Inclusion of Pesticide Transformation Products Is Key to Estimating Pesticide Exposures and Effects in Small U.S. Streams

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ABSTRACT: Improved analytical methods can quantify hundreds of pesticide transformation products (TPs), but understanding of TP occurrence and potential toxicity in aquatic ecosystems remains limited. We quantified 108 parent pesticides and 116 TPs in more than 3,700 samples from 442 small streams in mostly urban basins across five major regions of the United States. TPs were detected nearly as frequently as parents (90 and 95% of streams, respectively); 102 TPs were detected at least once and 28 were detected in >20% samples in at least one region—TPs of 9 herbicides, 2 fungicides (chlorothalonil and thiophanate-methyl), and 1 insecticide (flupyradritol) were the most frequently detected. TPs occurred commonly during baseflow conditions, indicating chronic environmental TP exposures to aquatic organisms and the likely importance of groundwater as a TP source. Hazard quotients based on acute aquatic-life benchmarks for invertebrates and nonvascular plants and vertebrate-centric molecular endpoints (sublethal effects) quantify the range of the potential contribution of TPs to environmental risk and highlight several TP exposure–response data gaps. A precautionary approach using equimolar substitution of parent benchmarks or endpoints for missing TP benchmarks indicates that potential aquatic effects of pesticide TPs could be underestimated by an order of magnitude or more.

INTRODUCTION

Pesticide degradation is often considered a beneficial attribute that can reduce threats posed by pesticide use to wildlife and people. The “disappearance” of a pesticide active ingredient (hereafter referred to as “parent”) in the environment, however, often is the result of biological and chemical degradation (transformation) that creates one or more transformation products (TPs). Biota may have greater exposure to TPs than to many parents, as TPs tend to be more mobile and more persistent in hydrologic systems than the parent compound. TP occurrence and potential toxicity in aquatic ecosystems remains limited. We quantified 108 parent pesticides and 116 TPs in more than 3,700 samples from 442 small streams in mostly urban basins across five major regions of the United States. TPs were detected nearly as frequently as parents (90 and 95% of streams, respectively); 102 TPs were detected at least once and 28 were detected in >20% samples in at least one region—TPs of 9 herbicides, 2 fungicides (chlorothalonil and thiophanate-methyl), and 1 insecticide (flupyradritol) were the most frequently detected. TPs occurred commonly during baseflow conditions, indicating chronic environmental TP exposures to aquatic organisms and the likely importance of groundwater as a TP source. Hazard quotients based on acute aquatic-life benchmarks for invertebrates and nonvascular plants and vertebrate-centric molecular endpoints (sublethal effects) quantify the range of the potential contribution of TPs to environmental risk and highlight several TP exposure–response data gaps. A precautionary approach using equimolar substitution of parent benchmarks or endpoints for missing TP benchmarks indicates that potential aquatic effects of pesticide TPs could be underestimated by an order of magnitude or more.
toxicologically relevant. Historically, the toxicological relevance of some TPs has been recognized only 20 to 30 years after commercial introduction of the parent. Measurement of TPs—known or hypothesized to exist—has been hampered by inadequate analytical capabilities and insufficient reference materials. The greater solubility and polarity and lesser volatility of TPs than parents make TPs less amenable to historical methods based on gas chromatography. Initial field studies (e.g., refs 2 and 34) measured 10 or fewer TPs, but that number has continued to increase with improved analytical methods. Liquid chromatography/tandem mass spectrometry analytical instruments now provide increased sensitivity and selectivity that allows for the determination of parents and their more polar TPs at environmentally relevant concentrations.

The U.S. Geological Survey (USGS), a pioneer in the measurement of pesticide TPs, evolved from measuring 8 TPs in surface water and groundwater in the 1990s to measuring 40 TPs in the 2000s. More recently, target methods, including those of the USGS, have expanded to measure more than 100 TPs. Newer target and suspect-screening methods measure hundreds to more than a thousand TPs, although their application in field studies has been limited to a relatively small number of samples. Despite these analytical advances, research regarding TP exposure and toxicological effects of TPs in stream communities continues to be the exception rather than the rule.

Here we present results of an extensive investigation of pesticide parents (n = 108) and TPs (n = 116) in 442 small streams across five major regions of the United States with basins that span a range of land uses. We test the hypotheses that TPs in small U.S. streams, especially in urban areas, are more widespread and diverse than previously known, that TPs in small U.S. streams, especially in urban areas, are more widespread and diverse than previously known, that groundwater is an important source of TPs to streams, and that the potential toxicity of TPs is underestimated. We use a groundwater is an important source of TPs to streams, and that the potential toxicity of TPs is underestimated. We use a groundwebapps.usgs.gov/rsqa/ to quantify the potential contribution of a large suite (>100) of TPs to potential instream biological effects at this scale, and the first to evaluate sublethal effects of TPs using molecular endpoints.

**METHODS**

**Site Selection and Study Design.** The USGS Regional Stream Quality Assessment sampled from 76 to 100 small (wadeable) streams in each of five regions of the United States (Figure 1; Supporting Information Figure S1A–E; https://webapps.usgs.gov/rsqa/#!/), about three-quarters of which are headwater streams (i.e., stream order 1–3) (Table S1). The five regions (Midwest, Southeast, Pacific Northwest, Northeast, and Coastal California) were selected to represent diverse natural climatic and geographic settings across the United States, but all contained extensive agricultural and/or urban development. Sampling-site basins within each region span gradients of agricultural and urban land use; Developed land in Midwest basins was largely agricultural and developed land in basins in the other four regions was largely urban (Table S1). One region was assessed each year (2013–2017) during the spring and early summer growing season; samples for analysis of water quality were collected weekly for 4–12 weeks, with the exception of 11 reference sites in the Northeast that were sampled once. Basin boundaries and site characteristics are available for download.

**Sample Collection and Analytical Methods.** Weekly discrete water samples were collected following USGS protocols. In brief, samples were collected using depth- and width-integrating methods in which subsamples were collected at 10 increments across the stream and composited into a single sample. Thus, one sample represents a single date and time. From this composited sample, 20-mL subsamples were filtered (0.7-μm pore-size glass fiber disk filter) into 40-ml amber glass vials, stored on ice, and shipped on ice within 1–2 days of collection to the USGS National Water Quality Laboratory (Denver, CO). A broad-spectrum direct-aqueous-injection LC/MS-MS method was used to quantitate concentrations of 108 parent pesticides and 116 TPs in water: 57 herbicide parent compounds and 66 herbicide TPs, 40 insecticide parent compounds and 47 insecticide TPs, and 11 fungicide parent compounds and 3 fungicide TPs. A list of analytes and analyte-specific information are provided in Table S2. This list largely comprises current-use pesticides; of the 108 parent pesticides analyzed, 6 had been canceled at the start of this study in 2013, 2 more were canceled by 2016 (Table S2), and no additional cancellations occurred during 2016–2020.

**Quality Control.** Laboratory quality control included reagent spike and blank samples and addition of surrogate compounds to every sample. Quality control for environmental samples consisted of field blanks, replicates, and spikes. Spike recoveries for 2013–2015 are published and interpreted. Results of quality-control samples are discussed and summarized in the Supporting Information text and Tables S3–S6.

**Data Handling and Analysis.** Analytes, their method detection levels, and laboratory reporting levels are reported in Table S2. Some censored data (i.e., reported nondetections) have raised reporting levels because of analytical or environmental circumstances such as interference in the sample, changed instrumental sensitivity during a run, or failure to meet one of the three required criteria for determining a detection. In cases where censored data had raised reporting...
levels that were above the next highest calibration standard, these results were dropped from the data set (i.e., considered to be missing data) because the analysis did not meet the desired laboratory analytical resolution (see the Supporting Information for details). This occurred in <4% of sample results.

Several compounds required additional consideration. Dichlorvos is both a parent insecticide and a TP of the insecticide naled; for this study, dichlorvos was categorized as a parent. Similarly, methamidophos is both a parent insecticide and a TP of the insecticide acephate, but because the use of methamidophos as a pesticide was canceled in 2009 we categorized it as a TP. Carbendazim (parent thiophanate-methyl) has industrial use in caulks and other building supplies, but its only conventional use is in tree injection; residues of carbendazim therefore are likely from transformation of the parent, and carbendazim was categorized as a TP. The TP 1H-1,2,4-triazole (parent propiconazole, a fungicide) had an extreme number of raised reporting levels; censoring at the calibration-standard reporting level affected 82% of the 3705 chemical determinations for this TP. The remaining results therefore were not considered representative, and 1H-1,2,4-triazole was excluded from this study, although it is included in Table S2. Three TPs, 2-chloro- and 1H-1,2,4-triazole was excluded from this study, although it remaining results therefore were not considered representative, and the median of the minimum basal flows was 0.2, and the median of the maximum basal flows was 1.0.

**Screening Assessments.** Screening-level assessments of potential parent and TP effects on instream biota were done for potential toxicity and for bioactivity at the molecular level and for individual compounds and cumulatively (i.e., mixtures). Individual parent and TP effects were screened by comparing detected concentrations to compound-specific acute-toxicity invertebrate and plant benchmarks using the most sensitive benchmarks available from the U.S. Environmental Protection Agency Office of Pesticide Programs at the time of assessment (Table S2). Chronic benchmarks, which are applicable to 21-day average concentrations, are included on some figures for context. Detected concentrations were divided by the acute invertebrate or nonvascular plant benchmark to obtain a toxicity quotient (TQ) for that pesticide compound in that sample toward invertebrates or nonvascular plants. Site-specific cumulative risk for pesticide mixtures under a maximum-exposure scenario was estimated as the sum of the maximum TQ measured for each compound at a site using the toxEval version 1.1.1 package of the open source statistical software R.

Sublethal effects were explored using exposure-activity ratios (EAR) based on the ToxCast database, which provides vertebrate-centric exposure-response metrics for more than 9000 organic chemicals to as many as 1100 molecular bioassay endpoints that include such groups as DNA binding and oxidoreductase. The approach is analogous to the comparison to screening-level benchmarks. For each compound detected in a sample that had corresponding ToxCast data, the EAR was computed as the ratio of the compound concentration to the activity concentration at cutoff (ACC, from the Toxicity ForeCaster, ToxCast; high-throughput screening data), which is the concentration at which measured bioactivity first exceeds a baseline. An EAR of 0.001 has been reported to be approximately equivalent to the widely used TQ screening-level threshold of concern of 0.1, in that a majority of chemicals with TQ > 0.1 also had EAR > 0.001. Site-specific maximum exposures for pesticide mixtures were defined as the sum of the maximum EAR for each pesticide compound at a site (ΣEARmax) (presumptive additive effects).

The storage-discharge relation for the basflow-separation model was calibrated at each site to maximize the basflow component of streamflow within the physical constraint that basflow cannot exceed streamflow, using daily streamflow records retrieved from the USGS National Water Information System for the 10-year period prior to and including the sample year at each site. Basflow as a fraction of streamflow (basflow fraction) was calculated for each sample day. The daily values of basflow fraction depend on the characteristic streamflow recession rate for a stream, antecedent streamflow conditions, and the magnitude of the event. Basflow fraction is generally low and may be close to 0 during rain events when basflow is a negligible portion of streamflow. Basflow fraction approaches 1 over sustained dry periods. Most sampling for this investigation was conducted during dry weather—across the 168 gaged sites, the median of the median basflow fractions was 0.7, the median of the minimum basflow fractions was 0.2, and the median of the maximum basflow fractions was 1.0.
RESULTS AND DISCUSSION

Pesticide and Transformation Product Occurrence. 

Parent pesticides were widespread in small streams in the five regions of the U.S., with 103 parents detected at least once, and at least one parent detected at least once at 418 of the 442 stream sites (95%) (complete parent and TP data provided in companion data release76). However, TPs were nearly as pervasive—102 were detected at least once, and at least one TP was detected at least once at 396 stream sites (90%).76 The number of unique TPs detected was highest in the Midwest (100), followed by the Pacific Northwest and the Northeast (77 each), the Southeast (74), and Coastal California (68), and were diverse: 55 herbicide TPs, 44 insecticide TPs, and 3 fungicide TPs (detection frequencies by region in Table S2, Figure S2). About one-half of detected TPs (48) occurred rarely, i.e., were detected in <1% of samples from any region. More than one-third occurred fairly commonly—38 occurred in ≥5% of samples from at least one region and 28 were detected in ≥20% of samples from at least one region. The latter primarily were TPs of the acetanilide and amide and triazine herbicide classes but also included TPs of the phenylpyrazole insecticide, isoxaflutole herbicide, sulfonylurea and urea herbicide, and fungicide (polychlorinated aromatic degrade) classes. These 28 compounds were termed “frequently detected TPs” (Tables S7 and S8). Parents chlorothalonil and thiophanate-methyl, both fungicides, of two frequently detected TPs (4-hydroxychlorothalonil and carbendazim, respectively) were not analyzed. The other 26 frequently detected TPs are TPs of 10 parents; those 10 frequently detected TP groups (TPs and parents) and the TPs 4-hydroxychlorothalonil and carbendazim are the focus of additional investigation.

Occurrence of TPs at 90% of the 442 sites studied across five regions of the U.S., just slightly less than the parents (95% of sites), demonstrates that TPs likely are widespread in small U.S. streams, including many headwater streams. The presence of pesticides and TPs in headwater streams is of particular interest because such streams comprise the majority of river network length77,78 and have a higher proportion of biodiversity than larger water bodies.79,80 Although the numbers of herbicide and insecticide TPs measured (66 and 46, respectively) and detected at least once (55 and 44, respectively) are relatively similar, herbicide TPs were detected substantially more frequently than insecticide TPs, comprising 9 of the 12 parent-frequently detected TP groups. The preponderance of herbicide-TP detections may result from the generally much higher rates of application of herbicides relative to insecticides and fungicides. For example, a maximum recommended rate for application of the herbicide atrazine on turf is 0.54 kg/ha,81 more than 40 times a recommended nonagricultural application rate for the insecticide fipronil of 0.014 kg/ha,82 and recommended atrazine application rates for agricultural uses are even higher.83 Acreage and frequency of application may also play a role. The less intensive application of insecticides does not translate, however, to lower potential toxicity, as insecticides tend to be acutely toxic to invertebrates at concentrations orders of magnitude lower than herbicides and in some cases similar to or lower than the analytical reporting level (e.g., dichlorvos) (Table S2).

Detection frequencies of TPs had distinct regional patterns. Herbicide TPs were detected most frequently in the Midwest, where the 21 TPs detected in >15% of samples all had herbicide parents (Table S2, Figure S2). The midwestern U.S. is characterized by intensive row cropping, and 96 of the 100 Midwest basins had >5% agricultural land use;44 in agricultural applications, herbicide use dwarfs insecticide use.44,84 Land use in the basins in the other four regions, however, generally spanned an urban gradient,44 and urban contributions of
fungicides and insecticides to surface waters often exceed those of agriculture.\textsuperscript{34,41,85} The two frequently detected fungicide TPs (4-hydroxychlorothalonil and carbendazim) were detected more frequently in the Southeast, Pacific Northwest, Northeast, and Coastal California regions than in the Midwest. Concentrations and detection frequencies of the insecticide fipronil and its frequently detected TPs were substantially higher in the Southeast than in other regions, likely related to the warm climate, which is conducive to numerous insect pests found in and around the home—including fleas, chiggers, ticks, mosquitoes, cockroaches, termites, and fire ants—for which fipronil is recommended.\textsuperscript{46}

For several frequently detected TP groups (parent and frequently detected TPs), the sum of TP molar concentrations ($\Sigma[TP]$, nondetections considered as zeroes) exceeded the parent concentration $[P]$ for the subset of samples in which either a parent or TP in that group was detected (Figure 2, Figure S3, Table S9). All regions combined, $\Sigma[TP] > [P]$ in $\geq$50% of this sample subset for 6 of the 10 frequently detected TP groups: the herbicides atrazine, metolachlor, acetochlor, isoxaflutole, and metribuzin, and the insecticide fipronil (Table S7). Other parent–TP groups that were less frequently detected also had $\Sigma[TP] > [P]$ for more than 50% of the samples with detections: of eight additional parent–TP groups with a parent or TP detected in >100 samples, $\Sigma[TP] > [P]$ for parent–TP groups alachlor and norflurazon (both herbicides), the insecticide diazinon, and the fungicide propiconazole (Table S2).

The prevalence of TPs relative to the parent (Figure S3) is affected by many factors, including persistence of the parent compound (persistence data from ref 87). For the herbicides diuron and hexazinone, the parent compound is persistent in soils, and $[P] > \Sigma[TP]$. Conversely, the parent herbicides acetochlor, metolachlor, and isoxaflutole (a propesicide) are relatively nonpersistent in soils, and $\Sigma[TP] > [P]$. And although the insecticide fipronil is persistent in soils, the persistence of its TPs is even greater\textsuperscript{48} and might explain why $\Sigma[TP] > [P]$. Additionally, the analytical method used here quantifies six TPs of fipronil, the most of any parent compound, increasing the likelihood that $\Sigma[TP] > [P]$. For atrazine, $\Sigma[TP]$ tended to exceed $[P]$, despite moderate persistence of the parent in soils. Here, however, changes in agricultural practices may play a role. Downward trends in atrazine concentrations in U.S. streams and upward trends in concentrations of the TP deethylatrazine from 2002–2012 have been attributed in part to changes in agricultural practices intended to limit runoff (including a decrease in application rates), resulting in more atrazine degradation in soils.\textsuperscript{89} Such changes could promote higher concentrations of TPs relative to the parent for other agricultural pesticides.

Changing patterns of pesticide use in response to regulation, pest pressures, or other factors may also contribute to relative concentrations of parents and TPs, as decreased use of the parent pesticide may result in greater proportional occurrence of the TP(s) over time, particularly in streams with a greater fraction of baseflow. Agricultural use of metribuzin decreased into the mid-2000s,\textsuperscript{84} when its use then began to increase primarily for soybeans, a major crop in the midwestern U.S.\textsuperscript{41} Consistent with use patterns, metribuzin $\Sigma[TP] > [P]$ for all regions except the Midwest. As of 2013 (when Midwest samples were collected), dimethenamid use on corn had decreased since peak use in 1996; parents and their TPs were detected primarily and at higher concentrations in the Midwest, where corn is the dominant crop\textsuperscript{41} than in other regions, and generally $\Sigma[TP] > [P]$. Use of the herbicide simazine on corn has remained relatively steady but has decreased on orchards and grapes and is considered moderately persistent in soils and stable in water, consistent with $[P] > \Sigma[TP]$ in all regions but Coastal California, where decreasing use on grapes may favor the relative occurrence of the simazine TP (agricultural simazine use in Coastal California counties during January–May 2017 was mostly on wine grapes (https://calpip.cdpr.ca.gov/main.cfm)).

The frequently detected TPs represent a wide variety of degradation pathways, including oxidation, reduction, dealkylation of an amino group, and chlorine substitution by a hydroxyl group. Fate processes involved in TP formation are equally numerous and include hydrolysis, anaerobic or aerobic soil metabolism, and aqueous and soil photolysis. Some of the frequently detected TPs can be formed by multiple processes; for example, DCPMU, a TP of the herbicide diuron, can be formed by soil photolysis, aerobic soil metabolism, and anaerobic and aerobic aquatic metabolism. Additionally, the fate processes leading to the transformation of a pesticide can shift with time. For example, the prevalence of hydroxyatrazine as the major TP of atrazine detected in the streams reflects the recent evolution and adaptation of soil microorganisms capable of hydroxylation pathways that lead to complete degradation.\textsuperscript{80,91} The relatively frequent occurrence of many of the TPs measured in this study therefore cannot be attributed to one or a few common degradation pathways or fate processes.

The fungicide chlorothalonil has a more complex story. Chlorothalonil was not measured for this study (insufficient ionization for electrospray), but one of its eight TPs, 4-hydroxychlorothalonil, was measured and was detected in $>20\%$ of samples in the Southeast and in $>10\%$ of samples in the Northeast and Coastal California. Given its high reporting level relative to other TPs measured for this study (98 ng/L, exceeding the 75th percentile reporting level for TPs [Table S2]), the presence of 4-hydroxychlorothalonil in the small streams studied may be underestimated. The frequent occurrence of 4-hydroxychlorothalonil indicates the probable occurrence of other TPs of chlorothalonil that were not measured for this study. A screening study of 31 samples of Swiss groundwater detected 8 TPs of chlorothalonil in at least 1 sample, including the first environmental detection of TP R471811, which occurred in every sample collected and had the highest concentration of the more than 1 400 compounds measured.\textsuperscript{37} Chlorothalonil is a high-production fungicide used on a number of specialty crops\textsuperscript{48} and is also commonly used on turf grass, including golf courses.\textsuperscript{92} In California, for which nonagricultural pesticide-use data are available, chlorothalonil is the most intensively used pesticide on “recreational areas.”\textsuperscript{93} A study of golf-course-green leachate reported that, although the parent compound chlorothalonil was rarely detected, the TP hydroxychlorothalonil occurred in 87% of leachate samples.\textsuperscript{94} In our study, 46% of all basins had at least one golf course but 82% of study basins with a detection of 4-hydroxychlorothalonil had at least one golf course (see Table S10 for breakout by region). This significant difference highlights the potential contribution of use of chlorothalonil on golf courses to occurrence of 4-hydroxychlorothalonil in small streams.

Relation to Flow. Occurrence and concentrations of frequently detected TP groups were investigated in samples collected during runoff conditions (“runoff”) and baseflow
conditions (“baseflow”) in the streams. Flow data were available for computation of baseflow fraction for 168 of the 442 study sites (Midwest, n = 25; Southeast, n = 53; Pacific Northwest, n = 12; Northeast, n = 49; Coastal California, n = 29) (Table S11). Detection frequencies for TPs were computed as the detection of any TP in a frequently detected TP group, i.e., \( \sum [TP] > 0 \). Parent compounds did not tend to occur more frequently in one or the other flow endmember: The detection frequency of the parent was significantly greater \((p < 0.05)\) during runoff for 3 of 10 frequently detected TP groups and greater during baseflow for 1 group; for the remaining 6 groups there was no significant difference between flow endmembers (Figure 3A) (detection frequencies by region shown in Figure S4). TPs, in contrast, tended to occur more frequently during baseflow than runoff: The detection frequency was significantly greater during baseflow for six frequently detected TP groups and during runoff for two.

The “typical” assemblage of concentrations of frequently detected TPs and associated parents differed between runoff and baseflow. This assemblage was evaluated by determining the 80th percentile concentration (i.e., the concentration exceeded in 20% of samples) of \([P]\) and \(\Sigma [TP]\) for each frequently detected TP group (Figure 3B) (80th percentile concentrations by region are shown in Figure S5) in runoff and baseflow stream samples. For all regions combined, this 80th percentile assemblage had detectable concentrations of five frequently detected TP groups (parent and at least one TP) during both runoff and baseflow, except metolachlor and simazine TPs, which were detected in less than 20% of runoff samples. For four of five TPs with 80th percentile concentrations that are detections, the 80th percentile concentration was significantly higher during baseflow than during runoff (permutation test, \(p < 0.05)\); the highest 80th percentile concentrations were for a TP of atrazine and of metolachlor in baseflow.

Contrasting patterns of pesticide and TP occurrence and concentration during runoff and baseflow are illustrated for the most commonly detected herbicide and insecticide groups (atrazine and fipronil and their TPs) (Figure 4; cumulative frequency distributions for the other frequently detected TP groups are provided in Figure S6). For the atrazine group, elevated concentrations of \(\Sigma [TP]\) in baseflow occurred more
frequently than in runoff and more frequently than elevated concentrations of the parent in either flow endmember. For fipronil, elevated concentrations of Σ[TP] in both baseflow and runoff occurred more frequently than those of [P], and distributions of Σ[TP] in runoff and baseflow were similar, especially at higher concentrations. Although concentrations of fipronil were orders of magnitude lower than concentrations of atrazine, a substantial number of samples contained fipronil at a concentration that exceeded the chronic invertebrate benchmark.

Relative proportions of parent and TP, particularly during runoff, will depend on timing of use, with more recent use reflected by a higher proportion of the parent compound in runoff and streams.95,28 Samples for this study were collected during the spring growing season and therefore may bias the TP/parent ratio low at the beginning of the season, particularly for herbicides. Pre-emergent herbicides (e.g., atrazine) are likely to be applied at the beginning of the growing season and post-emergent herbicides (e.g., glyphosate) are likely to be applied throughout the growing season, affecting the timing of peak concentration.59 Higher pesticide application during the spring growing season, however, pertains more to agricultural areas and to herbicides than to urban areas and to insecticides and fungicides.96 Additionally, timing of use is likely to have a greater effect on pesticide assemblages during runoff than during baseflow.28 Little attenuation of most compounds is expected to occur during transport downstream: A Lagrangian study of in-stream transport of wastewater-derived chemicals indicated that most of the >200 organic contaminants (including pesticides) had little attenuation relative to boron.97 Another Lagrangian study, however, noted rapid removal of the insecticide imidacloprid downstream from a wastewater treatment plant and attributed it to sorption to organic matter rather than transformation.98 Concentrations of pesticides and TPs in baseflow have several potential sources, including wastewater treatment plant discharges, distant (upstream) sources, atmospheric deposition, and groundwater discharge. Of the 168 gaged sites considered here, 111 do not have a wastewater treatment plant in the basin (Table S1),46 therefore effluent is unlikely to be a major source of pesticides and TPs in baseflow at most sites. Most of the basins are sufficiently small that flow originating as runoff at the uppermost end of the stream would rapidly arrive at the sampling point, i.e., before baseflow conditions are reached. The median basin area is 57 km² and has a stream length of about 15 km; assuming an average flow velocity during baseflow of 0.3 m/s, water starting at the top of the stream would arrive at the sampling point in about 14 h. The stream length of the 95th percentile basin-area stream (870 km²) is about 65 km, translating to a travel time of <3
days. We therefore conclude that runoff from distant sources likely is a minor contributor to pesticides measured during baseflow conditions. Pesticides can be delivered to the catchment by wet and dry deposition, particularly in areas of high pesticide use. The vast majority of this deposition, however, will not occur on the water surface, as stream surface area is negligible relative to basin area. As a result, we hypothesize that pesticides and TPs associated with atmospheric deposition might be associated with runoff conditions but not with baseflow conditions. We therefore conclude that groundwater discharge likely is a dominant source of pesticides and TPs in baseflow. This is consistent with the many studies that have documented occurrence of pesticides and their TPs in U.S. groundwater (e.g., refs 2 and 18). A 2021 study reported that about 30% of more than 1,204 groundwater wells across the U.S. contained a pesticide TP and that TPs were detected more frequently than parent compounds. Further, detection frequencies were substantially higher in shallow groundwater: at least one pesticide or TP was detected in 86% of 344 shallow monitoring wells in the study. The results of our study are consistent with those that have demonstrated groundwater discharge to be a source of pesticide compounds in baseflow, albeit at smaller geographic scales.

The occurrence of parent pesticides and TPs in baseflow has implications for chronic toxicity to aquatic organisms. Because baseflow comprises much of the flow in small streams, aquatic organisms are likely to be exposed to the pesticide-TP signature in baseflow throughout much of the year. Our results indicate that higher concentrations of many TPs are more likely to occur in baseflow than runoff (Figure 3B). Additionally, under the right geochemical conditions, baseflow can be contaminated by legacy pesticides in groundwater discharge, although in our study occurrence of TPs of the eight discontinued parents in baseflow was rare (two TPs of discontinued pesticides, fenamiphos sulfoxide and alachlor OA, were detected during baseflow, once each). Nonetheless, the frequent detection of TPs in baseflow indicates that chronic exposures of aquatic communities is one of many compelling reasons to improve our understanding of TP toxicity.

**Potential Toxicity to Aquatic Life.** Parent pesticides exceeded an aquatic life benchmark substantially more frequently than did TPs (Figure S7). Nine parent compounds had an acute invertebrate TQ > 1 (potential toxicity) in at least one sample, and seven had an acute nonvascular plant TQ > 1 in at least one sample, but only one TP (of the insecticide malathion) had an acute invertebrate TQ > 1 and none had an acute nonvascular plant TQ > 1 (Figures S7 and S8). Thirty-four parent compounds had at least one acute invertebrate or acute nonvascular plant TQ > 0.1 (threshold of concern); two TPs — of the insecticides acephate and terbufos — had an acute invertebrate TQ > 0.1. The contribution of TPs to potential acute toxicity, however, is underrepresented because only about one-quarter of the 116 TPs measured have an aquatic life benchmark, whereas 95% of the 108 parent pesticides have an acute invertebrate or acute nonvascular plant benchmark (Table S2). To explore the envelope of potential toxicity, TQs for those TPs that lack a published benchmark were computed using the benchmark for the parent (equimolar substitution), excluding 7 TPs likely to be substantially less toxic than their parent (Figures S7 and S8) (see the Supporting Information for detailed discussion). Using this upper bound, TPs of the insecticides bifenthrin and malathion also had at least one acute invertebrate TQ > 1 and an additional five had at least one acute invertebrate TQ > 0.1. Similarly, TPs of the herbicide acetochlor had at least one

![Figure 5. Comparison of maximum-exposure scenario hazard ratios for detected parent pesticides only and parents plus transformation products (TPs) for existing screening levels (top row) and with substitution of parent compound screening levels for those TPs lacking them (bottom row).](https://dx.doi.org/10.1021/acs.est.0c06625)
acute nonvascular plant TQ > 1, and an additional seven TPs had at least one acute nonvascular plant TQ > 0.1.

Vertebrate-centric sublethal effects were investigated using the in vitro EAR determined from the ToxCast database. An EAR > 1 indicates a concentration (exposure) reported to modulate a molecular target in vitro; an EAR ≤ 1 indicates a lower likelihood of biological reactivity. An EAR = 0.001 has been suggested to be equivalent to a ΣTQ = 0.1 (i.e., threshold of concern). Nine parent compounds had an EAR > 1 and 62 parents had an EAR > 0.001; no TP had EAR > 1, although 10 parents had at least one TP with an EAR > 0.001 (Figure S9). However, similar to TP TQs, EARs underrepresent potential sublethal toxicity because most TPs lack data in ToxCast: Acceptable ToxCast data were available at the time of access for 97 of the 108 pesticides (90%) but only 24 of the 116 TPs (21%). To explore an upper bound on potential sublethal toxicity, EARs were computed using the parent ACC (equimolar substitution) for those TPs that lack an ACC in ToxCast. The 7 TPs excluded from the TQ upper-bound analysis were included in the EAR analysis, as their bioactivity at the molecular level is unknown. Under this scenario, TPs of acetochlor and the fungicide chlorothalonil had at least one EAR > 1, and TPs of another 13 parents had an EAR > 0.001.

We explored the potential acute toxicity and sublethal effects of pesticide mixtures using a site-specific maximum-exposure scenario, i.e., summing the maximum acute invertebrate and acute nonvascular plant TQs for each compound (ΣTQ and ΣTQANVP) and maximum EARs (ΣEARmax) determined for all samples at each site (Figure S5, upper panels). Because of the under-representation of potential toxicity related to TPs, the upper bound of the toxicity envelope was estimated by computing ΣTQ and ΣEAR using the aquatic life benchmark or ACC of the parent for those TPs lacking them (Figure S5, lower panels). Under this upper-bound extrapolation, the number of sites with a ΣTQ > 1 was the same (56), but the number with a ΣTQANVP > 1 increased 8% from 87 to 94 and the number with an ΣEARmax > 1.0 increased 94% from 35 to 68 (Figure S5). Summed EAR and benchmark-based risk quotient results are summarized in Romanok et al. The paucity of data on toxicity of TPs to aquatic organisms results in an underestimate of potential toxicity associated with pesticide compounds as a group, unless assumptions are made to assign toxicity thresholds to TPs. The scarcity of acute nonvascular plant benchmarks and ACCs for TPs results in potential toxicity to nonvascular plants or biological reactivity for parent+TP that are nearly identical to those for the parent only and that differ at relatively few sites for toxicity to aquatic invertebrates (Figure S5). The ΣTQANVP, ΣTQAVPRmax, and ΣEARmax that used parent screening-level substitution for those TPs without screening levels (upper bound extrapolation) indicated substantially more potential acute toxicity to nonvascular plants and more vertebrate-centric molecular bioactivity than when only those TPs with an existing benchmark or ACC were considered (Figure S5, upper and lower panels on far right). There was little change, however, to predicted acute toxicity to aquatic invertebrates (Figure S5, upper and lower panels in center). We attribute this difference to the comparatively high availability of invertebrate benchmarks for fipronil TPs, the only insecticide TPs that were frequently detected (Table S2); the most frequently detected TPs were TPs of herbicides (Figure S2), which tend to be substantially less toxic than insecticides to aquatic invertebrates (Table S2). Existing acute invertebrate benchmarks therefore do a reasonable job of predicting acute toxicity to invertebrates for the suite of pesticides and TPs we measured. However, some insecticides that occurred frequently in our study, such as the neonicotinoid imidacloprid (38% of samples), have TPs that we did not measure but that may contribute to toxicity.

Further, despite our relatively extensive analyte list, there are some commonly used insecticides and their TPs, such as the neonicotinoid clothianidin and the ryanoid chlorantraniliprole, that we did not measure. This data gap highlights the importance—and challenge—of updating analyte lists to keep pace with changes in pesticide use.

**Implications.** Our assessment indicates that TPs may be contributing substantially more to instream toxicity than currently known. Additionally, although we estimated an upper-bound on toxicity associated with pesticides and TPs in the 442 small streams presented here, there are several reasons why potential instream toxicity (ΣTQ) or molecular response (ΣEAR) might be greater than our estimate. Some TPs are more toxic than the parent, therefore globally substituting the parent benchmark underestimates their contribution to toxicity. This will be counterbalanced to an unknown extent by TPs that are less toxic than the parent; the net result depends on which TPs are more prevalent. Second, because of the few existing toxicity tests for TPs, even existing TP benchmarks may not be adequately protective of the most sensitive species because of insufficient data. Standard test organisms (e.g., *Daphnia magna* for invertebrates) may be less sensitive toward some pesticides than species of benthic invertebrates present in many streams.

In a mesocosm experiment that investigated the toxicity of fipronil and its TPs, for example, the concentration values for three fipronil TPs protective of 95% of the aquatic invertebrates in the mesocosm were 10 to almost 3000 times lower than the EPA chronic invertebrate benchmarks. Similarly, the bioassay endpoints used in ToxCast for development of EARs are predominantly for vertebrate molecular effects and may not fully capture effects to aquatic invertebrates. Third, reporting levels for TPs in this study tended to be greater than those of the parents (Table S2); as a result, some TPs might be present but not detected and therefore are not included in ΣTU or ΣEAR. This becomes particularly important when toxic thresholds are in the same range as reporting levels, as for some insecticides. Fourth, although this study included a broad range of pesticides and TPs, it represents only a small slice of those pesticides and TPs we measured. However, some insecticides occurred frequently (e.g., imidacloprid), but their TPs were not measured for this study. In other cases, not all TPs of a parent were measured—for example, of the eight TPs of chlorothalonil that have been identified, only one was measured for this study. Finally, although samples were collected more frequently (weekly) for our study than for many monitoring studies, this timing nonetheless can miss a substantial number of ephemeral but potentially toxic "events." Thus, the results of weekly sampling at 442 small U.S. streams reported here may underpredict the maximum potential toxicity to aquatic organisms.

This continental-scale study of pesticide TPs, the most geographically extensive to date, highlights the near ubiquity of TPs in small U.S. streams in both urban and agricultural areas. TPs occurred nearly as frequently as parents, and the sum of concentrations of TPs of a given parent often exceeded those of the parent. Consistent with earlier studies, herbicide TPs were the most widely detected and occurred at the highest concentrations of TPs of a given parent often exceeded those of the parent. Consistent with earlier studies, herbicide TPs were the most widely detected and occurred at the highest
concentrations, but TPs of the insecticide fipronil and the fungicides chlorothalonil and carbendazim also occurred frequently and at concentrations of potential concern for aquatic life. The results reveal the presence of numerous TPs in baseflow at concentrations similar to those in runoff, indicating probable chronic exposures of aquatic organisms to TPs and, potentially, long-term exposure associated with TPs in groundwater discharging to streams. Finally, the lack of toxicity data and bioassay endpoints of biological activity for most TPs precludes a robust understanding of potential toxicity of pesticide compounds to aquatic organisms. Our quantitative estimate of an upper bound on toxicity, in some cases orders of magnitude higher than that based on existing benchmarks and biomarkers, exposes the importance of this data gap.

**ASSOCIATED CONTENT**

#### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.0c06625.

Description of analytical methods and reporting levels; results for laboratory and field quality control; description of the toxicity quotient approach; maps of the five study areas; figures illustrating detection frequency, 80th percentile concentrations, and cumulative frequency distributions of frequently detected TP groups under flow endmember conditions; and figures illustrating ranges of acute aquatic invertebrate toxicity quotients, acute nonvascular plant toxicity quotients, and exposure activity ratios as boxplots (PDF).

Tables providing site descriptions and a numerical key to sites shown on the maps; information on analytes and detection frequencies; quality-control summaries for surrogate compounds, field blanks, replicates, and spikes; detection frequencies and routes of formation for frequently detected TPs; sampling data for the frequently detected TPs and their parents; percentage of samples in frequently detected TP groups for which the sum of the TP molar concentrations exceeded that of the parent; data on golf course occurrence; and discharge and baseflow fraction for samples collected at gaged sites (XLSX).

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**Notes**

The authors declare no competing financial interest.

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Data and additional information on the Regional Stream Quality Assessments can be found at https://webapps.usgs.gov/rsqa/#/. Machine readable data files are available as USGS data releases at 10.5066/P9YV8DD1 (pesticide concentrations), 10.5066/P9PSU28Y (comparison to aquatic life benchmarks and ToxCast ACGs), and 10.5066/P9AIPH3P (baseflow separation).

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