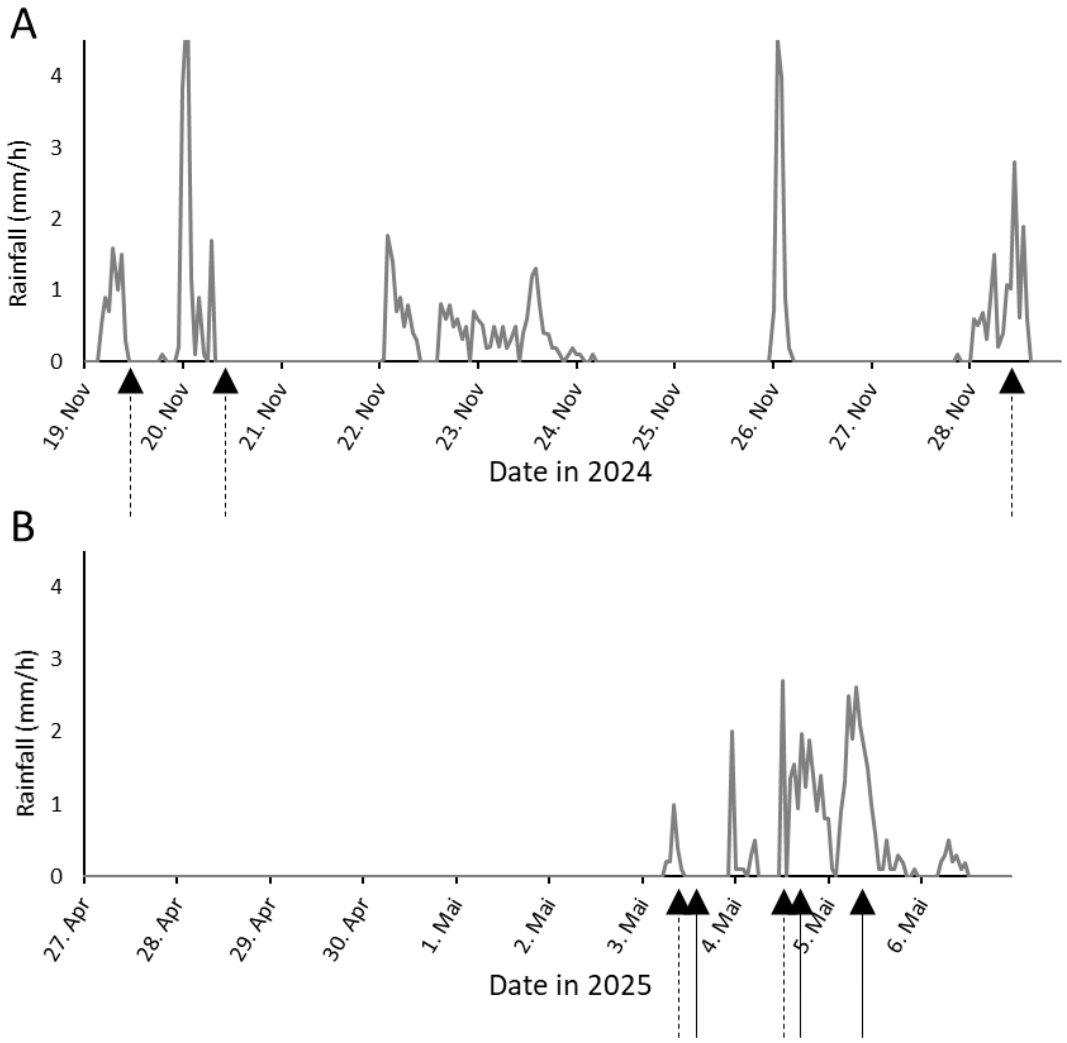

Figure 2. Sampling times during two campaigns overlaid on hourly rainfall (mm/h) at a Schwamendingen measurement station. (A) November 2024 sampling campaign. (B) Sampling times during May 2025 campaign. A week or more of dry weather preceded the sampling. Collection of grab samples of the stream waters was interspersed during the rain events. Dashed arrows indicate samples that were only analysed for chemical markers of road runoff and with HPTLC-bioassays. Solid arrows indicate samples that were analysed for additional parameters by Labor Veritas.

A sampling campaign was conducted in May 2025 to generate more refined time collection over a single rain event and evaluate spatial differences – or the effect of the urban area. In this sampling campaign, we focused on Schwamendinger Dorfbach and Spitalerbach and added upstream locations on the Brandbach and below the Zoologischer Teich.

2.1.2 Water sampling

Water samples were collected in 2 L glass Schott bottles. A clean glass beaker attached to a sampling pole was dipped into the flowing stream. The collected water was poured into a bottle until it contained about 1.2 L. To sample in the manhole at the Zoologischer Teich, a metal bucket was lowered on a cord into the shaft. The first water retrieved with the bucket was used to rinse the bucket and then discarded. The subsequent dips with the bucket were first poured into a clean glass beaker before transferring the water into a 2 L Schott bottle. The samples were transported to the Ecotox Centre laboratory and stored at 4°C until processing. The storage time was a maximum of two days, when all of the samples had been collected and could be processed in one batch. All glassware was pre-rinsed three times with acetone. A clean beaker was used for each sampling location. A field blank was collected near Schwamendinger Dorfbach and Spitalerbach by transferring nanopure water from the laboratory to a 2 L Schott bottle using sampling equipment and further processed in the laboratory in parallel with the other samples.

Water samples were extracted and concentrated 1000x with solid phase extraction (SPE). The water was filtered through 0.22 µm glass fiber filter. About 45 mL of filtered water was transferred to a falcon tube and directly frozen at -20°C. About 1 L of the remaining water was adjusted to pH 7.2 ± 0.1. The water was passed over a conditioned SPE cartridge Strata-XL (Phenomonex), then dried. The cartridge was eluted with methanol and acetone into a glass vial. This extract was filtered and concentrated to reach a final concentration factor of 1000 and stored at -20°C.

In parallel to water samples collected for LC-MS/MS and HPTLC analysis, water samples were collected in separate glass bottles for testing by Labor Veritas. These samples were analysed for total suspended solids (TSS), total phosphorus, total nitrogen, chemical oxygen demand, and for various pesticides and their metabolites.

2.2 Analysis by the Ecotox Centre

2.2.1 LC-MS/MS

Water samples were analysed for three tire markers with HPLC-MS/MS (Agilent). 1,3-diphenylguanidine (DPG), N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-Q), and hexamethoxymethylmelamamine (HMMM) are tire-associated chemicals that may indicate the presence of tire particles in road runoff.(6) These three chemicals were added to an existing method for herbicides and pharmaceutical chemicals, which were then evaluated as a secondary priority. Water sample extracts were injected (1-50 µL) on a C18 column. Detection occurred on the triple quadrupole mass spectrometer with electrospray ionization in positive mode. Internal standards labelled with deuterium or carbon-13 were added to each sample before injection. DPG-d10, 6PPD-Q-d5, and carbamazepine-d8 were used for DPG, 6PPD-Q, and HMMM, respectively. As part of the trial analyses with samples from November 2024, analysis with and without SPE was performed. Because DPG-d10 and an internal standard for HMMM were not yet available, this report shows results from direct analysis of water samples from November 2024. Chemical results for May 2025 samples are from SPE extracts and internal standards. This allows better comparison to bioassay results, which were performed only on the extracts.

2.2.2 HPTLC-bioassays

Water samples were analysed for bioactivity after separation on HPTLC according to previous publications.(5, 7) Up to 50 µL of each sample was applied to a silica gel plate and separated with multiple chromatographic development. The plates were dried and sprayed with genetically modified bacteria or yeast for the detection of bioactive chemicals. Bioactive chemicals then inhibit intrinsic luminescence of the bacteria, or induce the yeast to produce an enzyme. A substrate is then added that the enzyme cleaves to a colored or fluorescent chemical. Therefore, the presence

of a dark zone in the bacterial toxicity test, a blue fluorescent color in the yeast estrogen screen, or an pink or orange color in the yeast AhR screen indicate bioactive chemicals on the HPTLC plate.

3 Results and Discussion

3.1 General Observations

Visual observation of the water during the sampling indicated that the water level at Schwamendinger Dorfbach does not respond dramatically to rain events. However, Spitalerbach quickly changes flow and becomes turbid (See cover image).

3.2 LC-MS/MS

Preliminary Sampling Campaign November 2024

DPG, 6PPD-Q, and HMMM were detected in multiple samples (Figure 3). In some samples, notably Spitalerbach, the concentrations of these three chemicals seem to co-vary. Taken as markers of tire wear, these three chemicals together indicate a signature of road runoff. Ruff et al. 2025 detected DPG, HMMM, and 6PPD-Q in the inlet of a Strassenwasser Behandelungsanlage (SABA) up to 4, 5, and 0.2 µg/L, respectively.(2) This is a water body that probably is close in character to Spitalerbach, which receives a large portion of its flow from street drains. The concentrations were indeed in a similar range in the current study, up to about 8, 6, and 0.5 ug/L for DPG, HMMM, and 6PPD-Q, respectively (Figure 5 and supplemental Table A1).

In a case where the concentration of only one chemical is elevated (e.g. DPG in Schwamendinger Dorfbach on Nov. 28), we cannot infer an influence of road runoff. While DPG is an additive in tire rubber, it is also used in other products. This could indicate a different source of DPG to Schwamendinger Dorfbach than to Spitalerbach on Nov. 28, 2025. Additional chemicals were measured incidentally, in that they were already in the chemical analysis method, and not intended as markers of road runoff. Caffeine follows a similar trend as DPG, 6PPD-Q, and HMMM, and therefore might also be related to road runoff. Caffeine was observed in a previous study to be linked to urban density.(8) Diclofenac was detected just once in the Glatt, independent of the road runoff markers. This diclofenac detection exceeded the environmental quality standard (EQS) of 0.05 µg/L.(9) Diuron was detected in one sample at the limit of quantification.

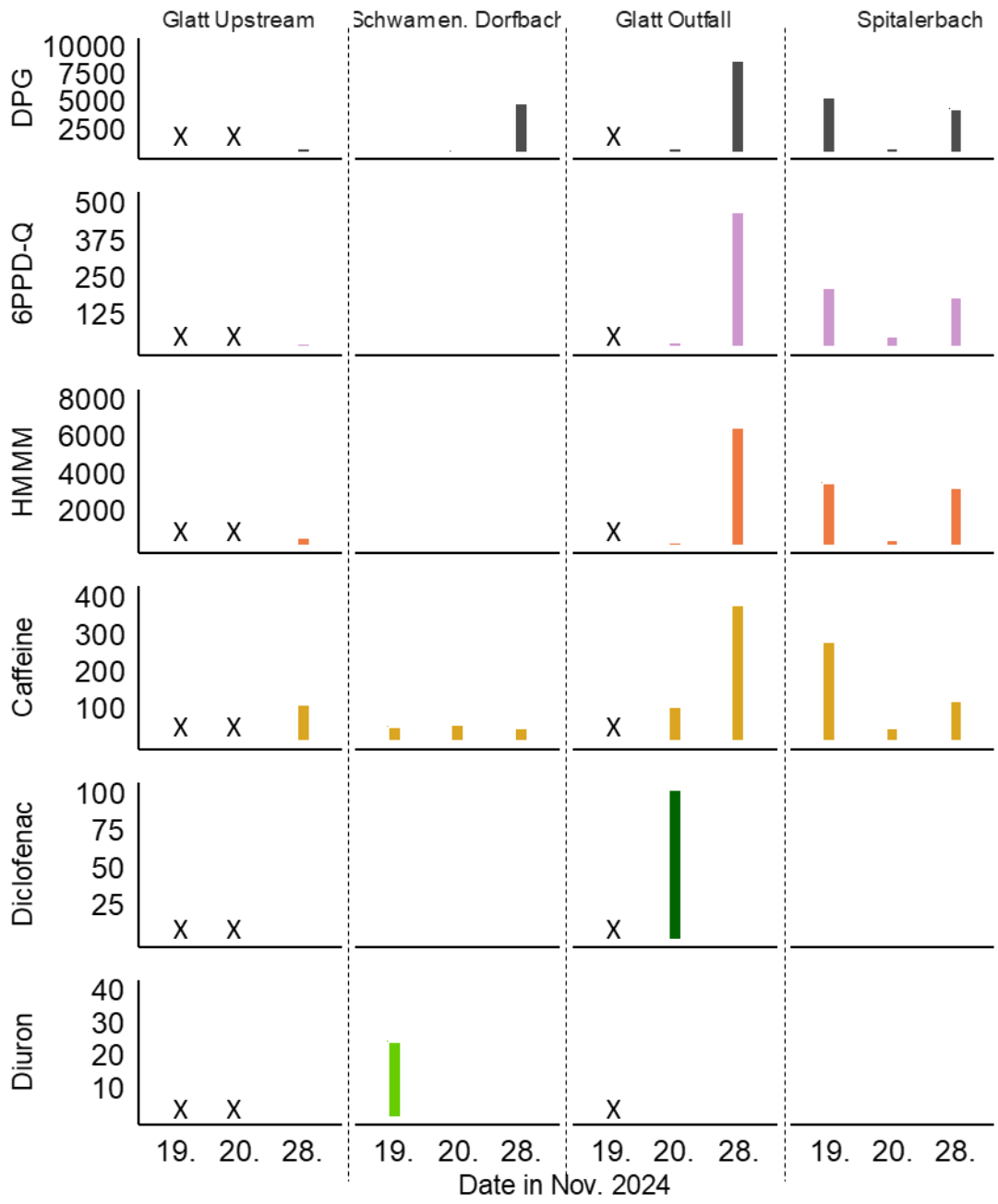

Figure 3. Tire marker chemicals, caffeine, diclofenac, and diuron (ng/L) detected in samples collected in November 2024. Samples marked with “X” were not collected.

Sampling Campaign May 2025

In the second sampling campaign, again Spitalerbach consistently had the highest chemical concentrations (Figure 4 and supplemental Table A2). No dry period sample was collected in this campaign because the rain arrived earlier than forecast. See supplemental Figure A1 for the chemicals plotted next to the rainfall in May 2025.

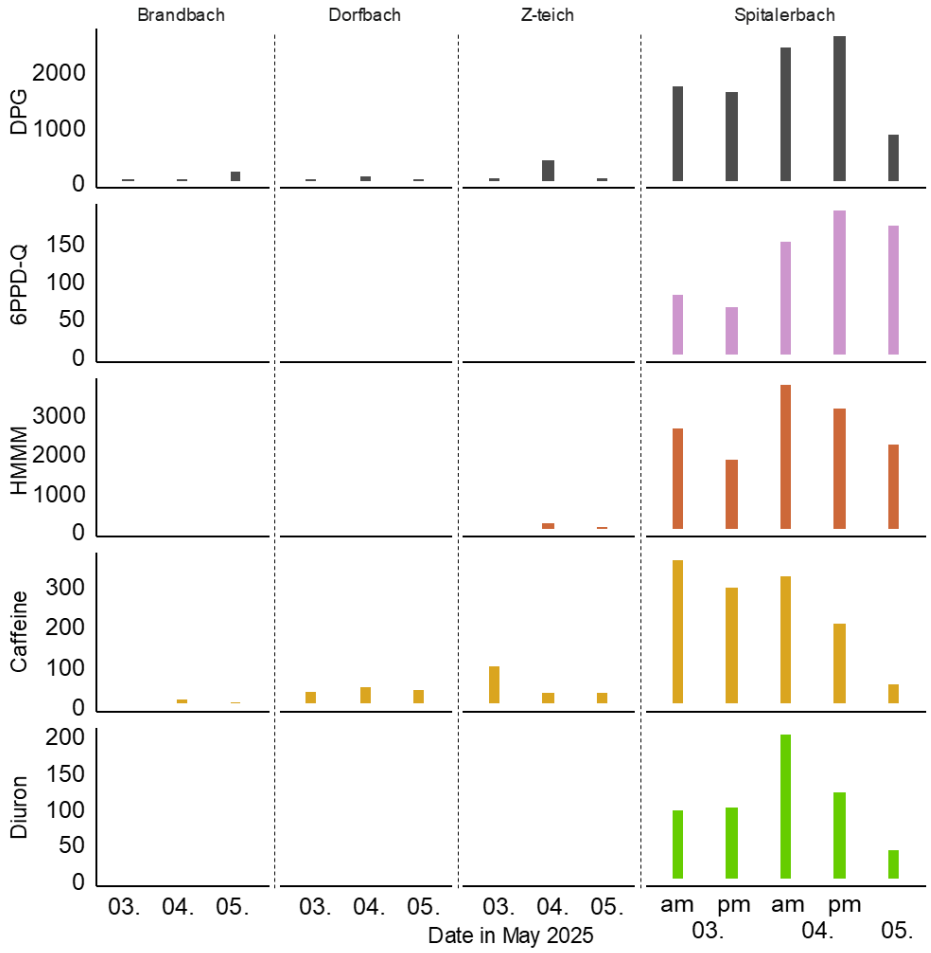

Figure 4. Tire marker chemicals, caffeine, and diuron (ng/L) detected in grab water samples from May 2025. Two samples were collected at Spitalerbach on May 3 and May 4 in the morning (am) and afternoon (pm). See Figure 2 for more details. Diclofenac was not detected in any sample. Z-teich: Zoologischer Teich Auslauf.

Total suspended solids (TSS) were measured by Labor Veritas as contracted by ERZ. The results of TSS compared to rainfall in Schwamendigen are shown in supplemental Figure A2. The TSS measurements follow a similar trend as can be visually seen for particles collected on filters before SPE of the water samples at the Ecotox Centre (supplemental Figure A3). The highest TSS concentration was observed in Spitalerbach on 04. May. This matches the result for tire chemicals. However, more frequent sampling would be needed to firmly establish a link between TSS and specific chemicals.

Diuron was detected almost exclusively in May 2025 in all samples from Spitalerbach. This chemical happened to be the only overlapping analyte with Labor Veritas. The results between the two laboratories agreed well (supplemental Figure A4). Differences in the measurements of these time-matched water samples can be expected because they were not homogenized before sending to the laboratories. Diuron is not allowed to be used in agriculture or gardens, but it is applied as a biocide in façade coatings.(10) These detections might indicate a specific connection to road

runoff that is seasonal. The detected concentrations of diuron reached levels between the acute and chronic EQS (0.25 and 0.07 µg/L, respectively).(11) Although the chronic EQS was exceeded in Spitalerbach, this stream discharges immediately into the Glatt, where it will be diluted. These chemical results allowed us to establish which samples have a signature of road runoff which helps to interpret which signals from the bioassays relate to road runoff.

3.3 HPTLC-bioassays

General statements about the bioassays

Bioactive chemicals can be seen in many of the water samples. Bacterial toxicity appears as dark zones on a light grey background. Estrogenicity is a blue fluorescent response. AhR activity is a pink color on a yellow background. Positive controls indicate that the bioassays were functioning properly. A field blank negative control assessed the presence of contamination from the sample collection and extraction processes. Some artifacts of the assays can be seem in some tracks. The bacterial toxicity assay has a constant background effect of dark lines across the plates at about retention factor (Rf) of 0.2. Finally, we do see some estrogenicity in the field blanks, indicating contamination occurred sometime during the sample collection or processing.

Preliminary Sampling Campaign November 2024

The initial sampling event in November 2024 confirmed that we would be able to detect bioactive chemicals in Spitalerbach and Schwamendinger Dorfbach. All four samples were active in each bioassay (Figure 5). We also found that we would be able to detect differences between samples. For example, Spitalerbach during a rain event had consistently higher bioassay responses (more HPTLC bands with greater intensity) than any other sample. This corresponds to higher concentrations of road runoff chemicals on 28.11 than 20.11. Samples without chemical signature of road runoff were also bioactive in every assay, indicating non-road runoff sources. Estrogen hormones are common contaminants in surface water(12) so for the second sampling campaign in May 2025, the HPTLC separation was tailored to better compare to positive control chemicals estriol, 17β-estradiol, 17α-ethinylestradiol, estrone. Likewise, the chromatography for the AhR bioassay was updated to better separate the positive controls, and possibly the unknown chemicals too.

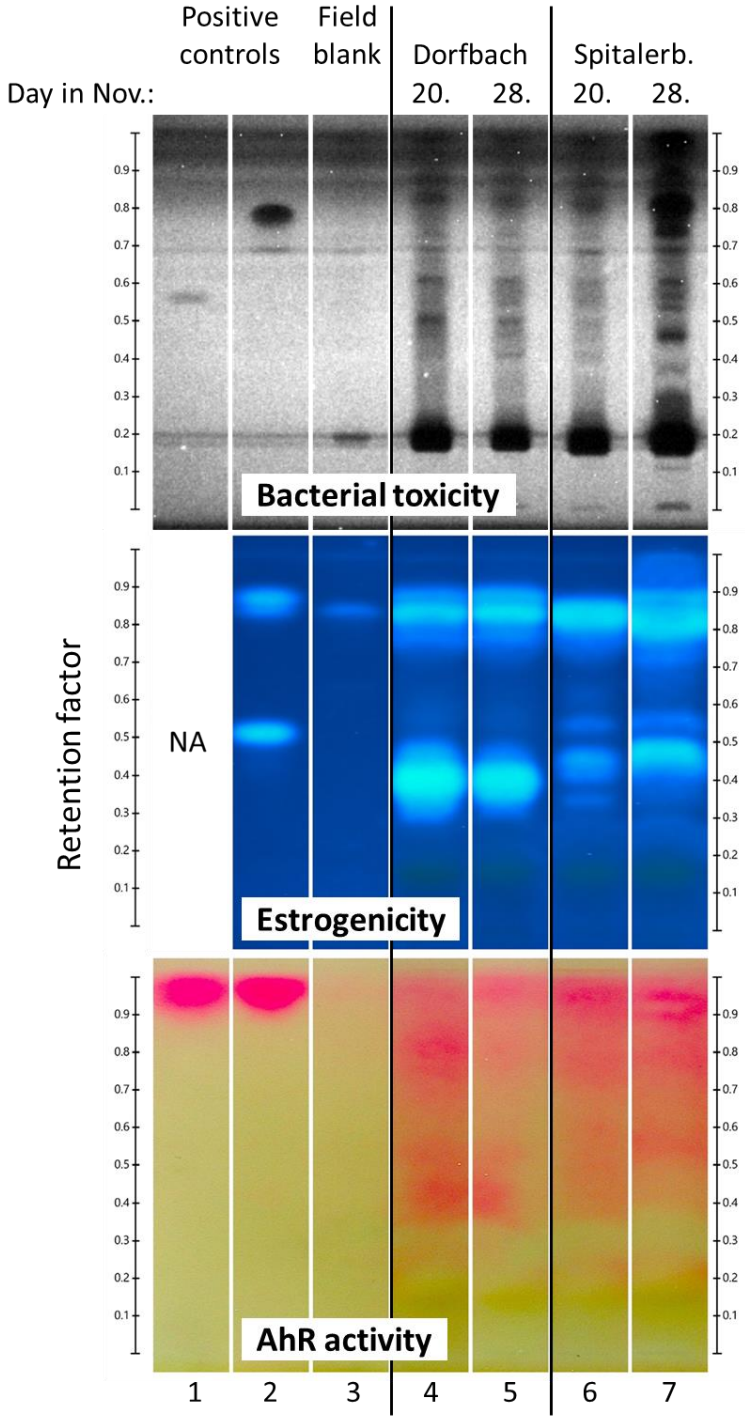

Figure 5. Bacterial toxicity, estrogenicity, and AhR activity of water samples collected in November 2024. Positive controls for each test were as follows. Bacterial toxicity: caffeine 630 ng (track 1), 1,2-dichlorophenol 63 ng (track 2). Estrogenicity: mixture of estriol 100 pg, 17β-estradiol 1 pg, 17α-ethinylestradiol 1 pg, estrone 10 pg (track 2). AhR activity: β-naphthoflavone 5 ng (track 1), benzo[a]pyrene 50 ng (track 2).

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Sampling Campaign May 2025

Spatial and temporal comparison

Spitalerbach samples had the highest activity in all three bioassays (Figure 6). In other words, there were more bands at greater intensity than the other samples. The downstream samples (Schwamendinger Dorfabach and Spitalerbach) were always more active than their respective upstream samples.

The profile of AhR activity for Z-teich and Spitalerbach on May 5 seems different from the other days, possibly indicating a different source of contaminants that is consistent upstream and downstream in this waterway. Bacterial toxicity was also lower on May 5 than the other days. Some chemicals (see DPG and caffeine) detected with LC-MS/MS are also lower than the other sampling times at Spitalerbach (Table 2). Estrogenicity does not seem to follow this pattern.

Possible contribution of tire particles to bioactivity

Tires are a potential source of chemicals to urban streams. Extracts of cryogenically milled tire tread (CMTT) that were generated and tested in previous work (5) were evaluated in parallel to the Zürich water samples. Some bioactive zones detected in CMTT match the retention factors of bioactive zones in the water samples. Many more bioactive substances do not match the HPTLC result of CMTT.

Comparison to positive controls

Positive control chemicals applied in the bioassays are used to check that the test organisms are functioning properly, and do not necessarily indicate substances that might be detected in the samples. However, it is plausible that known estrogens are present in water samples. Some of the bioactive bands in the water samples line up with the positive controls estriol, estradiol, and estrone. Chemical analysis for these estrogenic substances would support whether they are contributing to the bioactivity in Zürich streams.

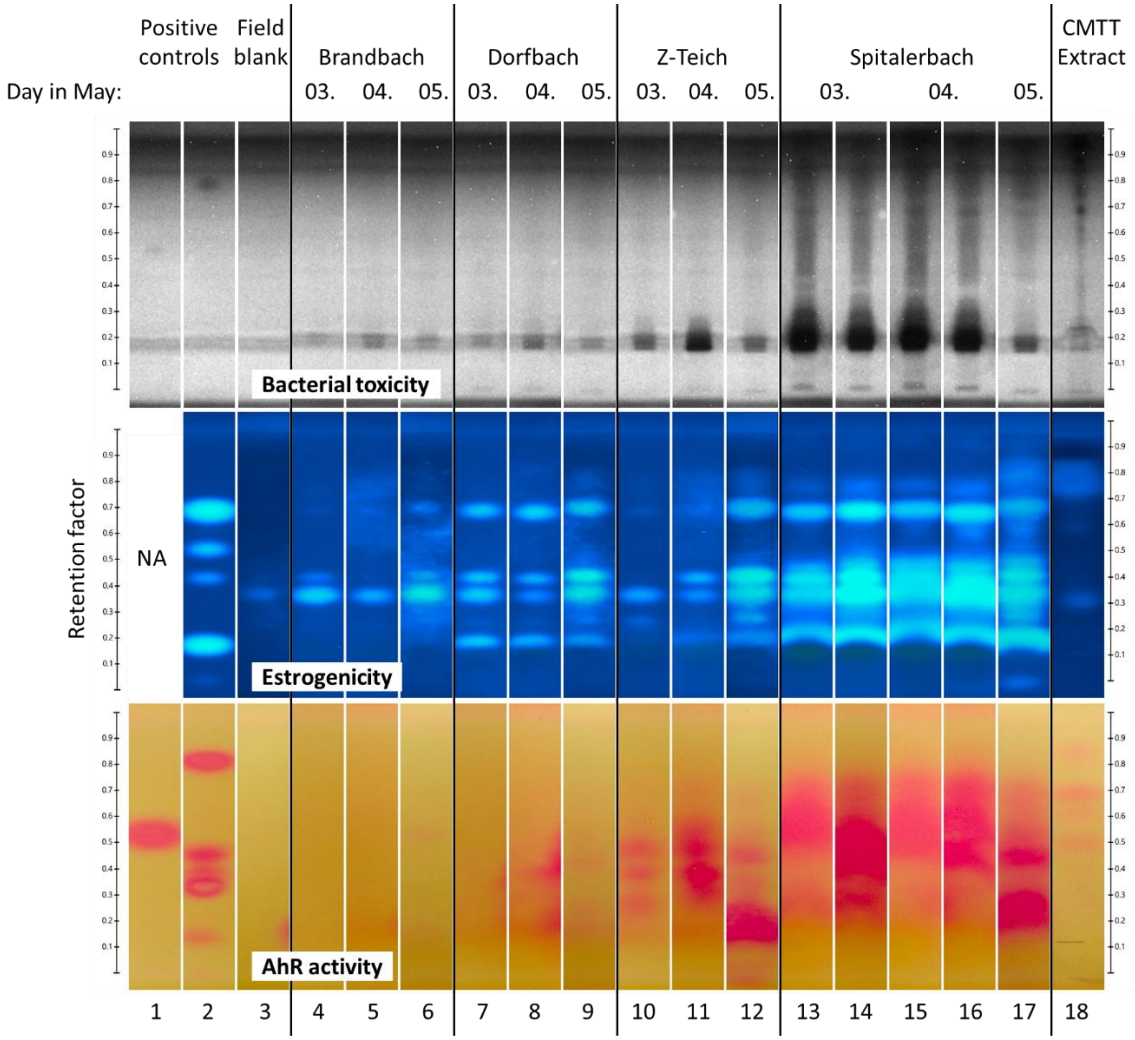

Figure 6. Bioactivity of water samples from sampling campaign in May 2025.The samples were processed with SPE to 1000 times concentration of the water. Samples applied at 10 µL for bacterial toxicity and 15 µL for estrogenicity and aryl hydrocarbon receptor (AhR) activity. CMTT extract applied at 15 µL. Some AhR activity encroaches from neighboring tracks, seen as activity on the edges of the tracks, and not centered like the positive controls. An example is track 8, Schwamendinger Dorfbach on 04. May. Applying 50 µL of sample often overloaded the HPTLC plates, creating distorted bands. Therefore, samples tested at lower doses are presented in Figure 6. Positive controls for each test were as follows. Bacterial toxicity: caffeine 630 ng (track 1), 1,2-dichlorophenol 63 ng (track 2). Estrogenicity: mixture of (from bottom) estriol 100 pg, 17β-estradiol 1 pg, 17α-ethinylestradiol 1 pg, estrone 10 pg (track 2). AhR activity: β-naphthoflavone 5 ng (track 1), benzo[a]pyrene 50 ng (track 2).

Overall, the number and intensity of bioactive bands in the HPTLC-bioassays were greatest in the samples from Spitalerbach. This matches the chemical results that the greatest concentrations were detected in Spitalerbach. Therefore, road runoff either brings these unknown bioactive chemicals into the streams, or otherwise correlates with unknown sources. While tire wear is possibly contributing to the total load of bioactive chemicals, there are chemicals that cannot be explained by tires.

However, the samples without a road runoff chemical signature also contained bioactive chemicals. Even the upstream locations are not pristine. They are near paved or gravel roads, which

carry foot and vehicle traffic. Contaminants from the traffic or other sources may settle in from the air or wash directly into the streams.

Additional results by Labor Veritas

The full results for the chemical parameters measured by Labor Veritas are provided in Table A3. Figures A5-A13 show each of the parameters except for TSS and Diuron, which presented in Figures A2 and A4. Typically, samples from Spitalerbach had the highest concentrations of the sum parameters and individual pesticides. This is consistent with the generally higher load of the road runoff markers measured by the Ecotox Centre, and the biological results on HPTLC. However, a few exceptions included mecoprop, which was detected at the highest concentration in the outlet of the Zoologischer Teich, the upstream location of Spitalerbach. DEET was detected at the highest concentrations in the Brandbach and Dorfbach.

These results may, for example, indicate different sources of chemicals that do not relate to widespread contaminants in road runoff. As discussed further in the limitations section, broader and more frequent sampling would help elucidate the sources and dynamics of these chemicals.

Limitations

This study is not able to cover all of the chemicals flowing in the streams during the studied rain events. Pulses of chemicals that peak in concentration in the water might not have been captured by grab sampling. Future work should consider automated sampling that continuously collects and composites water over time. This would still require solid-phase extraction which is selective and does not capture/enrich all compounds present in a sample. An alternative technique, passive sampling, would also capture chemicals during concentration peaks, but concentrate chemicals that preferentially bind to the passive sampler material and thus captures a different ratio of chemicals compared to environmental concentrations.

We compared the HPTLC-bioassay results of stream samples and CMTT, laboratory generated tire particles that were made from fresh tires and extracted with methanol and dichloromethane. The CMTT are not fully representative of tire particles in the environment because tires and tire particles experience aging processes (e.g. UV, heat, biodegradation) in the environment. Therefore, the profile of bioactive chemicals in CMTT may be different from tire-associated chemicals that enter streams.

While the HPTLC-bioassays detected chemical hazards in this study, we do not know if they pose a risk to wildlife or humans. To evaluate risk, we would need to identify the unknown substances, quantify them, and determine their potency. Furthermore, for the three quantified vehicle associated compounds, no quality criteria exist. However, diclofenac and diuron both exceeded their EQS for chronic toxicity. Therefore, there was risk from these two chemicals at least once.

The two streams in this study are quite small. Dorfbach is an open stream and is habitat for biofilm, invertebrate animals, and other wildlife. Spitalerbach is not ecologically relevant because it mostly flows in channels underground. Both of these streams flow into the Glatt River and combine with the outflow of Lake Greifensee, effluent from wastewater treatment plants, and other road runoff sources. The Glatt is home to even more wildlife including fishes, and is a well-visited fishing location for local anglers (https://www.fvz1883.ch/reviere/glatt-209). The dilution factor and the influence of other sources is unknown. However, the risk from diuron is almost certainly lower in the Glatt compared to Spitalerbach, depending on other sources to the Glatt. Further work is needed to investigate the hazard and risk of road runoff to ecology or human health in the Glatt and other rivers. A first step could be to collect more samples from the Glatt.

4 Conclusions

Chemicals associated with non-exhaust vehicle emissions were detected at highest concentrations in Spitalerbach. Schwamendinger Dorfbach and the upstream locations for both streams always had lower concentrations of these chemicals. This was expected because Spitalerbach directly receives drainage from roads in Schwamendingen. The concentrations vary with time as they are expected to be due to rain events. This allowed us to establish 6PPD-Q, DPG, and HMMM as indicators of road runoff in the samples collected for this study.

The greatest bioactivity was also observed in samples from Spitalerbach. Some of this bioactivity is likely due to the stormwater runoff. There are different bioactive chemicals (HPTLC bands) in Spitalerbach than in the samples without a chemical signature of road runoff. These samples also have some overlapping bioactivity with chemicals from ground car tire tread. Comparing these toxicity profiles suggests a contribution from tires but more work is needed to explicitly link the bioactive responses to tire wear or other components of road runoff.

With chemical and toxicity loads concentrated in Spitalerbach, it seems that infrastructure targeting road runoff reduces inputs to the small, open streams like Schwamendinger Dorfbach. However, risk from contaminants, including vehicle-related chemicals, in the receiving water requires further study.

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6 Indexes

6.1 List of Figures

6.2 List of Tables

Appendix 1 Supplemental tables and figures

Table A1. Water concentrations (ng/L) of selected chemicals analysed in samples collected in November 2024. BLOQ: below limit of quantitation.

	Target chemical (LOQ in ng/L)						
	Date in 2024, time	DPG (9)	6PPD-Q (17)	HMMM (7)	Caffeine (22)	Diclofenac (73)	Diuron (23)
Glatt up-stream	28.11. 11:00	540	17	350	99	*BLOQ*	*BLOQ*
Schwamen. Dorfbach	19.11. 12:00	78	*BLOQ*	15	42	*BLOQ*	23
	20.11. 11:00	90	*BLOQ*	12	46	*BLOQ*	*BLOQ*
	28.11. 11:00	4500	*BLOQ*	79	37	*BLOQ*	*BLOQ*
Glatt outfall	20.11. 11:00	530	22	190	94	100	*BLOQ*
	28.11. 11:00	8400	460	6400	370	*BLOQ*	*BLOQ*
Spitalerbach	19.11. 12:00	5000	200	3400	270	*BLOQ*	*BLOQ*
	20.11. 11:00	540	39	320	34	*BLOQ*	*BLOQ*
	28.11. 11:00	4100	170	3100	110	*BLOQ*	*BLOQ*
Lab blank		*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*

Table A2. Water concentrations (ng/L) of selected chemicals analysed in samples collected in May 2025. BLOQ: below limit of quantitation.

	Target chemical (LOQ in ng/L)						
	Date in 2025, time	DPG (3)	6PPD-Q (8)	HMMM (4)	Caffeine (3)	Diclofenac (18)	Diuron (7)
Brandbach	03.05. 12:50	22	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*
	04.05. 16:45	6.6	*BLOQ*	*BLOQ*	11	*BLOQ*	*BLOQ*
	05.05. 08:40	160	*BLOQ*	*BLOQ*	3.5	*BLOQ*	*BLOQ*
Schwamen. Dorfbach	03.05. 13:45	23	*BLOQ*	*BLOQ*	31	*BLOQ*	*BLOQ*
	04.05. 15:50	81	*BLOQ*	24	42	*BLOQ*	*BLOQ*
	05.05. 09:15	28	*BLOQ*	25	34	*BLOQ*	*BLOQ*
Zoologischer Teich, Auslauf	03.05. 12:00	42	*BLOQ*	11	95	*BLOQ*	*BLOQ*
	04.05. 16:20	370	*BLOQ*	150	28	*BLOQ*	*BLOQ*
	05.05. 08:20	44	*BLOQ*	47	27	*BLOQ*	*BLOQ*
Spitalerbach	03.05. 10:10	1700	80	2600	360	*BLOQ*	95
	03.05. 13:30	1600	63	1800	290	*BLOQ*	100
	04.05. 13:10	2400	150	3700	320	*BLOQ*	200
	04.05. 15:30	2600	190	3100	200	*BLOQ*	120
	05.05. 09:10	830	170	2200	50	*BLOQ*	39
Field blank		9.7	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*
SPE blank		*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*	*BLOQ*

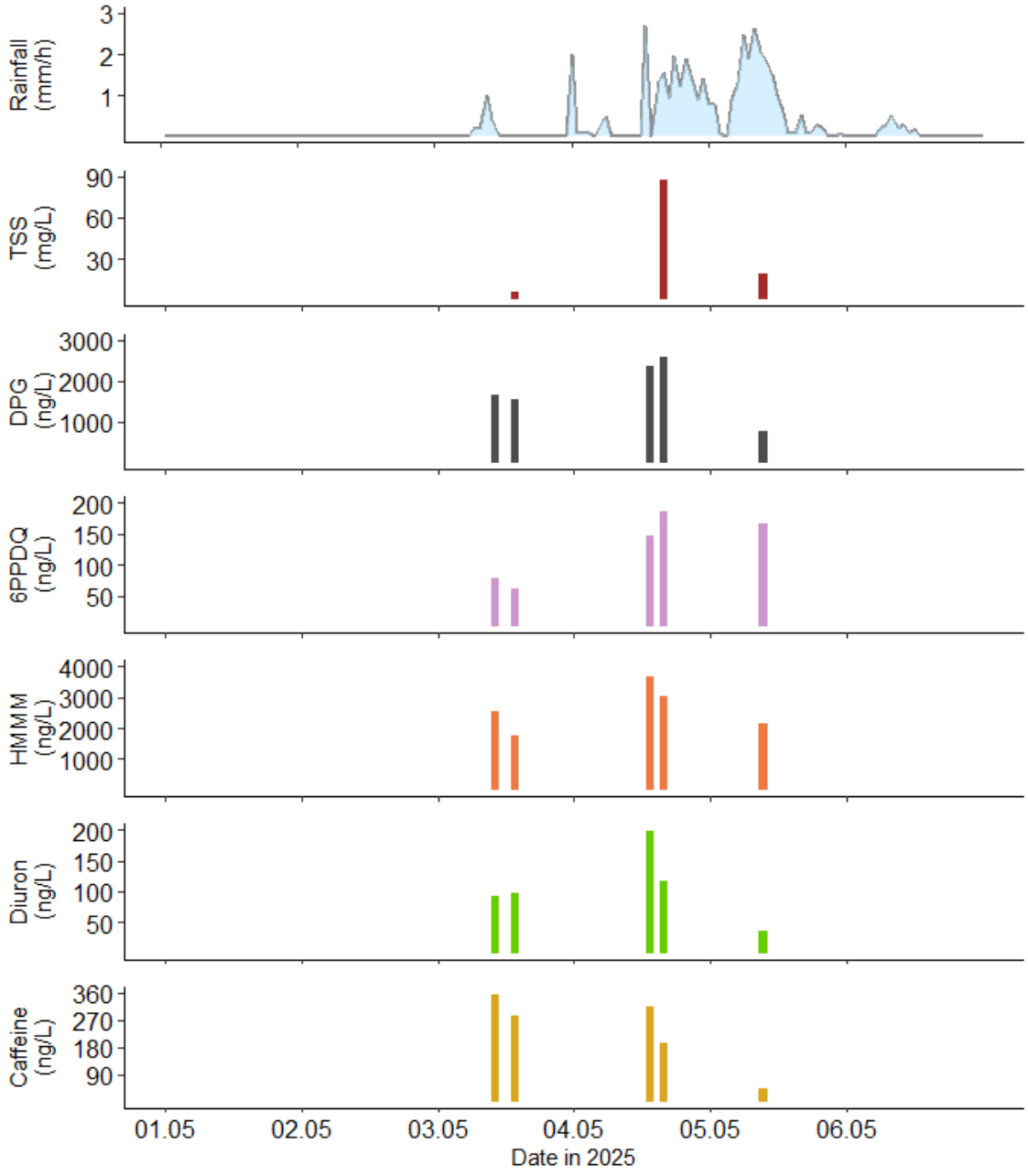


Figure A1. Selected chemical concentrations and TSS in Spitalerbach compared to rainfall in Schwamendingen. Only 3 samples collected for TSS. Chemicals measured by Ecotox Centre, TSS measured by Labor Veritas.

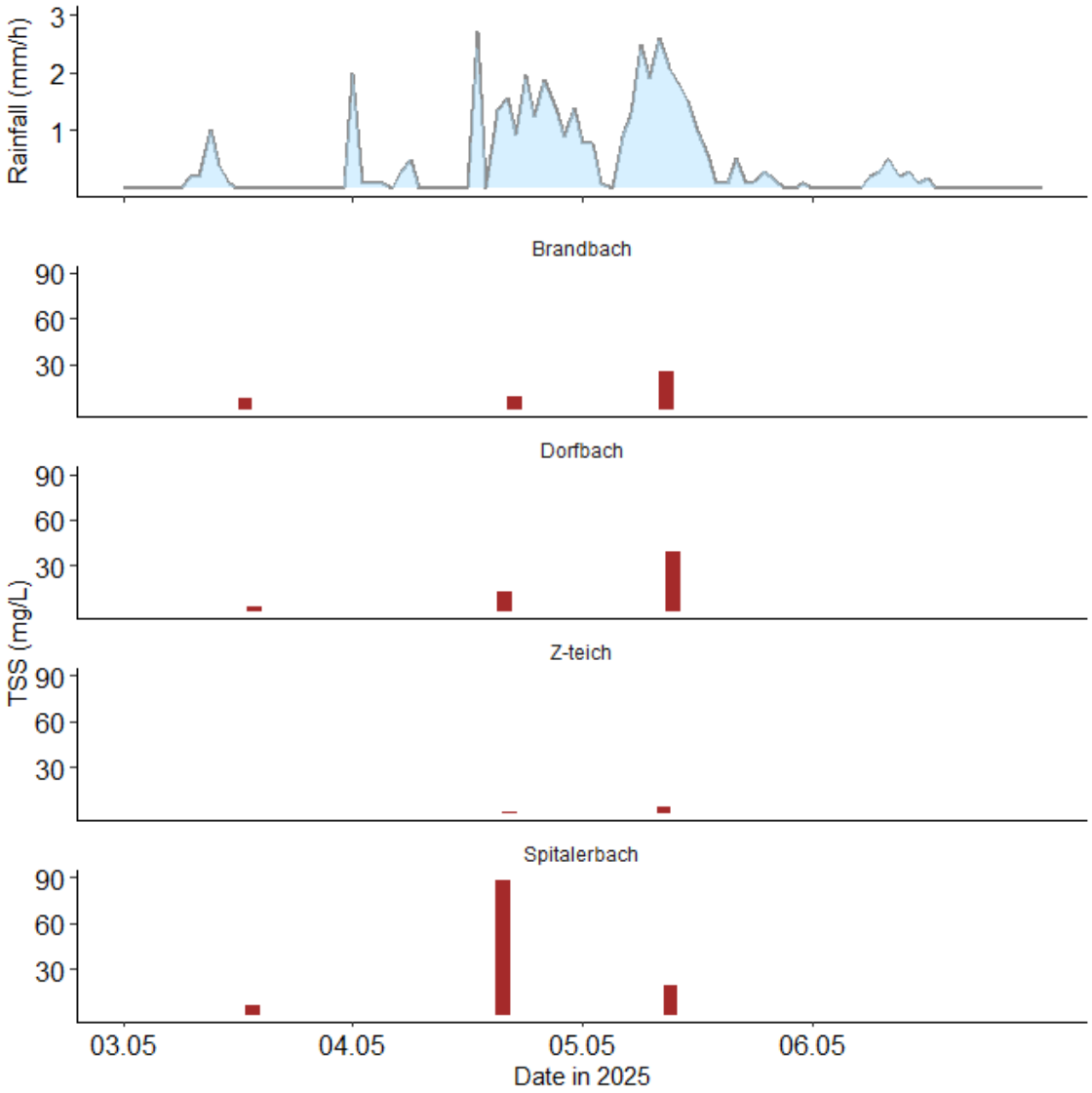


Figure A2. Total suspended solids (TSS) compared to rainfall in Schwamendingen. TSS was measured at three timepoints at four locations. Bars are offset between sites, which shows the time required to travel between sites.

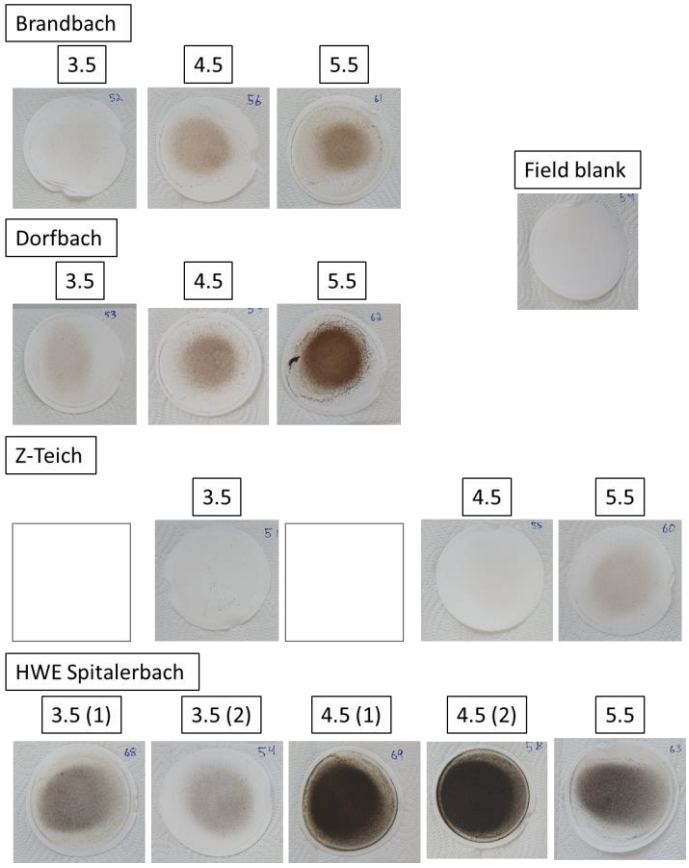


Figure A3. Particles filtered out of water before solid-phase extraction of samples collected in May 2025. Samples collected from Z-Teich and Spitalerbach are aligned to correspond to the same nominal time points. Samples from Spitalerbach "3.5 (1)" and "4.5 (1)" were not analysed for TSS by Labor Veritas.

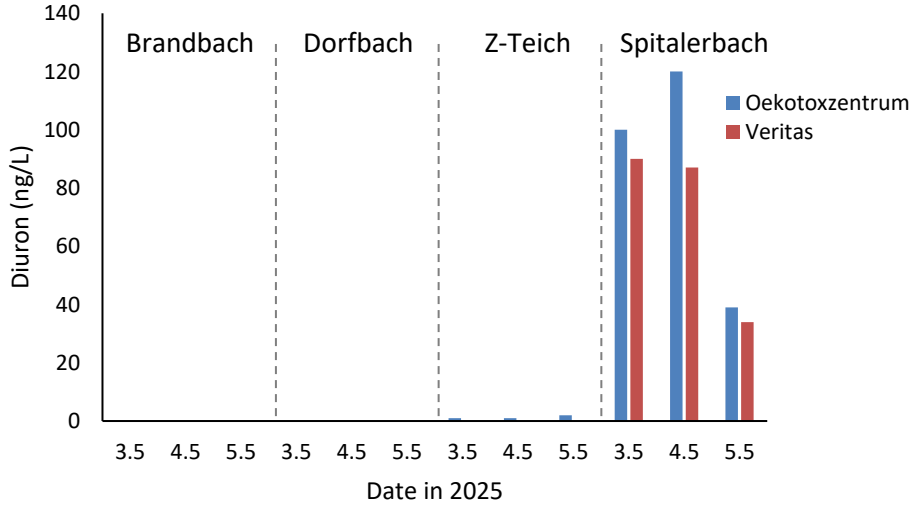


Figure A4. Interlab comparison of results for Diuron. Diuron was the only detected chemical that was measured by both the Ecotox Centre (Oekotoxzentrum) and Labor Veritas. The samples were collected at the same time in May 2025 but were not mixed together before sending to the laboratories.

Table A3. Parameters reported by Labor Veritas, which correspond to figures AX-AX. TSS: total suspended solids. BLOQ: below limit of quantitation

		Target Parameter (LOQ)										
	Date in 2025, time	TSS (2 mg/L)	Gesamt-phosphor (0.003 mg/L)	Gesamt-stickstoff (0.1 mg/L)	Chem. Sauer-stoffbedarf (CSB) (2 mg/L)	2,6-Dichlorbenzamid (0.01 µg/L)	Carbendazim (0.01 µg/L)	DEET (0.01 µg/L)	Desethyl-atrazin (0.01 µg/L)	Dichlorprop (0.01 µg/L)	Diuron (0.01 µg/L)	Mecoprop (0.025 µg/L)
Brandbach	03.05. 12:50	9	0.005	1.1	BLOQ	BLOQ	BLOQ	0	BLOQ	BLOQ	BLOQ	BLOQ
	04.05. 16:45	10	0.014	1.2	BLOQ	BLOQ	BLOQ	0.117	BLOQ	BLOQ	BLOQ	BLOQ
	05.05. 08:40	26	0.022	1.2	4	BLOQ	BLOQ	0.012	BLOQ	BLOQ	BLOQ	BLOQ
Dorfbach	03.05. 13:45	4	0.016	1.1	BLOQ	BLOQ	BLOQ	0.053	BLOQ	BLOQ	BLOQ	BLOQ
	04.05. 15:50	14	0.023	1.2	2	BLOQ	BLOQ	0.044	BLOQ	BLOQ	BLOQ	BLOQ
	05.05. 09:15	40	0.051	1.3	BLOQ	BLOQ	BLOQ	0.135	BLOQ	BLOQ	BLOQ	BLOQ
Z-teich	03.05. 12:00	BLOQ	0.019	1	BLOQ	BLOQ	BLOQ	0.031	BLOQ	BLOQ	BLOQ	0.063
	04.05. 16:20	2	0.02	2.1	10	BLOQ	BLOQ	0.036	BLOQ	BLOQ	BLOQ	0.989
	05.05. 08:20	5	0.021	0.8	4	BLOQ	BLOQ	0.029	BLOQ	BLOQ	BLOQ	0.234
Spitalerbach	03.05. 13:30	7	0.095	3	21	0.012	0.063	0.057	0.013	0.076	0.09	0.026
	04.05. 15:30	89	0.227	2.2	60	0	0.046	0.041	0	0.094	0.087	0.236
	05.05. 09:10	20	0.06	1.1	16	0	0.051	0.021	0	0.035	0.034	0.068

Figures A5-13. Labor Veritas results for various parameters.

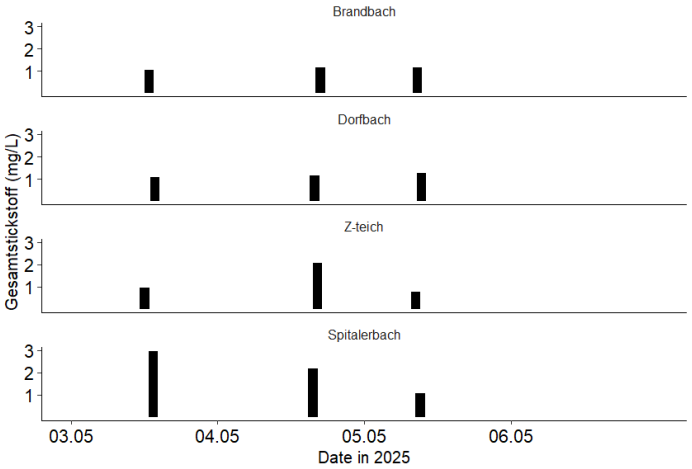


Figure A5. Total nitrogen

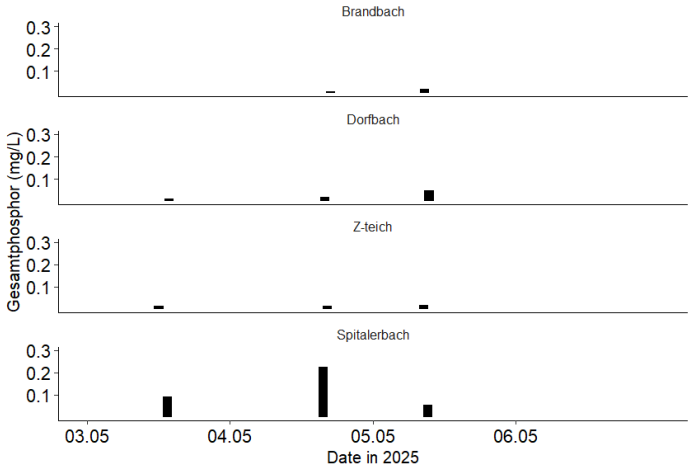
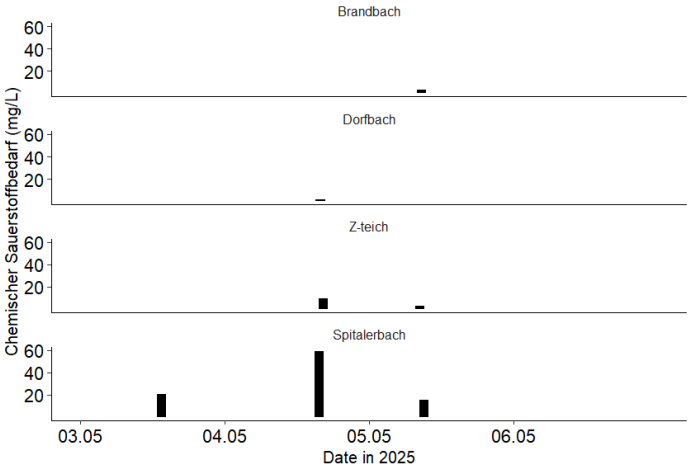


Figure A6. Total phosphorus



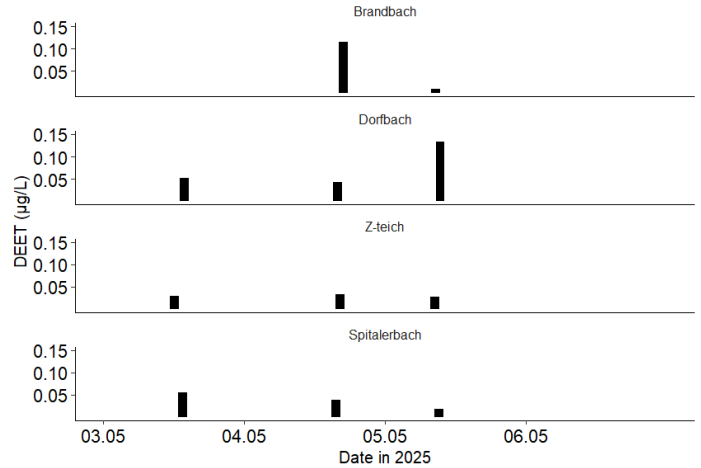


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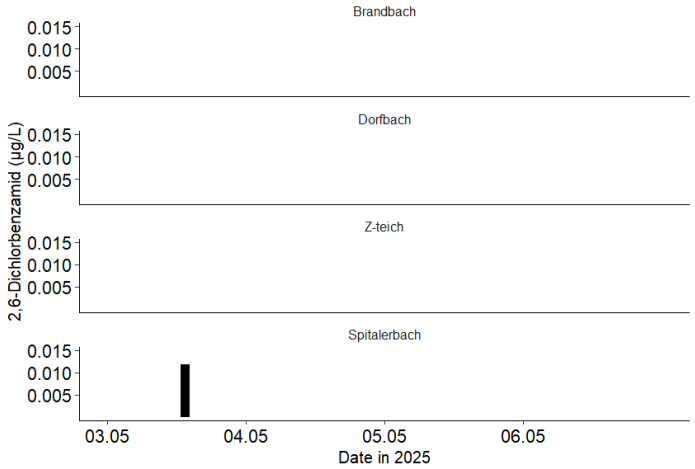
Figure A7. Chemical oxygen demand

Figure A8. DEET



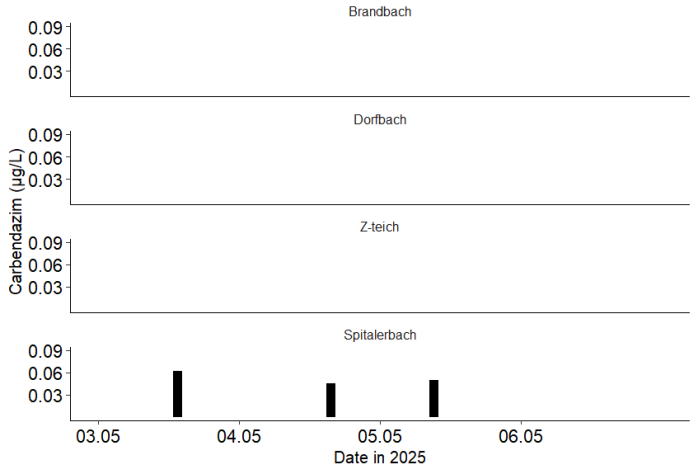
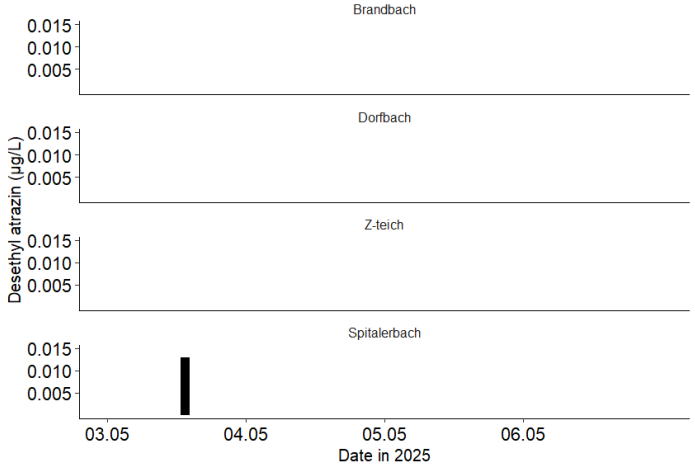


Figure A9. 2,6-Dichlorbenzamide

Figure A10. Carbendazim



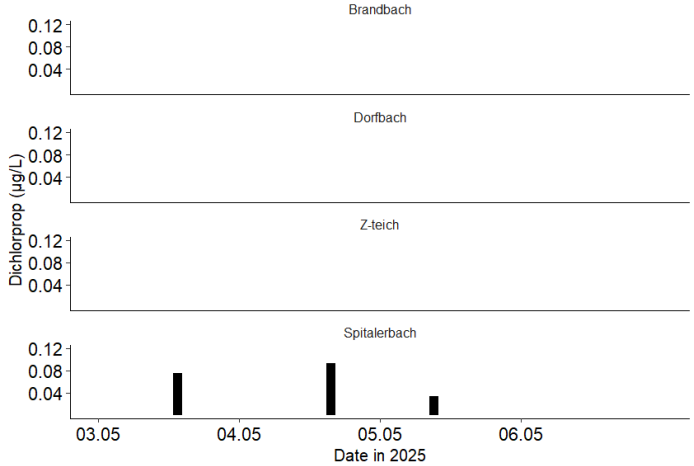


Figure A11.Desethyl-atrazine

Figure A12. Dichlorprop

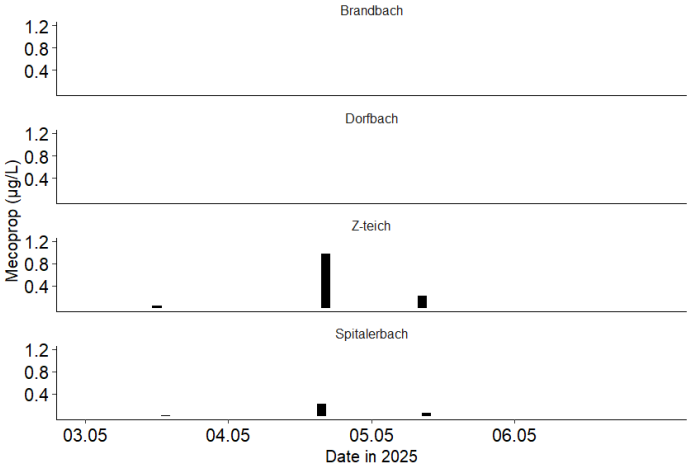


Figure A13. Mecoprop