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Kim A. Anderson

Assessing the risk from exposure to a chemical mixture in the environment can seem prohibitively challenging. Most components of the mixture are not readily identifiable, chemicals may interact to cause other-than-additive toxicity, and the number of potential combinations of environmental contaminants is enormous. These challenges can make it seem impossible to accuractely assess risks associated with chemical mixtures. In reality, not all chemicals in the environment are accessible for organismal uptake, there are a limited number of predictable combinations of chemicals in the environment, and a minority of chemicals are likely responsible for the majority of toxicity in any given sample. This dissertation addresses the seemingly daunting challenge of assessing mixture toxicity by strategically adapting and implementing a collection of sampling and analytical methods to reduce complex environmental mixtures into manageable components. In the first study, personal exposure to chemical mixtures in rural Peru was assessed with silicone wristbands. The samples naturally clustered into groups that were defined by distinct classes of chemicals and were associated with broad demographics of the study participants. The results revealed chemical patterns in wristbands that are possibly indicative of common mixtures in the personal environment and suggest regional sources and routes of chemical exposure. The second study simplified environmental mixtures from a contaminated urban waterway into the most hazardous components. Effect-directed analysis of passive sampling device extracts using a zebrafish bioassay, chromatographic fractionation, and various chemical analyses, eliminated priority pollutants as suspect toxicants and identified responsible toxicants. Specifically, fatty acids and possibly dithiocarbamates were previously unrecorded components of LDPE extracts that likely drive the toxicity of the whole mixtures. Indeed, a minority of chemicals in the whole mixture was responsible for the majority of effects. In total, this dissertation demonstrates that complex mixtures are not unsolvable by applying passive sampling and compatible techniques for simplifying exposure to, and effects of, environmental mixtures.

‘Tis a Gift to be Simple: On Passive Sampling and Compatible Techniques for Reducing the Complexity of Environmental Mixtures

by
Alan J. Bergmann

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APPROVED:

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Alan J. Bergmann, Author

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CONTRIBUTION OF AUTHORS

Alan J. Bergmann is the primary author and responsible for all components of this dissertation except as follows.

Paula E. North is the Principal Investigator on the project that funded the research presented in Chapter 2. She participated in study design, data collection, interpretation of results, and writing. Luis Vasquez helped conceive of the study, recruited volunteers and collected wristbands for the study. Hernan Bello helped to select sampling locations and communicate with study participants. He also helped with data interpretation. Maria Gastanaga was critical to obtaining IRB approval in Peru, select sampling locations, and interpreting results.

Robert L. Tanguay is the director of the Sinnhuber Aquatic Research Laboratory that conducted the zebrafish bioassays described in Chapter 3. He also helped with data interpretation.

Kim A. Anderson contributed to the study design, data interpretation, and writing of Chapters 2 and 3.

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For my grandfathers.

‘Tis a Gift to be Simple:

On Passive Sampling and Compatible Techniques for Reducing the Complexity of Environmental Mixtures

Chapter 1 Introduction

Environmental contamination is the presence of undesired substances in natural systems, commonly as a result of human activity. Intentional or incidental release of chemicals accompanies many activities that directly benefit humanity. Pesticides help to control disease vectors. Vehicles move people and goods around the world. Such activities have unintended effects on human health and the environment which are often not captured in the cost. Toxicology is the study of chemicals that have adverse effects on biological systems. By identifying toxic chemicals and their effects, we can begin to evaluate their risks to wildlife and people. Therefore, one role for toxicology is to contribute to the study of unforeseen costs associated with human activity.

1.1 The problem of mixtures

Toxicology is practically implemented through the process of risk assessment, in which hazard and exposure identification are necessary components. It is common for the toxicity of chemicals to be assessed one at a time. In reality, organisms simultaneously engage with many chemicals from a variety of sources.[1] The toxicity of the mixture is often assumed to be equivalent to the additive sum of the individual components. However, chemicals may interact to enhance or attenuate each other's toxicity, violating the assumption of additivity. To investigate such other-than-additive effects, one can construct binary, tertiary, or more complex mixtures of chemicals for toxicity assessment, a "bottom-up" approach. Complex mixtures of chemicals in the environment are additionally challenging to study because the composition of the whole mixture is often largely uncharacterized and can vary spatially and temporally. Toxicity of known mixture components may only explain a fraction of effects associated with the whole mixture due to the presence of unknown (non-target) compounds.[2] With hundreds of thousands or more chemicals in the environment, the number of possible combinations makes a bottom-up approach impractical without severe prioritization of the mixture components.

Starting with a whole environmental mixture is a "top-down" approach that can overcome the challenges of building representative samples from authentic standards. Every environmental sample could be individually evaluated for its unique composition and toxicity but in fact the number of realistic combinations of chemicals are much fewer than what is mathematically possible. Common sources of environmental contaminants result in typical, reoccurring, mixtures that exhibit predictable environmental fate. Polycyclic aromatic hydrocarbons (PAHs), are commonly released from petroleum and combustion sources illustrating how mixtures can be predictable. PAHs are typically generated as mixtures so where you find one PAH, you will find many others. Additionally, different sources generate different profiles

of individual PAHs, *e.g.* oil spills and wood burning.[3] These are typical mixtures that can be used in forensic profiling to help determine the source of contamination. Similar techniques are available for other contaminants including pesticides like *p,p'*-dichlorodiphenyltrichloethane (DDT).[4] PAHs and pesticides co-occur in the environment with many other chemical mixtures but only a part of the mixture may cause toxicity. Therefore there is ongoing need to identify predictable and relevant mixtures, and to prioritize hazard assessment, within the total multi-class mixture.

1.2 The mixtures toolbox

There are many tools available for evaluating the environmental mixtures. They are drawn from many disciplines and cannot all be enumerated here.[1,5] Considerations for the selection of tools to evaluate mixtures are described in this section.

A challenge in assessing mixtures using a top-down approach is collecting a relevant sample. Due to spatial and temporal variability in the environmental distribution of chemials, directly collecting environmental media for analysis may miss episodic events and therefore underestimate exposure. One method for overcoming this challenge is to collect a time integrated sample. For compounds that may bioconcentrate, a time-integrated sample may also be more representative of the exposure mixture. Additionally, not all compounds in the environment are available to interact with organisms (*e.g.* strongly sorbed to sediment) so even if they have hazard potential, they will not cause risk.

Passive sampling devices (PSDs) collect chemicals via diffusion over the course of contact with their environment providing a time-integrated mixture. Specifically, polymeric PSDs such as low-density polyethylene and silicone sample the freely dissolved aqueous or vapor phase of lipophilic organic compounds. Because, in the absence of transformation and elimination, the same fraction of chemicals can bioconcentrate PSDs are generally considered to capture the bioavailable fraction. PSDs exclude chemicals that are not available for direct absorption by lipid tissue such as those inaccessibly bound to particulate matter and those with low lipophilicity. Thus, PSDs can provide a first step in simplifying complex environmental mixtures by capturing a time integrated sample that contains only bioavailable components.

However, even a PSD-simplified mixture is still complex. Targeted chemical analysis can detect major components of interest but, even when screening against large chemical libraries, may only identify a fraction of what drives toxicity.[2] Non-targeted approaches can be effective to identify components but

require advanced instrumentation and are not amenable to high sample throughput. A compromise between increasing the number of possible chemical detections and the time of analysis is needed.

Bioassays are a type of analytics that can describe the potential effect of a mixture with fewer assumptions about its composition than chemical analysis. Bioanalytical measurements target modes of toxicity instead of specific chemical structures. Options range from *in vitro* bioassays that target specific mechanisms of action to *in vivo* assays that test whole organism responses. Given the many known toxic modes of action and the number of species in the world, there are many bioassays at the disposal of researchers.

Alternatively, the effects of simple combinations of chemicals can be modeled based on the mechanism of the mixture components and whether the components interact to cause other-than-additive effects, *i.e.* synergy or antagonism.[1,6] For environmental samples, in which there are many unknown components, describing the mixture is more challenging. The toxic equivalency approach is commonly used to characterize an environmental sample's response in a bioassay with respect to the toxicity of a reference compound.[1,2] If the toxicity of components has not been evaluated, then quantitative structure activity relationships can be used to estimate the response. An epidemiological approach is to look for associations between sample chemistry and effects. Through deconvolution of many samples with multivariate analyses a researcher can associate measured effects with target analytes.[7,8] Mathematical approaches are only as good as the chemical and bioanalytical data that go into the analyses. Tools from many different disciplines (*e.g.* bioassays in combination with modeling) complement each other and are strategically integrated in successful evaluations of environmental mixtures.

1.3 Specific aims and hypotheses

This dissertation presents two approaches for simplifying complex mixtures into manageable components. In both, we employ PSDs in novel applications and adapt techniques from the mixtures toolbox that are fit for our purposes. The first chapter simplifies personal exposure into typical mixtures using silicone wristbands. In the second, we empirically simplify environmental mixtures to the components with greatest hazard potential using a variety of laboratory tools.

1.3.1 Simplifying exposure

Chapter 2 of this dissertation combines tools described above to investigate exposure to complex mixtures of chemicals through multiple pathways. The 'exposome' has been defined as the total exposure a person

experiences during her lifetime.[9] This huge concept is impractical to address directly. There are many definitions of exposure but for human health risk assessment, the U.S. EPA has used the definition of exposure as ”contact between an agent and the visible exterior of a person (*e.g.* skin and openings into the body).”[10] Personal exposure is the cumulative result of surroundings and behavior that influence chemical encounters so exposure is highly individualized.[11] Therefore, despite an enormous number of chemicals in the environment, a person can only be exposed to subsets of the total mixture. Under the resulting premise that combinations of personal factors drive differences in exposure, we should be able to identify mixtures that are typical of different groups in a population.[8]

Recent advances in passive sampling have adapted common silicone wristbands into personal PSDs.[12] Worn on the wrist, the wristband samples from a person’s external environment, and captures chemical exposure in a portion of the visible exterior. A wristband potentially captures dermal and inhalation routes of exposure and does not reflect the composition of internal exposure (*i.e.* is not representative of the mixture of chemicals at the target site). Wristbands are promising for the identification of typical mixtures in the personal external environment but, until this dissertation, have not been used in such a broad application. Additionally, wristbands present a simple method to determine personal exposure in areas that are difficult to access. The technology is non-invasive, does not require electricity, and is considered quite fashionable by some. We aimed to take advantage of these properties to assess personal exposure in a remote region of Peru – where data on chemical use and exposure is in its infancy. We hypothesized that by having participants wear wristbands we would be able to identify typical mixtures in their personal environments explained by differences in demographic information. By addressing this hypothesis, we aid the development of health research in Peru and advance wristband passive sampling.

1.3.2 Simplifying effects

Chapter 3 builds on the results of Chapter 2 by further simplifying representative mixtures into the hazardous components. Effects-directed analysis (EDA) is a framework for strategically combining techniques from the mixtures toolbox. Bioassays are used to determine potential effects of environmental samples. Through iterative simplification of the sampled mixtures, potential toxicants are reduced to a number that is manageable for targeted and non-targeted chemical analyses. Tentatively identified toxicants can be confirmed through the testing of authentic standards in the same bioassay. Thus EDA leads the researcher to identify hazards among many chemicals in a complex mixture. EDA also has the ability to identify interaction effects. By separating chemicals into distinct fractions, one can compare the toxicological effect of each component to the total mixture and use mathematical tools described above to

identify other-than-additive toxicity. EDA is similar to EPA's toxicity identification evaluation process and is also a candidate for use in regulatory toxicology.[5,13] Studies expanding on the tools available for EDA, and successful case studies, benefit future applications of EDA.

We aimed to apply the EDA framework to empirically determine what is causing toxicity in PSD samples from the Portland Harbor Superfund Megasite. We hypothesized that a minority of chemicals in the complex mixture is responsible for the toxicity. Based on previous work, we expected PAHs to be at least partially responsible for the developmental effects of the environmental mixtures. In addressing these hypotheses, we demonstrate the first EDA coupling our unique combination of compatible tools, low-density polyethylene passive sampling and a zebrafish embryo bioassay.

The work presented in this dissertation addresses one of the biggest challenges in toxicology: how to deal with mixtures. Our approach is to reduce complex mixtures into manageable components and through a combination of top-down and bottom-up strategies, prioritize components for further study.

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MULTI-CLASS CHEMICAL EXPOSURE IN RURAL PERU USING SILICONE WRISTBANDS

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Chapter 2 Multi-class chemical exposure in rural Peru using silicone wristbands

2.1 Abstract

Personal silicone wristband samplers are demonstrated in Peru in four agriculture and urban communities where logistic and practical constraints hinder use of more traditional approaches. The wristbands and methods enabled quantitation of 63 pesticides and screening for 1397 chemicals including environmental contaminants and personal care products. Sixty-eight wristbands were worn for nominally one month by volunteers from four communities of Alto Mayo, Peru. We identified 106 chemicals from eight chemical classes among all wristbands. Agricultural communities were characterized by pesticides and PAHs, while the capital city had more personal care products. Multiple linear regressions explained up to 40% of variance in the wristbands from: chlorpyrifos, cypermethrin, and DDT and its metabolites (DDx) (r^2 = 0.39, 0.30, 0.40 respectively). All three pesticides were significantly different between communities and cypermethrin and DDx were associated with participant age. The calculated relative age of DDT suggested some communities had more recent exposure than others. This work aids health research in the Alto Mayo and beyond by identifying typical mixtures and potential sources of exposure to organic chemicals in the personal environment. Silicone wristband sampling with chemical screening is a candidate for widespread use in exposure monitoring in remote areas.

2.2 Introduction

Exposure to exogenous chemicals is a substantial contributor to disease risk[1,2] and adverse effects may be exacerbated by other health states such as malnutrition.[3] Many factors influence a person's total exposure to toxicants[4] which can result in highly individualized chemical environments.[2] By characterizing the external personal environment we can prioritize individual and mixtures of chemicals for toxicity and risk assessment. Techniques to measure personal exposure include internal and external tissue sampling,[4,5] personal air monitoring[6] and hand wipes.[7] Silicone wristbands were recently introduced as personal passive sampling devices (PSDs).[8]

PSDs are frequently used as stationary monitors of the environment.[9-11] They operate by concentrating organic compounds over time, which improves environmental detection limits and captures episodic exposures. By sampling through passive diffusion into a hydrophobic polymer, such as low-density polyethylene (LDPE) or polydimethylsiloxane (silicone),[12] many PSDs mimic uptake of chemicals by lipid tissue.[9] Although silicone PSDs predominately sample lipophilic compounds, O'Connell *et al.* identified chemicals with logK_{ow}s (log octanol-water partition coefficient) from -0.07 to 9.5 in extracts of silicone wristbands.[8] Simply worn on the wrist, the silicone samples from any media it contacts. Therefore the wristbands capture chemicals from inhalation and dermal routes of exposure but do not account for ingestion. Recent work showed that concentrations of organophosphate flame retardants and polycyclic aromatic hydrocarbons (PAHs) in wristbands correlated with urinary metabolites of those compounds.[7,13] These studies support a link between the ambient personal environment sampled by the wristbands and the internal environment. As mixed-media samplers, the calculation of environmental concentrations is difficult but wristbands can be used to evaluate relative exposures.[8,14,15] PSDs are well suited for sampling in remote regions. A previous study showed that PAHs and pesticides are stable in low-density polyethylene PSDs transported in polytetrafluoroethylene (PTFE) bags for multiple weeks at ambient temperatures.[16]

The current study examines wristbands worn by volunteers in the Alto Mayo, a heavily deforested region with developing agriculture in the Department of San Martin in Northeast Peru. Much of Latin America is increasingly urbanized and industrially developed, exposing residents to more anthropogenic chemicals, while still experiencing the hazards of infection and malnutrition.[1] Land use intensification of low-middle income countries is associated with pesticide use[17] but little information is available about the Alto Mayo. A series of reports surveyed pesticide application, environmental distribution, and blood concentrations of farm workers from Tarapoto, located 80 km southeast of the Alto Mayo.[18-20] They described farm workers

who mixed and applied pesticides with no personal protection equipment, storage of pesticides in residences, and that children would sometimes help in agriculture fields.[20] These practices have been documented elsewhere[21,22] and could distribute pesticide exposure among community members.

Here, we determine the distribution of pesticides and many other chemicals in the personal environment of Alto Mayo residents using silicone wristbands. We hypothesized that wristbands would accumulate different suites of chemicals in different communities, providing evidence for differential exposure based on lifestyle. We anticipated that pesticides would be greater in wristbands worn by farm workers and that in non-farming communities we would observe more urban signature such as elevated personal care products. By identifying typical mixtures of exposure we provide needed preliminary data for health research in the Alto Mayo and demonstrate the applications and limitations of silicone wristbands as monitors of the personal environment.

2.3 Methods

2.3.1 Materials

All solvents were purchased from Fisher Scientific (Pittsburgh, PA). Methanol, isopropanol, dimethyl sulfoxide (DMSO), and hydrochloric acid (HCl) were ACS grade. Ethyl acetate was Optima and n-hexane was GC-Resolv. Standards were purchased at >99% purity. Tetrachloro-*m*-xylene (TCMX) was from Supelco (Bellefonte, PA, USA), polychlorinated biphenyl (PCB)-100, PCB-209, and *p,p'*-dibromooctafluorobiphenyl were from Accustandard (New Haven, CN, USA), and PCB-65-d5, PCB-115-d3, and PCB-156-d3 were from C/D/N Isotopes (Pointe-Claire, QC, Canada). Water was filtered through an in-house 18MΩ*cm purification system (Millipore, Merck, Darmstadt, Germany).

2.3.2 Environmental passive sampling

To get an overview of what pesticides and other compounds might be present, we conducted an initial survey of bioavailable contaminants in surface water of the Alto Mayo in August 2013. Methods followed previous deployment and analysis of LDPE passive sampling[23,24] and are described in detail in the supporting information.

2.3.3 Personal passive sampling

Silicone wristbands were prepared as described previously.[8] Briefly, wristbands were cleaned with three rounds of soaking in 1:1 ethyl acetate:n-hexane, then two rounds in 1:1 ethyl acetate:methanol. Cleaned

wristbands were vacuum-dried and transferred to individually sealed PTFE bags for transport to Peru. Two sizes of wristbands were used and were weighed prior to solvent cleaning. Large and small wristbands were 5.68 g (sd = 0.03 g) and 4.64 g (sd = 0.03 g), respectively.

Volunteers were recruited in four communities of the Alto Mayo overlapping the areas targeted for water sampling (Figure 2.1). Institutional Review Board approval was obtained from three collaborating institutions: Medical College of Wisconsin, Oregon State University, and the Instituto Nacional de Salud of Peru. Moyobamba is the capital of the Department of San Martin with a population of > 40,000. Rioja is an agriculturally oriented town and is home to approximately 20,000 people. Yantaló is a small farming village with fewer than 5000 people. Yantaló is also the home campus of the Yantaló Peru Foundation clinic, diagnostic center, and environmental health research base that formed the impetus for this study. Tingana is a very small village that has an ecological reserve and is a destination for ecotourism, but many of its residents are also farmers. Each participant wore a wristband for 30-34 days in February and March, 2014. Demographic information was collected from participants: community of residence, occupation, gender, and age. Wristbands were collected in their original PTFE bags and returned to Oregon State University and coded with unique identifiers.

2.3.4 Chemical analysis

Each wristband was cleaned of particulate matter by rinsing with 2 rounds of 18 MΩ*cm water, and once with isopropanol. Surrogate standards (TCMX, PCB-100, and PCB-209) were pipetted onto the wristbands immediately before extraction. The wristbands were individually extracted in two rounds, 12 hours followed by two hours, with 100 mL ethyl acetate at ambient temperature.[8,14] Aliquots of the wristband extracts were spiked with the internal standard *p,p'*-dibromooctafluorobiphenyl and analyzed with gas chromatography. Extracts were diluted 1:10 to reduce analytical interferences of background lipids and siloxanes.

We screened for compounds using retention time locked full scan gas chromatography mass spectrometry (GC-MS) paired with automated mass spectral deconvolution and identification system (AMDIS, National Institute of Standards and Technology, Gaithersburg, MD, USA) and deconvolution reporting software (DRS, Agilent Technologies, Santa Clara, CA, USA) as described elsewhere.[23] The deconvolution package compares potential analytes to a list of PCBs, pesticides, PAHs, personal care products and pharmaceuticals (PCPPs), and more, which was compiled from commercially available mass

spectral and retention time libraries (Agilent) and further expanded in-house to a total of 1397 compounds. The complete list of compounds is available in Appendix C.

Wristband extracts were quantitatively analyzed for 63 pesticides on a dual column gas chromatograph (Agilent 6890N) with dual micro-electron capture detection (GC-μECD, or "ECD") as described in Donald *et al.*[14] Of 63 pesticides, 44 are classified as insecticides, 9 herbicides, 7 fungicides, and 5 pesticide degradation products. Our method reflects interest in insecticides for their potential human health impacts and includes 26 organochlorine, 7 organophosphate, and 7 pyrethroid insecticides. Three wristband extracts had backgrounds that obscured the internal standards in the ECD chromatograms so quantitative results are reported for the remaining 65 wristbands. Concentrations of pesticides in the wristband extracts were corrected for dilution, surrogate recovery, and mass of the wristband. Final results are presented as ng/g wristband.

2.3.5 Data analysis

Statistical analysis was performed with the software package JMP Pro 12 (SAS, Cary, NC, USA). The 106 chemicals detected in wristbands with the GC-MS screen were grouped into 8 chemical classes prior to analysis. Ideally, compound classification would be based on known chemical application in the participating region, but it is impossible to collect that information for hundreds of chemicals *a priori*. We aimed to be as robust as possible by assigning chemical class before performing analyses and by adjusting the significance level for multiple comparisons. The proportion of those chemicals detected in wristbands from each demographic group was compared for each class (Appendix equation A1). Chi-square likelihood ratio test was used to compare between the four communities and Fisher's Exact Test was used for comparisons of gender and occupation. The p-values were compared to a Bonferroni adjusted significance level for eight chemical classes, $\alpha = 0.05/8 = 0.0063$. Because there were very few fungicides detected in some communities, the Chi-square test was suspect so Fisher's Exact Test was also performed for the 4 X 2 contingency table of fungicides and community yielding the same results (data not shown).

Because chlorpyrifos, cypermethrin, and DDx were quantified in more than 50% of the wristbands, we explored the effects of community, occupation, gender, and age on these pesticides. Observations below detection were set to zero ng/g and nonparametric statistical tests were used. Differences between communities were assessed using analysis of variance with Wilcoxon-rank sum for multiple comparisons. Wilcoxon rank sums test was used for occupation and gender. Age was evaluated with a simple linear regression with a robust model fit using the Huber M-estimation method. Test statistics were assessed at a

Bonferroni adjusted significance level for the three analytes, $\alpha = 0.05/3 = 0.0167$. These comparisons were also performed with parametric tests after substituting zero or the limit of detection for non-detects, or omitting the non-detects altogether. Some differences in the significance were found but the overall conclusions did not change.

To account for interactions between the demographic variables, we constructed multiple linear regressions of chlorpyrifos, cypermethrin, and DDx. The concentrations were left-centered so the natural log of the wristband concentration was used in the standard least squares models. Community, occupation, gender, and age were used to build the models using Moyobamba, non-farm workers (other), and female as reference levels. Model terms were added and subtracted to minimize Akaike's information criterion. During initial model assessment, non-detects were identified as influential outliers, even when substituting the detection limits. These cases were omitted and we restricted our analysis and inference to cases where concentrations in wristbands were above detection limits. Six, 19, and two cases were removed for regression of chlorpyrifos, cypermethrin, and DDx, respectively. The major conclusions were the same with and without non-detects (Table A3).

To estimate relative time since application of DDT, the ratio of *p,p'*-DDT/(*p,p'*-DDE + *p,p'*-DDD) was calculated as first suggested for wristbands by Donald *et al.*[14] and follows similar diagnostics in environmental media[25] and human biomonitoring.[26,27] The DDT ratios of different communities were compared with Wilcoxon sum rank tests.

2.3.6 Quality control

Sample handling, analysis, and quantitation were performed as defined by laboratory data quality objectives and standard operating procedures. Trip, field, and numerous in-laboratory blanks mirrored the handling and processing of a deployed PSD. Surrogate standard compounds accounted for any loss during extraction and analysis of PSDs. Instrument blanks and continuing calibration checks were analyzed regularly during chromatography. During ECD analysis, the formation of *p,p'*-dichlorodiphenyldichloroethane (DDD) from *p,p'*-dichlorodiphenyltrichloroethane (DDT) was observed in some calibration check samples so DDT and its metabolites *p,p'*-dichlorodiphenyldichloroethylene (DDE) and DDD are reported as the sum of the three related compounds (DDx).[28]Samples were randomized for chemical analysis, and data was collected blind to the sample identity.

Average surrogate recoveries (relative standard deviation) were 76% (36%) for TCMX, 75% (27%) for PCB-100, and 99% (17%) for PCB-209. As trip blanks, two constantly sealed wristbands accompanied

researchers during deployments and one field blank was exposed to the air at Rioja and Tingana each. Non-deployed wristbands were concurrently cleaned and extracted with deployed samples. Two pesticides were measured in the field blanks (Tingana: 12 ng DDT /g wristband; Rioja: 17 ng chlorpyrifos /g wristband). Given the absence of these compounds in the rest of the QC samples, and that they are two of the most frequent and abundant compounds at the respective deployment locations, the presence of chlorpyrifos and DDT likely reflect the field blanks' brief exposure to the environment. Similarly, DEET was detected in one field blank with the GC-MS screen but was not omitted. Some phthalates were detected in field and laboratory QC. However, the relative abundances in deployed wristbands were orders of magnitude higher than in QC samples, so they were included in analyses.

2.4 Results

2.4.1 Environmental passive sampling

Chemicals detected in the surface water of the Alto Mayo are presented in the appendix including Table A1 and Figure A1.

2.4.2 Compliance and participant demographics

Sixty-nine wristbands were returned to researchers. One wristband was returned without a demographic survey, so data analysis was performed on 68 complete sets of paired wristbands and surveys. Participant demographics are provided in Table 2.1. Participants most frequently reported "farm worker" in their occupation description (25/68). Other occupations included housewife (16/68), student (12/68), and public employee (4/68).

2.4.3 1397 compounds by GC-MS Screen

A total of 106 unique compounds were detected among 68 wristbands. The most commonly observed compounds, in almost every sample, were four phthalate acid esters and two musks galaxolide and tonalide. All compounds were grouped into one of eight classes: 3 fungicides, 4 herbicides, 26 insecticides, 5 flame retardants, 13 industrial compounds, 29 PAHs, 15 PCPPs, and 11 plasticizers. Figure 2.2 shows the distribution of each chemical class between communities, gender, and occupation.

Figure 2.3 displays the profile of insecticides in each community. Additionally, the fungicides trifloxystrobin and tebuconazole were detected in 7 of the 15 wristbands from Rioja, and usually detected in the same wristbands. While herbicides were not significant as a class, 9 of 15 Rioja wristbands contained butachlor comprising 72% of the butachlor detections across the study. PAHs were detected in

greater proportions in wristbands from Rioja, Tingana, and Yantaló than Moyobamba. In contrast, samples from Moyobamba had the greatest proportion of PCPPs seemingly due to more detections of musk ketone, benzophenone, and benzyl benzoate.

In confirmation of the above results, a hierarchical cluster analysis identified six clusters of samples (Figure A2) that largely resembled differences identified in Figure 2.2. Together these results demonstrate patterns of potential chemical exposure between groups.

2.4.4 Pesticides by ECD

Among the compounds common to both the GC-MS screen and ECD methods we identified 61 of the same detections. We identified 532 more pesticide peaks in the ECD chromatograms and only three peaks were identified solely by the GC-MS screen. In all, 16 pesticides: 15 insecticides and one herbicide, were identified among 65 wristband samples. Three compounds were detected in more than 50% of wristbands: chlorpyrifos (59/65 wristbands, 17-9000 ng/g), cypermethrin (46/65 wristbands, 77-7000 ng/g), and DDx (63/65 wristbands, 8.8-5400 ng/g). The other compounds measured in wristbands were (in decreasing frequency) fipronil sulfide, dieldrin, dacthal, deltamethrin, permethrin, dimethoate, prophos, o,p'-dicofol, β-hexachlorohexane, α-chlordane, endosulfan-I, fipronil sulfone and λ-cyhalothrin. The quantitative method did not target DEET which was commonly detected in the GC-MS screen.

Differences between demographics for the three most common pesticides are presented in Figure 2.4 and results of optimized multiple linear regressions in Table 2.2. Accounting for everything else, age had the most significant effect on cypermethrin and DDx concentrations but was not associated with chlorpyrifos. Occupation had no effect on the concentrations of these compounds although, for chlorpyrifos there was a significant interaction between occupation and community. Gender was not a significant parameter for any of the three models but it was included in the optimized model for DDx, probably due to being near the 0.05 significance level. After optimization, the model fits were better than age by itself but did not surpass an r^2 value of 0.40.

DDT ratios ranged from 0 to 9.5 (Figure A3). Thirty-one wristbands (48%) had DDT ratios greater than one, reflecting relatively recent DDT exposure. The median DDT ratio in wristbands from Tingana was significantly greater than each of the other communities (Wilcoxon sum rank test, alpha = 0.05). While the median DDT ratio in Yantaló wristbands was less than one, several participants had elevated ratios suggesting that there are mixed sources of DDT in that community.

2.5 Discussion

The return rate of wristbands (92%) was quite good considering that one month is the longest wristbands have been worn for a study. Similar compliance success with silicone wristbands has been documented in studies with shorter deployment periods.[14,15] The participants were well distributed in gender and age but those from Tingana and Moyobamba were either all "farm worker" or "other," respectively. Moyobamba is the capital of the Department of San Martin and the largest community in this study. Residents of Moyobamba may have more diverse occupations than small farming villages like Tingana. Therefore, the occupations of participants in this study may reflect real differences between communities.

2.5.1 Distribution of diverse chemicals among wristbands

When grouped by chemical class, patterns of exposure appear among the wristbands (Figure 2.2). The strongest differences were observed between communities, suggesting regional patterns of exposure. Relatively low numbers of PAHs and a high proportion of PCPPs distinguished wristbands worn in Moyobamba from the other communities. As the largest city in this study, the pattern of chemical classes detected in Moyobamba wristbands may represent an urban signature of the Alto Mayo. The other three communities had more PAHs and pesticides than Moyobamba which may generally represent rural exposures.

The most dramatic difference between communities was the detection of trifloxystrobin and tebuconazole in wristbands from Rioja. Trifloxystrobin and tebuconazole are commonly used together in several pesticide formulations registered for use on rice in Peru.[29] Although not significant, Rioja also had markedly more hits of herbicides than other regions, the majority of which were butachlor. Participants from Rioja probably contacted products or residues containing trifloxystrobin, tebuconazole, and butachlor while wearing the silicone wristbands. Participants with these compounds were of mixed gender and occupation so more information is necessary to understand which community members in Rioja were handling these chemicals.

More insecticides were detected in wristbands from Tingana and Yantaló than the other communities. The profile of specific insecticides detected in wristbands from each community may reflect different use patterns (Figure 2.3). DEET, DDE, and DDD were detected in every community. DDT and piperonyl butoxide were only detected in wristbands from Tingana and Yantaló. Chlorpyrifos was detected at the highest rate in Rioja wristbands (50% of insecticides in Rioja compared to 8.8 and 11% in Tingana and

Yantaló, respectively) but was never detected in Moyobamba. Pirimiphos-methyl was uniquely identified in Moyobamba. We speculate that the capital city Moyobamba may use some pesticides, such as pirimiphos-methyl, for residential pest control while the agricultural communities of Rioja, Tingana, and Yantaló may use chlorpyrifos occupationally. Indeed, farm workers' wristbands had more insecticides than other occupations (Figure 2.2).

Wristbands worn by non-farm workers contained more PCPPs than wristbands worn by farmers. Nineteen of the 25 farm workers were men so insecticides and PCPPs were also significantly different between genders. Previous studies showed greater concentrations of benzophenone in urine of women than men,[30,31] which supports the generalization that PCPPs such as perfumes and lotions are more commonly used by women. Regardless of gender, more PCPPs were detected in wristbands from Moyobamba than other communities (Figure A4). Although not significant, we also observed more flame retardants in wristbands worn by non-farm workers. Therefore PCPPs, and potentially flame retardants, may also be chemical signatures of urban living in the Alto Mayo.

We observed weak evidence ($p = 0.0167$), although not significant, that women had more PAHs in their wristbands than men (Figure 2.2). By examining each region individually, we observed more PAHs in wristbands of women than men from Rioja and Yantaló but not in Moyobamba or Tingana (Figure A5). PAHs can come from many sources but one gender-specific hypothesis is that women are more likely to work with cooking fires resulting in disproportionate exposure to PAHs.[32,33]

Plasticizers were the most commonly detected compounds in all groups. Small amounts phthalates may come from laboratory contamination or the wristband itself but chromatographic responses of phthalates were 100 to 10,000 times greater in deployed samples than trip, field, or laboratory blanks. Phthalates have been measured in skin wipes,[34] as metabolites in urine,[35] and nails,[36] and identified as some of the most pervasive environmental contaminants.[37] Perhaps because of pervasive phthalate exposure, we did not observe any trends in the detection of these compounds. Quantitative assessment of phthalates may resolve differences between groups.

Hierarchical clustering identifies closely related samples without forcing associations between samples or analytes. Therefore, several of the major chemical groups that were identified in Figure 2.2 also cluster naturally (Figure A2). For example, the wristbands from Rioja that contained the fungicides trifloxystrobin and tebuconazole were clearly distinguished. Wristbands worn in Moyobamba only grouped in two of the six clusters. Those clusters were generally characterized by either PCPPs or the

absence of defining chemicals. Two clusters of wristbands that seemed to have the most PAHs were comprised of mostly female non-farm workers from Tingana, Yantaló, and Rioja. A group of mostly male farm workers from Tingana and Yantaló that clustered together were more likely to have DDT, DDE, DDD, and piperonyl butoxide, a finding corroborated in Figure 2.3. These results further demonstrate chemical signatures associated with lifestyle.

2.5.2 Distribution of individual pesticides

Of the compounds studied here, pesticides may pose the greatest health risks to residents in the Alto Mayo because of potentially high exposures and specific toxic modes of action, for example acetylcholinesterase inhibition by chlorpyrifos and other organophosphate insecticides. Additionally, because pesticides are intentionally applied, they might be the most manageable chemical exposure through education and improving access to personal protection equipment.[21,38,39] Therefore, we further evaluated the distribution of pesticides among the wristbands.

Some trends observed in the screening results were also apparent for the masses of chlorpyrifos, cypermethrin, and DDx in wristbands. For example, Moyobamba tended to have the lowest concentrations of those pesticides, even after accounting for other variables (Table 2.2). Occupational differences were not significant for the top pesticides (Figure 2.4), although chlorpyrifos and DDx would be significantly greater in farmer's wristbands at a more lenient significance level. Overall, broad demographic factors explained a surprising amount of differences in chemical exposure, but while we were able to explain up to 40% of the variability ($r^2 \leq 0.40$), most still remains unexplained. Naturally, a person's exposure is not only determined by the size of her community or her occupation. Many factors contribute to differences in pesticide exposure. Participants who did not self-describe as a farm worker may have also helped to mix or apply pesticides, and exposure could be distributed through pesticide handling and storage,[21,22] and medicinal uses.[40] Anecdotes from Tarapoto, near the Alto Mayo, reported that pesticides are often stored in the home,[18] as has been documented for rural farmers in Africa,[21,22] and that other family members will help mix and apply pesticides.[19] It is also possible that pesticides applied domestically would confound differences between occupations.

Consistent with screening results that show more insecticides with increasing age (Figure A6), cypermethrin and DDx were significantly associated with age (Figure 2.4) although the correlation coefficients were low. Correlation of wristband concentrations with age may reflect legacy contamination. Lasting exposure to lipophilic chemicals could accumulate in skin and become a source to the wristband as O'Connell *et al.* postulated.[8] This effect would be greatest for chemicals that are persistent and those

that are applied directly to the skin. Pyrethroids and DDT can be used in topical medicines, for example to treat scabies,[40] so there is potential for especially extensive dermal contact which could accumulate over time. DDT has a long history of use in Peru for malaria control but ceased *circa* 1990.[41] Lange[18] detected DDE, the main metabolite of DDT, in the blood of farm workers of Tarapoto, a city near the Alto Mayo. The DDE plasma concentrations were also correlated with the age of participants. Because DDT itself was not detected in blood, Lange concluded that DDT is no longer used in the region, but persists in the tissue of the farm workers.

The diagnostic ratio of DDTs in wristbands suggested that there are recent applications of DDT in the Alto Mayo. Participants from Tingana some participants from Yantaló had DDT ratios much greater than one (Figure A3). Tingana and Yantaló are neighboring villages so some community members may share exposures that the Rioja and Moyobamba do not. This forensic technique was first applied to silicone wristbands by Donald *et al.*[14] who addressed potential differences in sampling rate between DDT and its metabolites. Degradation of DDT during GC analysis could also influence the DDT ratio,[28] an artifact that we minimized with randomized sample analysis. Both would decrease the apparent DDT ratio so, if anything, bias the results toward a historical signature and our conclusions would not change: wristbands from Tingana had the greatest DDT ratios, suggestive of recent application.

2.5.3 Context

To get an overview of what pesticides and other compounds might be present in the environment in the Alto Mayo, we conducted an initial survey of bioavailable contaminants in the surface water of the Alto Mayo in August 2013. Details on methods and results are presented in the Appendix. Many of the compounds detected in wristbands were also identified in surface water. Chlorpyrifos, DDTs, cypermethrin, λ -cyhalothrin, butachlor, tonalide, cashmeran, biphenyl, sulfur and PAHs were measured at sampling locations downstream of significant agriculture, *i.e.* every location except Aguas Verdes. The fungicide cyclafuramid was the only compound detected in surface water but not in wristbands. Chlorpyrifos was especially pervasive in the Alto Mayo as it was the most concentrated pesticide in every water sample downstream of agriculture and in 91% of wristbands. This likely reflects widespread application of chlorpyrifos for agricultural or domestic pest control.

Several of the pesticides detected in this study were reportedly used in the nearby city of Tarapoto: tebuconazole, cypermethrin, methamidophos, permethrin, and butachlor.[18] Chlorpyrifos was not among the chemicals reportedly used in Tarapoto and it was not a target analyte in analyses of surface water and

blood samples there.[18,19] Most of the pesticides detected in the current study, including chlorpyrifos but not DDT, are registered for agricultural use in Peru.[29] However, some pesticides may also be used in medicine or vector control. Pesticide labels might not accurately reflect the contents, instead containing contraband material. Donald *et al.* found that approximately half of the pesticides detected in silicone wristbands worn by West African farmers were reportedly applied[14] so we expect to observe chemicals not explicitly used. Additionally, Donald *et al.* found many of the same compounds as reported in the current study including cypermethrin as one of the most commonly detected. However, chlorpyrifos and DDT were detected in less than 50% of wristbands. Instead, deltamethrin and λ-cyhalothrin were more prevalent compounds in West Africa.

2.5.4 Wristband applications and limitations

As passive sampling devices, silicone wristbands sample organic compounds in a partition-based method, similar to partitioning into lipid tissue. Because each chemical has its own affinity for the silicone polymer, we have intentionally refrained from comparing the presence or concentrations of different chemicals. By incorporating chemical-specific partitioning to silicone we can perform some simple comparisons. Chlorpyrifos, cypermethrin, and DDT have $\log K_{ow}$s of 4.96, 6.60, and 6.91 respectively, and therefore have high affinities for silicone wristbands and lipid tissue. They are by design preferentially sampled over polar compounds. With a $\log K_{ow}$ of 6.8, λ-cyhalothrin has similar affinity for silicone wristbands, but was only detected in a few cases. Therefore we know that participants in this study were more commonly exposed to chlorpyrifos, cypermethrin, and DDT than they were to λ-cyhalothrin. However compounds with $\log K_{ow} < 0$, *e.g.* caffeine and methamidophos, were also detected in wristbands deployed in the Alto Mayo and the ability of silicone wristbands to capture hydrophilic compounds has been observed previously.[8] Methamidophos was reportedly used in nearby Tarapoto[18] and an empty bottle was observed in the field during this study but was only detected in one of 68 wristbands in the Alto Mayo. Methamidophos is likely much more pervasive than the wristbands are able to detect.

Silicone wristbands, as deployed in this study, are multi-media samplers. Therefore we cannot infer to specific routes of exposure. We assume that internal exposure is underestimated because wristbands do not account for ingestion, an important route of exposure to chemicals such as organophosphate pesticides.[42] Finally, the current work was limited in the number of participants and relied on non-random recruitment so we cannot infer to the population level.

2.6 Conclusions

Using silicone wristbands, we can identify chemical mixtures in the personal environment that reflect lifestyle of participants and we are able to infer about potential sources of exposure. These multi-class profiles are important identifiers of typical mixtures that people of similar lifestyles would experience. Silicone wristbands are an excellent complement to health research, especially in remote regions where practical constraints might hinder use of other techniques. Future work can use wristbands to associate elevated wristband exposure with adverse health effects such as between insecticides and acute pesticide poisoning or neurobehavioral development.[3] More development is needed to relate the wristband results to environmental concentrations and internal exposure.

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2.8 Conflict of interest statement

Kim Anderson, an author of this research, discloses a financial interest in MyExposome, Inc. which is marketing products related to the research being reported. The terms of this arrangement have been reviewed and approved by Oregon State University in accordance with its policy on research conflicts of interest.

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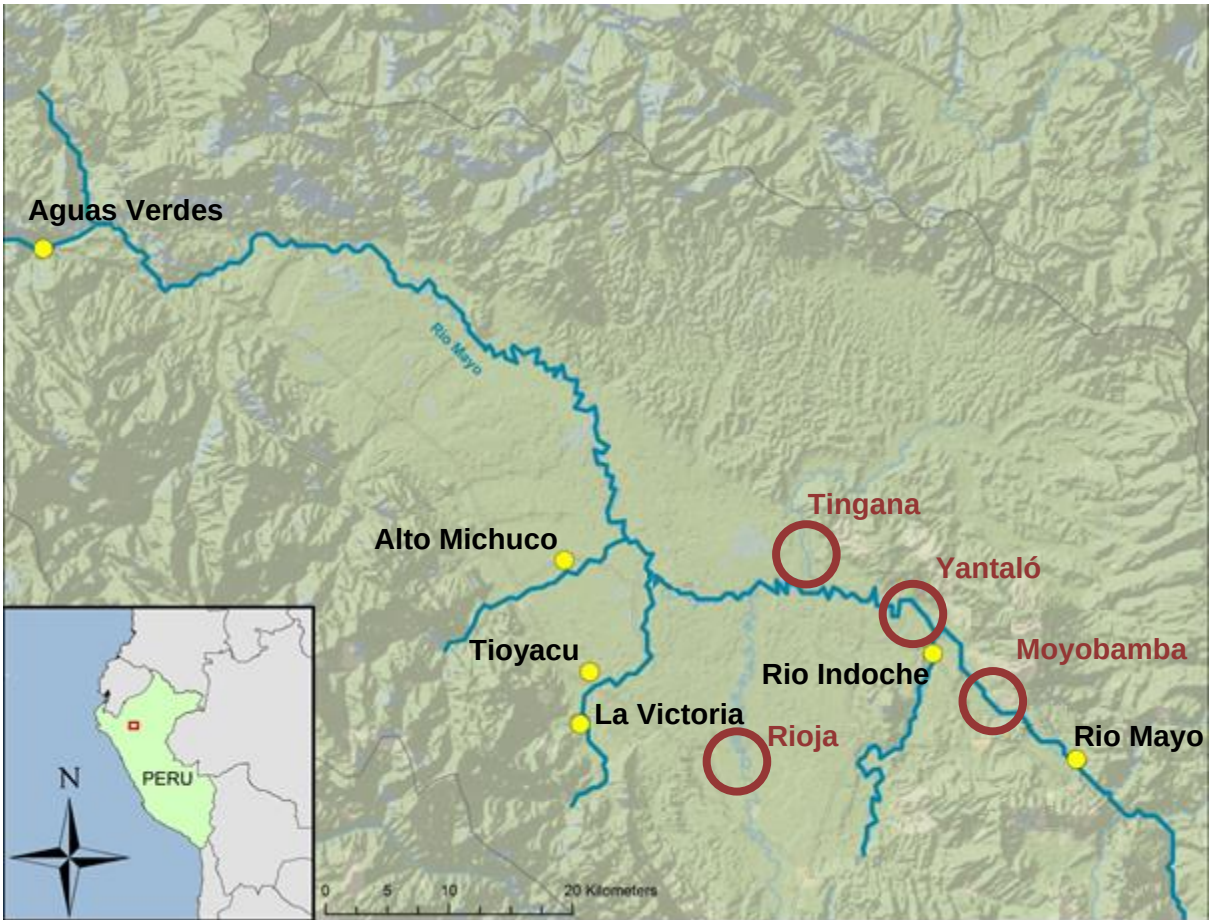

Figure 2.1. Alto Mayo region of Peru. Closed yellow circles (●) indicate the locations of stationary low-density polyethylene passive samplers that were deployed in 2013 (see Appendix). Silicone wristbands were distributed in four communities in 2014 (open red circles, ○). The Rio Mayo flows to the Southeast.

Table 2.1. Major demographics of participants who wore silicone wristbands.

		gender		age			occupation	
		female	male				farm worker	other
location	n	(%)	(%)	min.	median	max.	(%)	(%)
Moyobamba	15	66.7	33.3	6	36	63	0.0	100.0
Rioja	15	53.3	46.7	12	28	73	33.3	66.7
Tingana	13	30.8	69.2	19	50	59	100.0	0.0
Yantaló	25	52.0	48.0	8	45	70	28.0	72.0
total	**68**	**51.5**	**48.5**	**6**	**39**	**73**	**36.8**	**63.2**

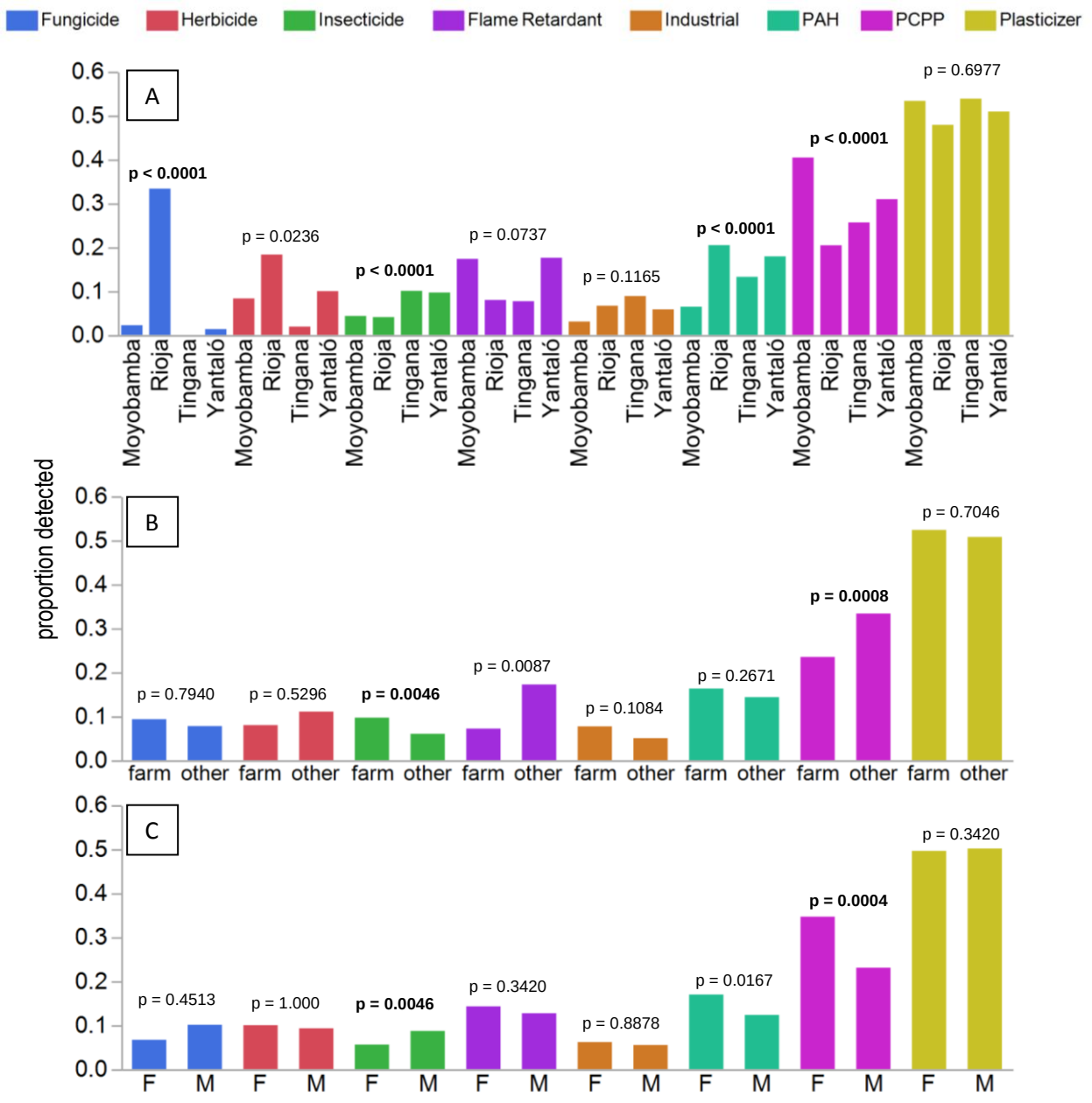

Figure 2.2. Proportion of positive detections for 8 chemical classes detected in silicone wristbands worn by residents of the Alto Mayo. Differences between communities (A) were evaluated with chi-square likelihood ratio test: n = 15,15,13,25 for Moyobamba, Rioja, Tingana, and Yantaló, respectively. Gender (B) and occupation (C) were compared with Fisher's Exact Test. P-values in bold are less than the significance level of 0.0063, adjusted for multiple comparisons. farm: farm worker (n=25), other (n = 43), M: male (n = 33), F: female (n = 35). The proportions are out of 3 fungicides, 4 herbicides, 26 insecticides, 5 flame retardants, 13 industrial compounds, 29 PAHs, 15 PCPPs, and 11 plasticizers.

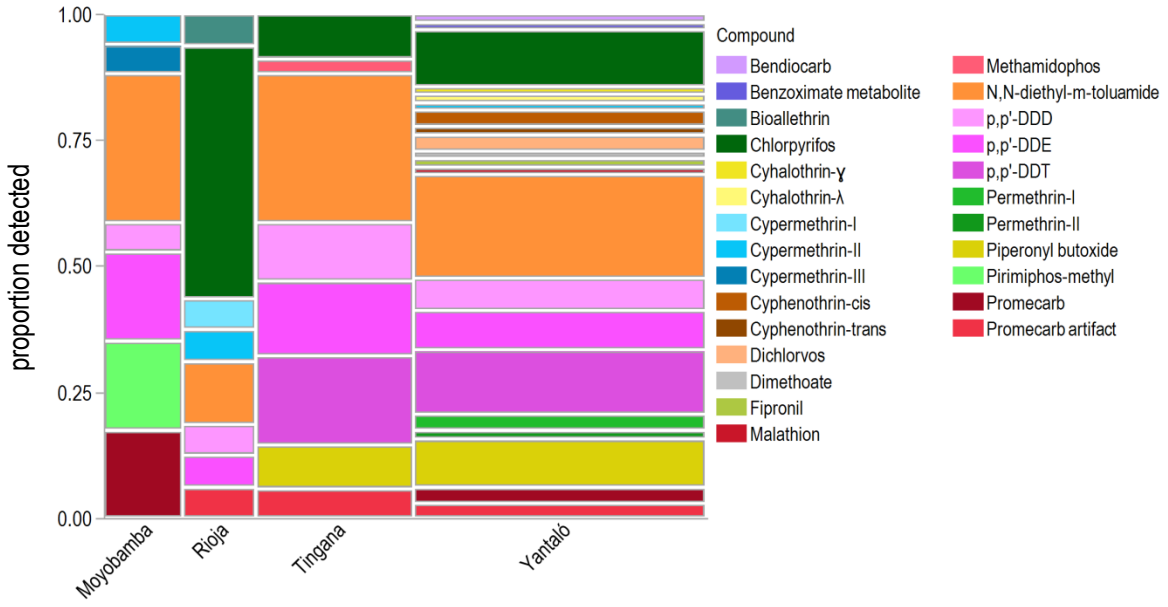

Figure 2.3. Relative contribution of individual insecticides to the total number of detections in silicone wristbands worn in each of four communities in the Alto Mayo. The width of the columns is proportional to the number of detections in wristbands from that community; Moyobamba: 17 detections in 15 wristbands, Rioja: 16 detections in 15 wristbands, Tingana: 34 detections in 13 wristbands, and Yantaló: 63 detections in 25 wristbands. The height of each colored box represents the proportion that an individual compound contributes to the total detections in that community. Isomers of cypermethrin and permethrin are not specified in the DRS library so are listed in retention order with roman numerals.

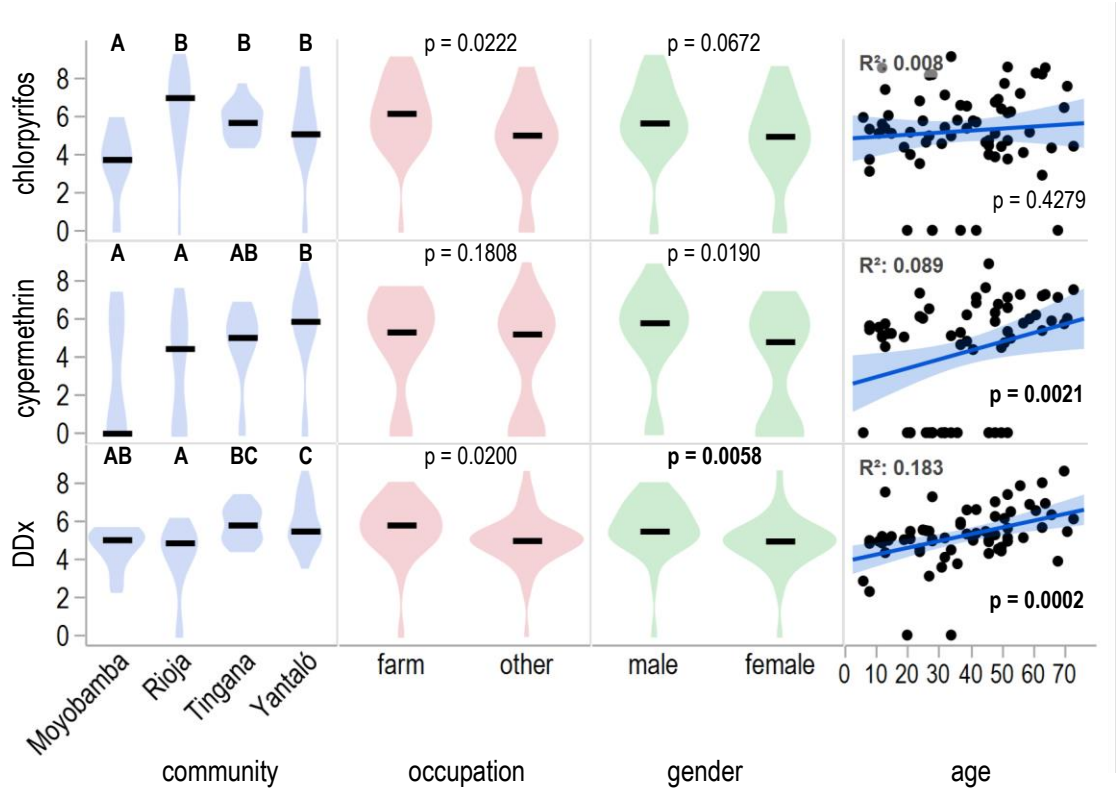

Figure 2.4. Differences in *ln*(concentration +1) of the most common and abundant pesticides measured in silicone wristbands between major demographic groups in the Alto Mayo. Horizontal bars indicate the median and shaded regions represent the distribution of the data. Community was compared using ANOVA with Wilcoxon rank sum tests; letters connect groups that are not statistically different (for statistical details, see Table A2). Occupation and gender were compared with Wilcoxon rank sums test, and the slope of age was assessed with a robust line of best fit. P-values are bold if lower than the adjusted significance level of 0.0167.

Table 2.2. Effect estimates from multiple linear regressions modelling the concentration of three pesticides in wristbands (ng/g wristband).

	Parameter[1]	Estimate	SE[2]	P[3]	r^2
ln(chlorpyrifos)	Intercept	5.45	0.21	**<0.0001**	0.39
	Community				
	Yantaló-Moyobamba	0.78	0.33	**0.0221**	
	Tingana-Moyobamba	0.52	0.56	0.3645	
	Rioja-Moyobamba	1.30	0.31	**<0.0001**	
	Occupation (farm worker)	-0.26	0.36	0.4835	
	Occupation*Community	1.10	0.47	**0.0239**	
ln(cypermethrin)	Intercept	5.08	0.30	**<0.0001**	0.30
	Community				
	Yantaló-Moyobamba	0.41	0.20	**0.0497**	
	Tingana-Moyobamba	-0.51	0.24	**0.0415**	
	Rioja-Moyobamba	-0.23	0.27	0.3854	
	Age	0.02	0.01	**0.0056**	
ln(DDx)	Intercept	4.37	0.30	**<0.0001**	0.40
	Community				
	Yantaló-Moyobamba	0.54	0.19	**0.0071**	
	Tingana-Moyobamba	0.29	0.25	0.2341	
	Rioja-Moyobamba	-0.39	0.24	0.1148	
	Gender (male)	0.24	0.13	0.0746	
	Age	0.02	0.01	**0.0016**	

[1] The parameters community, occupation, gender, and age were used in building these regressions. Reference level is female non-farm workers from Moyobamba.
[2] SE: standard error
[3] p = p-value at significance level of 0.05.

USING PASSIVE SAMPLING AND ZEBRAFISH TO IDENTIFY DEVELOPMENTAL TOXICANTS IN COMPLEX MIXTURES OF BIOAVAILABLE HYDROPHOBIC ORGANIC COMPOUNDS

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Chapter 3 Using passive sampling and zebrafish to identify developmental toxicants in complex mixtures of bioavailable hydrophobic organic compounds

3.1 Abstract

Using effects-directed analysis (EDA), we investigate associations previously observed between PAHs and embryotoxicity in field-deployed low-density polyethylene (LDPE). We conducted EDA using a zebrafish embryo assay and iterative fractionation of extracts of LDPE that were deployed in Portland Harbor Superfund Megasite, Oregon. Whole extracts induced toxicity including mortality, edema, and notochord distortion at EC_{20}s of approximately 100, 100, and 10 mg LDPE / mL, respectively. Through fractionation, we determined that PAHs at concentrations similar to previous research did not contribute markedly to toxicity. We also eliminated pesticides, phthalates, musks, and others identified in toxic fractions by testing surrogate mixtures. We identified free fatty acids as lethal components of LDPE extracts and confirmed their toxicity with authentic standards. We have chromatographic evidence that dithiocarbamates are responsible for notochord and other sub-lethal effects, although exact matches have not been obtained. Fatty acids and dithiocarbamates were previously unrecorded components of LDPE extracts and are likely contributing to the toxicity of the whole mixture. This work demonstrates the success of EDA in non-targeted hazard identification using the zebrafish embryo test as a self-contained battery of bioassays that allows identification of multiple chemicals with different modes of action. This is the first EDA to combine LDPE and zebrafish, approaches that are widely applicable to identifying developmental hazards in the bioavailable fraction of hydrophobic organic compounds.

3.2 Introduction

Countless chemicals are present in the environment as a result of natural and anthropogenic activities. As Neale *et al.*[1] have demonstrated, targeted analysis can often only describe a fraction of the toxicity of environmental mixtures. It is impractical to identify and investigate the toxicity of every chemical in the environment so there is a need to prioritize analysis to specific potential hazards.

A first tier approach for toxicant identification is to use mathematical approaches to deconvolute associations between toxicity and chemistry. For example, Allan *et al.*[2] compared the developmental toxicity of 50 samples collected with low density polyethylene (LDPE) passive sampling devices (PSDs) from the Portland Harbor Superfund Megasite (PHSM), in Portland, Oregon. Using multivariate statistics, they observed some correlations between toxicity and polycyclic aromatic hydrocarbons (PAHs), but could not explain the majority of toxicity. In this study we expand on their methods, applying an effects-directed approach to empirically identify responsible toxicants in PSD extracts.

Effects-directed analysis (EDA) is a framework for simplifying complex mixtures to identify specific toxicants through iterative fractionation and toxicity testing.[3] The concept of bioassay-directed fractionation is established,[4,5] but recent advances in chromatographic techniques and *in vitro* toxicity testing have resulted in a customizable and growing toolbox.[6] PSDs accumulate freely dissolved compounds (C_{free})[7] by passive diffusion from the same pool of compounds that is available to concentrate in organisms. Thus they address the need to incorporate bioavailability into toxicity assessments of hydrophobic organic compounds (HOC) mixtures and provide an ideal starting place for EDA which has been described as analogous to a phase II toxicity identification evaluation. [8,9] PSDs capture a time-integrated sample including episodic events that might be missed in a grab sample, and accumulate trace contaminants throughout deployment, lowering the environmental detection limits in chemical or bioanalytical tests and eliminating the need to extract and concentrate hundreds of liters of water.[1] Semipermeable membrane devices (SPMDs)[7,10] and silicone rubber[11,12] have been among preferred configurations for testing HOCs because they sample sufficient mass for the iterative fractionation and bioassays characteristic of EDA. Triolein-free LDPE is overtaking SPMDs as preferred configurations for chemical analysis[13-15] but few studies have applied bioanalytical tests to LDPE samples.

Zebrafish (*Danio rerio*) are a well-established model organism for early life stage toxicity testing.[16-18] They have high fecundity, are transparent during development, and their genome is fully sequenced.[19] The zebrafish embryo test (ZFET) is well suited as a bioanalytical screen because it can be performed in multi-well microtiter plates, requires relatively small sample volumes, and much of the embryo and liquid

handling can be automated.[20,21] As an *in vivo* assay, many endpoints corresponding to different modes of action can be assessed in one exposure. Simply observing morphological changes of the transparent embryos can identify toxicity and generate mechanistic hypotheses. Passive sampling devices (PSDs) and the zebrafish bioassay have been shown to be compatible and informative tools [2,22], but have not been employed together in EDA.

PAHs are historically of concern for potential carcinogenicity[23] and are re-emerging contaminants of concern for their developmental toxicity.[24-26] PAHs are contaminants of concern at the PHSM in the Willamette River, Oregon, where they drive remediation at several locations.[27] As products of incomplete combustion and as components of petroleum, PAHs inevitably occur as mixtures of unsubstituted and substituted (*e.g.* alkylated, oxygenated) compounds, further complicating toxicological profiling. We focus on PAHs because they were implicated as possible contributors to developmental toxicity of LDPE extracts from PHSM.[2] However, PAHs occur *in situ* in complex mixtures with many other HOCs, possibly with their own significant teratogenic potential.

In the present study, we further investigate the toxicity of LDPE extracts from the PHSM. Using the framework of EDA, our objectives were to (1) empirically determine the contribution of PAHs to developmental toxicity of LDPE extracts from PHSM, (2) identify and reject other specific compounds as contributors to toxicity and (3) demonstrate that coupling LDPE and zebrafish are effective tools in EDA. We report simple fractionation and screening methods to identify responsible toxicants in LDPE extracts and, when possible, we confirmed toxicity with authentic standards. These methods are approachable and can be broadly applied to identify potential hazards among HOC mixtures in the environment. To our knowledge, this is the first EDA performed using LDPE passive samplers and zebrafish embryos to isolate components of toxicity.

3.3 Methods

3.3.1 Chemicals

All solvents were purchased from Fisher Scientific (Pittsburgh, PA). Isopropanol, dimethyl sulfoxide (DMSO), and hydrochloric acid (HCl) were ACS grade. Dichloromethane (DCM) was Optima and n-hexane was GC-Resolv. Standards used for instrument calibration, *in situ* calibration of PSDs, and extraction surrogates were fluorene-d10, pyrene-d10, acenaphthene-d8, phenanthrene-d10, fluoranthene-d10, benzo[a]pyrene-d12, benzo[ghi]perylene-d12, perylene-d12 purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and benzo[b]fluoranthene-d12, naphthalene-d8, chrysene-d12

from C/D/N Isotope Inc. (Quebec, Canada) at >99% purity. A gel permeation chromatography (GPC) calibration solution containing corn-oil, methoxychlor, phthalate, perylene, and sulfur was purchased from Restek (Bellfonte, PA, USA). Bioassay medium was prepared by dissolving 0.3g/L Instant Ocean (Blacksburg VA, USA) in reverse osmosis water. Free fatty acids (FA), n-hexadecanoic acid (palmitic acid, PA) and n-octadecanoic acid (stearic acid, SA), were purchased (>99%) from Nu-chek Prep (Waterville, MN, USA). Water was filtered to 18 MΩ*cm through an in-house Milli-Q (Merck; Darmstadt, Germany) purification system. Additional compounds used for toxicant confirmation are listed in Table B1.

3.3.2 Site description

The PHSM, on the Willamette River, Oregon, extends from 1.9 to 11.8 miles (3 to 19 km) from the confluence with the Columbia River (Figure B1). For this study, four sampling sites were chosen within and near the PHSM to maximize differences in PAH concentrations and bioactivity. River mile (RM) 18.5 is upstream of the PHSM but is downstream of other agriculture and industry, and is near the confluence with Johnson and Kellogg creeks which drains much of Southern Portland. Within the PHSM, RM 11 East (E) and 3.5 West (W) were selected because historically they are high in polychlorinated biphenyls or PAHs, respectively. RM 1 Northwest (NW) is downstream of PHSM, close to the confluence with the Columbia Slough.

3.3.3 PSD preparation and deployment

As described in Anderson *et al.*,[13] PSDs were constructed from LDPE tubing (2.5 cm wide by 70 µm thick) obtained from Brentwood Plastics, Inc. (St Louis, MO, USA). Cut to 100 cm, LDPE strips weighed 4.82 ± 0.08 g.[28] LDPE was placed in amber jars and washed with three rounds of hexane with gentle agitation for 48 hours. Clean LDPE was dried under vacuum for 48 hours, placed in pre-cleaned air-tight metal cans and stored at -20°C until deployment preparation.

PSDs that were used to calculate C_{free} were prepared with PRCs. The LDPE strips were heat-sealed in a loop at one end of the strips, PRCs (fluorene-d10, pyrene-d10, benzo[b]fluoranthene-d12) were pipetted to the inter-membrane space, air removed, and the open end heat-sealed in a loop. Prepared PSDs were stored in amber glass jars at -20°C until transport to the field. PRC-containing PSDs were deployed on steel frames, five of which were housed in a steel cage (Environmental Sampling Technologies; St. Joseph, MO, USA). One cage was deployed at each of RM 1NW, 11E, and 18.5. Method variability was

assessed by deploying triplicate cages at RM 3.5W. Details of PRC methods, dissipation, and the calculation of water concentrations are available in the Appendix.

The LDPE for bioassays was prepared as above but did not include PRCs. These strips were sealed at the ends and with a loop in the center. Forty strips were deployed at each site by stringing the center loops on pre-cleaned steel cable attached to cages. The co-deployed LDPE strips were suspended three meters below the surface of the water. The deployment configurations are shown in Figure B2.

After 21 days, PSDs were retrieved and transported in amber jars (PRCs) or metal cans (no PRCs) to the laboratory and stored at -20°C. Table B2 describes the deployment and site conditions. The LDPE had little visible biofouling. To remove any periphyton or particulates, the LDPE strips were cleaned in the laboratory by light scrubbing and sequential washing in 1 N HCl, 18 MΩ*cm water, and twice with isopropanol, then dried. Five dry PSDs were stored in amber glass at -20°C until extraction. PSDs were extracted twice at room temperature with 200 mL n-hexane. For quality control and PRC-containing samples, surrogate standards were added immediately before the first addition of n-hexane. No standards were added to the extracts intended for bioassay testing. The extracts were reduced to 1 mL at 40°C in Turbo Vap closed cell concentrators (Biotage, Uppsala, Sweden), then transferred to conical centrifuge tubes, rinsing the 500 mL vessel three times with small volumes of n-hexane and adding that material. The extracts were reduced to 1 mL under a gentle stream of nitrogen and transferred to amber glass vials, combining extracts from the same RM such that 8 mL represented 40 LDPE strips. Extracts were stored at -20°C.

3.3.4 Fractionation

LDPE extracts were first fractionated by GPC using a Waters (Misford, MA) 515 HPLC pump, 717 plus autosampler, 2487 dual-wavelength absorbance detector, and Fraction Collector II. Separations were performed using an Envirosep ABC column (350 x 21.20 mm with 60 x 21.20 mm guard column; Phenomenex, Torrance, CA) flowing 100% DCM at 5 mL/min, absorbance read at 254 nm, and a 1.5 mL injection volume. Three fractions were defined using a reference injection of 16 PAHs and a GPC calibration solution (Figure B3). Fractions collected, in general, large molecular weight compounds (fraction F1), PAHs and other semi-volatile compounds (fraction F2), and elemental sulfur (fraction F3). Fractions were collected in 500 mL Turbo-Vap vessels and concentrated as described for the whole extracts. The final volume of each fraction was 1.5 mL, matching the injection volume.

Second tier fractionation was conducted on an Agilent (Santa Clara, CA, USA) 1100 series HPLC with a diode-array detector attached to a Waters Fraction Collector II. Normal phase (NP) separation was achieved with a cyano-propyl column (4 x 250 mm, 5 µm pores; Phenomenex), and a mobile phase of 100% n-hexane flowing at 0.8 mL/min for 1 min followed by a gradient to 100% DCM over 1.5 min, holding for 15 min before returning to n-hexane and re-equilibrating. As observed with similar NP separations,[29] most of the separation of PAHs occurred prior to the DCM solvent front (Figure B4). Four 2-minute fractions were collected during fractionation of F2 because of limits on the resolution of PAHs on the analytical size cyano-propyl column. F1 of RM 3.5W was separated into eight 1-minute fractions collected from 4.5 to 12.5 min. Fractions from triplicate injections of 0.2 mL were combined and concentrated to 0.6 mL at 40°C under nitrogen. Samples were protected from light throughout the process.

3.3.5 Zebrafish

For use in bioassays, PSD extracts, fractions, and control samples were evaporated under a constant stream of nitrogen and exchanged to DMSO. Zebrafish husbandry, exposures, and evaluations were performed by the Sinnhuber Aquatic Research Laboratory according to methods modified from Truong *et al.*[30] Embryo chorions were removed at 6 hours post fertilization and for each treatment 32 zebrafish were individually exposed in 96-well polystyrene plates. Exposure solutions were delivered to each well using an automated liquid handler (D300e Digital Dispenser, Hewlett Packard, Palo Alto CA, USA). Toxicity results were evaluated as a function of the mass of LDPE represented by the exposure solutions. Final exposure solutions represented 60 to 480 mg LDPE/mL in 1% DMSO. A 1% DMSO control was included on every plate. The plates were covered to reduce evaporation and were shielded from light with aluminum foil. The assay plates were maintained at 28 ± 1°C. A technician observed mortality and 20 sub-lethal effects including pericardial edema and notochord distortions at 24 or 120 hours post fertilization.

3.3.6 Chemical analysis

We quantified 62 PAHs on a modified Agilent 7890 gas chromatograph (GC) and Agilent 7000 triple quadrupole mass spectrometer (MS/MS) as described previously.[31] The internal standard, perylene-d12, was added to each sample or parallel aliquots of bioassay samples immediately prior to analyses.

We screened for 1397 compounds with methods adapted from Allan *et al.*[2] The updated method used an Agilent 7890A GC with 5975C MS scanning from 50 to 500 mass to charge (m/z) for 29 min, 100 to 660

m/z between 29 and 39 min, and 200 to 975 m/z from 39 to 54 min and our library contained >200 more compounds. The total ion chromatograms were analyzed with automated mass spectral deconvolution and identification system (AMDIS, National Institute of Standards and Technology, Gaithersburg, MD, USA) and deconvolution reporting software (DRS, Agilent). The deconvolution software compared detected spectra to a customized list of polychlorinated biphenyls, pesticides, pharmaceuticals, personal care products, industrial compounds, and more. The complete list and additional AMDIS parameters are provided in the Appendix.

3.3.7 Toxicant confirmation

Authentic standards of PA and SA were used to confirm retention times and create an external calibration (Figures S5 and S6). PA and SA were tested for developmental toxicity as a binary mixture in the zebrafish embryos assay in a fixed ratio of 2:1 SA to PA based on early estimates of relative concentration in the LDPE extracts. Concentrations ranged from 5.78 to 92.5 μM total PA and SA, levels chosen to span concentrations of total measured in the whole extracts (Figure B7).

Surrogate mixtures of F1 and F2.3 were created with compounds identified with AMDIS. AMDIS also estimates the concentrations of tentatively identified compounds. We purchased compounds identified in toxic fractions (Table 3.1) and verified the AMDIS generated concentrations within an order of magnitude using a three point concentration series. Then, representative mixtures (Table 3.1) of those compounds identified in toxic fractions F1 and F2.3 were evaluated in the zebrafish assay.

3.3.8 Data analysis

Concentrations that would elicit 20% response (LC_{20} or EC_{20}) for mortality, pericardial edema, and notochord were predicted from a log-logit model fit of the concentration response (JMP Pro 12). Sub-lethal effects were not included in analyses for treatments with greater than 80% mortality.

3.3.9 Quality control

The relative standard deviation (n=3) for the C_{free} ΣPAHs at RM 3.5W was 2.36%. Field and trip blanks, each composed of one LDPE strip, accounted for chemical artifacts during deployment and retrieval of PSDs. Naphthalene and a few other low molecular weight PAHs were measured at low concentrations in the field blanks. The background was subtracted from extract concentrations before calculating C_{free}.

Recovery of PAHs during fractionation was assessed by analyzing each fraction and comparing the total PAHs among the fractions to their parent sample. Among the GPC fractions, an average of 94% (SD = 7.61%, n = 4) of the PAHs in the whole LDPE extracts was recovered. Average recovery of PAHs after NP-HPLC fractionation of F2 was 120% (SD = 4.13%, n = 4).

In the ZFET, the average response for any single endpoint in the DMSO controls was 5.1% (SD = 5.5%). Non-deployed LDPE has been shown to be free of toxic artifacts [22]. Fractionation blanks were tested for toxicity artifacts. Each elicited less than 20% effect for any recorded endpoint (Figure B8). Toxicity in individual fractions generally recapitulated individual endpoints, indicating no loss due to fractionation for those samples. Reconstitution also tests that no loss of toxicity was due to the sample manipulation. Using the automated liquid handler, fractions were recombined for the ZFET. Toxicity of reconstitutions was compared to the parent samples: either the whole extract for GPC or F2 for NP-HPLC. The maximum tested concentration for the reconstitutions was the equivalent of 160 or 120 mg LDPE/mL for GPC and NP-HPLC, respectively. This was sufficient to recapitulate notochord distortion and pericardial edema for every sample except RM 1NW. Mortality was a less sensitive endpoint and predicted LC_{20} values were beyond the maximum concentration for tier one reconstitutions. For every sample in the NP fractions, LC_{20}s of the reconstitutions were within tested concentrations but the upper bounds of the 95% prediction intervals were extrapolated.

3.4 Results and discussion

3.4.1 Characterization of PAHs in LDPE extracts

The extracts of the LDPE deployed in PHSM contained an order of magnitude difference in total PAH concentrations. The lowest PAH concentrations were from RM 18.5, upstream of the PHSM. The highest concentrations were at RM 3.5W, within the Superfund and downstream of PAH priority clean-up zones.[27]

The bioassay exposure solutions represented up to 480 mg LDPE/mL and contained maximum nominal concentrations of total PAHs ranging from 127.2 to 898 μg/L (0.638 to 4.40 μM, Figure 3.1A). The most abundant PAHs were fluoranthene (0.173 to 1.38 μM) and pyrene (0.089 to 1.23 μM), which together comprised at least 62% of total PAHs, followed by retene and phenanthrene at 0.0676 to 0.195 μM and 0.0248 to 0.238 μM, respectively. Environmental concentrations of PAHs were calculated based on dissipation of PRCs from caged LDPE (Table B4). The total PAH C_{free} ranged from 7.79 (RM 18.5) to 48.7 ng/L (RM 3.5W). Bioassay concentrations of individual compounds were greater than environmental

levels by factors of approximately 100 to $4.5x10^4$. The total PAH concentrations in the exposure solutions were greater than the environmental concentrations by factors from 1.1×10^4 to 2.6×10^4.

In preceding work, Allan *et al.* reported the developmental toxicity of 50 LDPE samples from the PHSM in 2009 and 2010.[2] To evaluate our results in context with previously tested samples from the PHSM, we compared the 33 PAHs common to the analyses of this study and Allan *et al.* (Figure B9). The range in magnitude of Σ_{33}PAHs is similar between the studies and principal components analysis (discussed in detail in the Appendix) suggests that the distribution of PAHs is also similar between the two studies. Given the similarity in PAH content of samples collected in 2014 to those in the larger sampling campaigns of 2009-10, we anticipate that we can infer to the PHSM at large using our conclusions regarding the potential developmental toxicity of PAHs in LDPE extracts.

All four LDPE extracts elicited concentration-dependent mortality and sub-lethal effects including pericardial and yolk sac edemas, distorted notochord, bent axes, and craniofacial malformations. Many of the observed endpoints are highly correlated so three representative endpoints evaluated at 120 hours post fertilization were chosen to demonstrate the toxicity results: mortality, pericardial edema (edema), and notochord distortions. Cardiotoxicity, including edema, has been observed as an effect of embryonic zebrafish exposure to individual PAHs including retene,[25] pyrene,[24] and mixtures of PAHs as in crude oil[32] so was selected as a potential indicator of PAH toxicity. Distortion of the notochord was the most sensitive endpoint, observed in this study in almost 100% of fish exposed to the lowest concentrations tested. Variation in toxicity did not correspond to PAH concentrations. The EC_{20}s for mortality, notochord, and edema were similar between RMs (Figure 3.1, whole extract) as indicated by overlapping 95% prediction intervals.

3.4.2 Fractionation

In the first tier fractionation, the LDPE extracts were separated into three fractions with GPC. On average, 97% (SD = 1.15%) of recovered PAHs eluted in F2 (Table B5). Toxicity was observed in F1 and F2, but not F3. F1 induced edema and notochord distortions at levels that closely matched the whole extract, but was not lethal at any concentration (Figure 3.1). The PAH-containing fraction, F2, caused mortality at concentrations similar to the whole extract for all sites except RM 11E. Some edema was also observed in F2 for RMs 1NW and 3.5W. While PAHs are major components of F2, other compounds identified with AMDIS and observed in the chromatogram could have been contributing to toxicity, warranting further fractionation.

F2 was separated into four fractions by NP-HPLC. We observed elution of PAHs from low to high molecular weight as previously described.[33] Fractions containing the majority of total PAHs, *i.e.* F2.1 and F2.2, were not toxic to zebrafish embryos (Figure 3.1). In contrast, NP fractions with predominately non-PAH components, *i.e.* F2.3 and F2.4, were lethal to zebrafish embryos although at slightly higher EC_{20}s than the whole extracts or F2. There was one instance each of edema and notochord in the second tier fractionation that the predicted EC_{20} was within the tested concentration range but the upper 95% prediction interval was beyond the maximum tested concentration so these observations cannot be considered statistically significant. RM 11E, which did not elicit toxicity in F2, regained toxicity in F2.4. This could be within the variability of the assay as the effect concentrations are nearing the maximum tested concentration. Some edema that was observed in F2 remains unexplained. Regardless, the levels of edema observed in F1 (discussed below) supersedes any that PAHs may contribute, indicating that PAHs are relatively impotent developmental toxicants in LDPE samples from PHSM.

In addition to PAHs, oxygenated PAHs, polychlorinated biphenyls, some personal care products, and sulfur were eliminated by eluting in non-toxic fractions. Manual investigation of the total ion chromatograms revealed that F2.1 consistently contained two unidentified compounds with comparable abundances to pyrene and fluoranthene. Because F2.1 was not toxic, we did not work to identify these compounds.

The fractions F1, F2.3, and F2.4 elicited toxicity similar to the levels observed in the whole extracts. Several phthalates and fragrance compounds that were consistently detected in F1 along with a few others are listed in Table 3.1. Bis (2-ethylhexyl) phthalate is a contaminant of concern in Portland Harbor [27] and was consistently detected in these LDPE extracts. Some phthalates may also originate as laboratory contamination. However, an order of magnitude separated the relative abundances of phthalates in environmentally deployed samples from quality control. While bis (2-ethylhexyl) phthalate was detected in every fraction, only in F1did its abundances recapitulate what was observed in the whole extracts. Some of the identified compounds have documented toxicity to zebrafish embryos. For example, di-n-butyl phthalate has been shown to induce axis defects in developing zebrafish[34] and the toxicities of galaxolide and tonalide to zebrafish embryos have also been documented.[35] However, there were many other major peaks apparent in the chromatograms of F1, warranting further fractionation. As there seemed to be very little variation in toxicity between RMs, a representative F1 from RM 3.5W was separated with NP-HPLC. Of eight fractions, only F1.6 affected zebrafish development, closely resembling the toxicity of F1 (Figure B10). F1.6 corresponded to the elution of the DCM solvent front. The majority of

compounds identified in F1 eluted in F1.6 but most of the non-target peaks were collected in non-toxic fractions, further implicating phthalates, musks and others listed in Table 3.1.

F2.3 contained 4,4'-DDD, benzanthrone, and benzophenone (Table 3.1). No compounds, except for some phthalates (discussed above), were identified with the DRS screen in F2.4 for any sample. Manual examination of the F2.3 and F2.4 chromatograms revealed free fatty acids as major components. In five of the six toxic NP fractions, the largest and most common peaks in the chromatograms of these fractions were PA and SA (Chemstation match factors = 99). The consistent toxicity and apparent isolation of FAs in F2.4 suggest that FAs are responsible toxicants.

3.4.3 Toxicant confirmation

Surrogate mixtures representing F1 and F2.3 caused no effect in the zebrafish bioassay. Those compounds were therefore deconfirmed as responsible toxicants. The multi-tier fractionation isolated the toxic responses of notochord distortion and edema in F1.6 of RM 3.5W (Figure B10) so there are a limited number of compounds left to be discovered. An overview of the components of F1.6 is diagrammed in Figure B11. The notochord distortion observed in samples from PHSM is a distinct corkscrew shape as documented previously for dithiocarbamates (DTCs).[30,36] Neither of two DTCs in our screening library was detected in samples from PHSM but several peaks in F1.6 display mass spectra that resemble DTCs, albeit with match factors that were typically less than 60 compared to entries in the NIST 2008 mass spectral library (Figure B12). DTCs are particularly potent toxicants to developing zebrafish and the combination of several related compounds may result in the effects observed here. More work is necessary to identify the specific chemicals causing toxicity in F1.

A surrogate mixture containing 12 to 190 μM total FA (PA + SA) was used to confirm fatty acid toxicity in the ZFET. Figure 3.2 compares the mortality rate of the surrogate mixture to that of the whole LDPE extracts in terms of their PA and SA concentrations. PA + SA was lethal to zebrafish embryos with an LC_{20} of 25 μM (95% prediction interval: 16-35). The LC_{20}s of whole LDPE extracts were generally lower than the surrogate mixture ranging from 5.5 μM (4.0-6.9, RM 3.5W) to 16 μM (13-19, RM 18.5). As a surrogate mixture, PA + SA demonstrated that FAs are capable of inducing mortality but did not recapitulate the magnitude of effects observed in the LDPE extracts. Free FAs are not well-suited to GC-MS. Although they have good linearity in the ranges measured in our samples, we observed high detection limits and fronting peak shapes. Therefore, undetected FAs are probably also present in the toxic fractions. Undetected non-FA components may also contribute to the toxicity of the whole mixture,

resulting in lower LC_{20}s. Concentrations of PA and SA decreased with fractionation (Figure B7) mirroring lower mortality rates in fractions compared to the whole extracts. Additionally, neither PA + SA nor FA-containing fractions, F2.3 and F2.4, induced sub-lethal effects, augmenting the evidence that FAs are responsible. Additional discussion about the distribution and plausible mechanisms of FA toxicity is presented in the Appendix B.

3.4.4 Possible sources of tentatively identified toxicants

PA and SA are essential FAs in plants and animals and are common compounds in organic matter excreted by algae.[37] Human activities can increase levels of FAs by inducing eutrophication, a common occurrence in urban waterways, and through discharge of wastewater effluent of which PA and SA are major components.[38] As surfactants, although FAs are highly hydrophobic and pre-dominantly in the anionic form, a neutral fraction would exist in the freely dissolved phase and be available for uptake into the PSD polymer. Haftka *et al.*[39] demonstrated that solid phase microextraction fibers can accumulate the neutral fraction of some FAs. PA and SA would be similarly available for uptake by LDPE. It may be impractical to regulate FAs because they are naturally pervasive and generally innocuous. While here we strived to expand on the methods of Allan *et al.*, other researchers may choose to remove FAs prior to toxicological evaluation. As for DTCs, they have many applications including as fungicides and as accelerators in rubber manufacturing[40] so can be present in the environment as a result of agriculture or leachate from rubber products.

Sabaliunas *et al.*[41] identified oleic acid as a major contributor to the toxicity of SPMDs to *V. fisherii*. They attributed the presence of oleic acid to the hydrolysis of methyl oleate impurities of triolein by periphyton communities that often grow on PSDs. This artifact of SPMDs is not possible when using triolein-free LDPE as in this study. Fatty acids may be used as lubricants during LDPE manufacturing[42] so could pose a source of artefactual toxicity if the LDPE is not properly conditioned. LDPE used in this study did not have residual fatty acids. Interestingly, after identifying FAs and DTC-like spectra as possible toxicants, we observed some similar peaks in some process blanks associated with post-deployment cleaning. As with phthalates, FAs and DTCs may be common components of laboratory equipment[43] so would be useful target analytes for researchers designing studies with embryonic zebrafish. Regardless of the source, FAs and DTCs were previously unrecorded components that likely contributed to the toxicity of LDPE samples from the PHSM.[2,22]

Although PAHs were previously associated with toxicity in LDPE samples from PHSM, in the current study, the isolated PAH component was relatively impotent. Toxicity was attributable to FAs and, we postulate, to DTCs. In the process, we eliminated many other target analytes. LDPE collects a predictable suite of chemicals so similar fractionation and analysis techniques could be applied to LDPE samples from any site. While PAHs were relatively non-toxic, it is possible that they drive other endpoints such as mutagenicity. However, we were able to incriminate multiple toxicants with different modes of action using zebrafish embryos, demonstrating the ZFET as a self-contained battery of bioassays. It would be possible to improve the assay and lower bioanalytical detection limits by adopting methods, such as passive dosing,[44] for better control of aqueous concentrations of HOCs. With considerations discussed in this paper, LDPE and ZFET are widely applicable to testing mixtures of hydrophobic organic compounds and would complement a comprehensive approach using a battery of PSDs[12] with a battery of bioassays.[1]

3.5 Acknowledgements

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Figure 3.1. Tracking PAH content and developmental toxicity during the fractionation of LDPE passive sampling device extracts. (A) Nominal maximum concentrations of Σ_{62} PAHs in exposure solutions tested in zebrafish bioassay. (B1) Mortality, (B2) pericardial edema (edema), and (B3) notochord distortion in zebrafish embryos exposed to LDPE extracts and fractions. EC_{20}s were calculated with logistic regression of a 6 point concentration series with n = 32 fish per treatment. All fractions were tested from 0 to 480 mg LDPE/mL; Open triangles indicate fractions for which the predicted EC_{20} was beyond the tested concentration range. Error bars are the 95% prediction interval. Dashed lines separate fractions; heavy dashed lines separate tiers of fractionation.

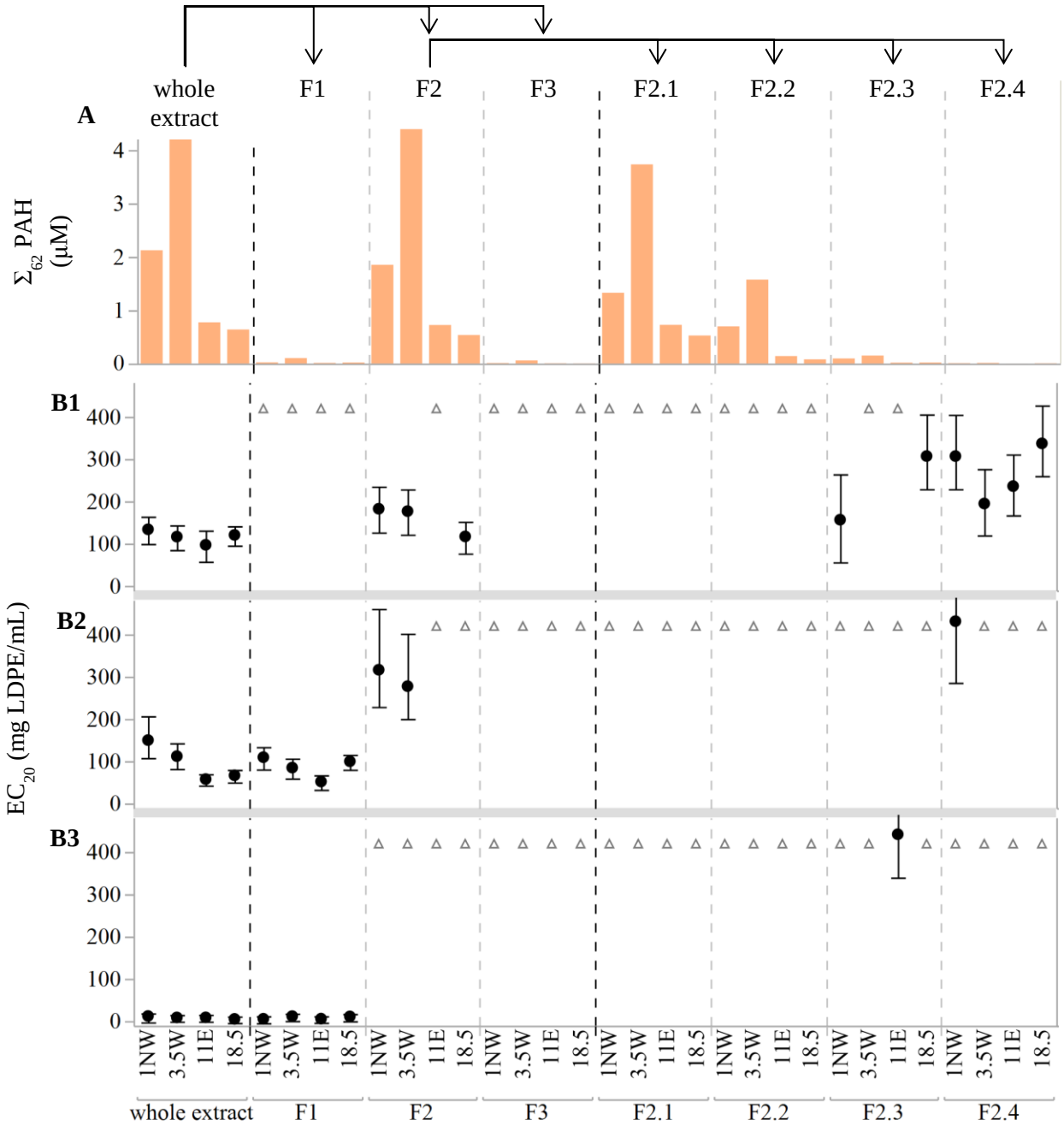
whole extract
F1
F2
F3
F2.1
F2.2
F2.3
F2.4
A
Σ_{62} PAH (μM)
B1
B2
B3
EC_{20} (mg LDPE/mL)
1NW
3.5W
11E
18.5

Figure 3.1

Table 3.1. Compounds identified in toxic fractions were compiled in surrogate mixtures for toxicity confirmation.[a]

Compound	CASRN	method[b]	fractions that caused edema and notochord					fractions that caused mortality						surrogate mixtures	
			F1				F1.6	F2.3		F2.4				maximum conc. (uM)	zebrafish results
			1NW	3.5W	11E	18.5	3.5W	1NW	18.5	1NW	3.5W	11E	18.5		
bis(2-ethylhexyl) phthalate	117-81-7	DRS	P	P	P	P	P	P	P	P	P	P	P	50	
diethyl phthalate	84-66-2	DRS	P	P	P	P	P	*ND*	*ND*	*ND*	P	*ND*	*ND*	0.9	
diisobutyl phthalate	84-69-5	DRS	P	*ND*	P	P	P	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	0.7	
di-n-butyl phthalate	84-74-2	DRS	P	P	P	P	P	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	7	
ethiolate	2941-55-1	DRS	*ND*	*ND*	P	P	P	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	1	no effect
galaxolide	1222-05-5	DRS	P	P	P	P	P	*ND*	P	*ND*	*ND*	*ND*	*ND*	80	
methoxychlor	72-43-5	DRS	*ND*	*ND*	P	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	NT[c]	
tonalide	1506-02-1	DRS	P	P	P	P	P	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	8	
tri-p-tolyl phosphate	1330-78-5	DRS	*ND*	P	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	NT[c]	
4,4'-DDD	72-54-8	DRS	*ND*	*ND*	*ND*	*ND*	*ND*	P	*ND*	*ND*	*ND*	*ND*	*ND*	6	
benzanthrone	82-05-3	DRS	*ND*	*ND*	*ND*	*ND*	*ND*	P	P	*ND*	*ND*	*ND*	*ND*	9	no effect
benzophenone	119-61-9	DRS	*ND*	*ND*	*ND*	*ND*	*ND*	P	P	*ND*	*ND*	*ND*	*ND*	11	
palmitic acid	57-10-3	TIC	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	P	P	P	P	P	2000	
stearic acid	57-11-4	TIC	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	P	P	P	P	P	700	mortality

[a] Compounds reported as present (P) or below detection limits (not detected, *ND*)
[b] Compounds detected with AMDIS and deconvolution reporting software (DRS) or identified by manual examination of the total ion chromatogram followed by retention time confirmation (TIC)
[c] Not tested

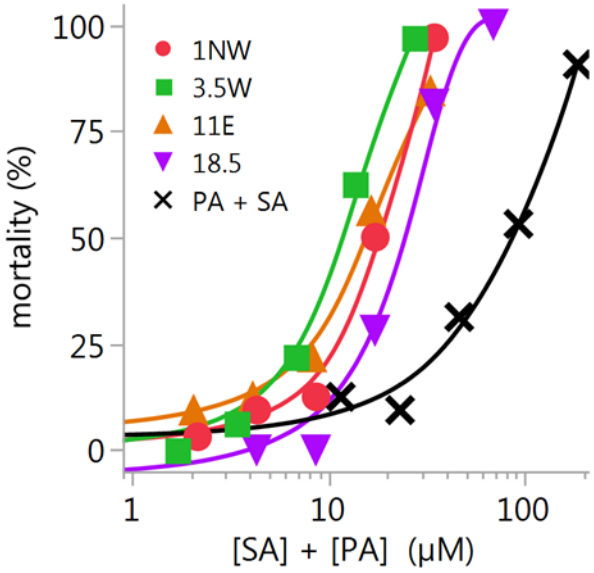

Figure 3.2. Concentration-dependent mortality of the whole LDPE extracts from four river miles (1NW, 3.5W, 11E, 18.5) in terms of nominal palmitic acid (PA) and stearic acid (SA) compared to a binary mixture (PA + SA). Lines are drawn to aid the eye.

Chapter 4 Conclusions

This dissertation demonstrates approaches for simplifying complex environmental mixtures into manageable components. In Chapter 2, we identified hundreds of chemicals in the personal environment of residents of the Alto Mayo, Peru. Analyses revealed that the chemical mixture in participants' wristbands differed depending on their community location, occupation, gender, and age. The distribution of chemicals may be indicative of typical mixtures in the Alto Mayo. Thus, silicone wristband passive sampling devices paired with a qualitative screening method successfully identified distinct mixtures of chemicals in people's environments. Quantitative pesticide analysis of the wristbands provided additional insight into differences among wristband contents, demonstrating distinct patterns of pesticide exposure and suggesting differences in sources based on participant community. These findings are directly applicable for health researchers in the Alto Mayo and aid the development of wristbands as an efficient and easy to use technology for monitoring personal exposure. Future work in the Alto Mayo could pair wristband sampling with more detailed information about health status to establish associations between chemical exposure and disease. Mixtures identified in the wristbands could help identify disproportionate disease risks in certain communities, or they could be used to create artificial mixtures for hazard assessment in bioanalytical tests.

In Chapter 3, we thoroughly investigated mixtures collected from surface water of a contaminated site with LDPE passive sampling devices. Use of the EDA framework eliminated PAHs and many other suspected compounds. Non-target components were identified as suspect toxicants through purifying the mixtures, a top-down approach, and confirming toxicity with authentic standards, a bottom-up approach. We ruled out any obvious other-than-additive effects between mixture components because the combined toxicity of the fractions consistently recapitulated the effects of the whole parent extract. In addition, by comparing to extensive sampling campaigns in PHSM,[1] we have evidence that our results are representative of other LDPE samples from this model urban waterway. EDA can be time and resource-intensive due to iterative fractionation and testing. The future of EDA lies in miniaturizing and accelerating steps to reduce cost and increase throughput. For example, the automation of many steps of the zebrafish embryo bioassay used in this work is a critical step toward making EDA a more feasible tool outside of academic research.[2]

Both chapters in this dissertation relied on combinations of tools for assessing mixtures. We achieved initial simplification of the total mixtures by using PSDs to collect only the fractions of mixtures that were available for biological uptake. Silicone wristbands are multi-media samplers that capture a large

portion of person's external chemical exposure. Future work could improve quantitative exposure assessment with wristbands by incorporating chemical-specific partitioning information, possibly with PRCs, to determine the fraction to equilibrium of sampled compounds. This would allow comparison of concentrations in wristbands that were deployed for different durations and might enable researchers to parse wristband concentrations into the environmental source media. Future studies should also assess the contribution of inhalation, dermal, and/or hand-to-mouth exposures to chemical concentrations measured in wristbands. By further elucidating the connection between environmental sources and internal exposures, wristbands can be incorporated into risk assessment or holistic frameworks like the Aggregate Exposure Pathway.[3,4] PSDs inherently sample the bioconcentratable fraction so, ignoring *in vivo* toxicokinetics, hazard identification is weighted toward compounds with greater potential to accumulate in organisms. LDPE tubing, used to sample surface water in Chapter 3, collected enough sample volume for the iterative analyses of EDA but does not come to equilibrium for all compounds within reasonable deployment periods. Therefore the ratios of chemicals tested in bioassays are in part dependent on deployment duration, which may especially impact results if toxicological interactions occur in the mixture. Complete equilibrium, and therefore consistent enrichment factors (concentration in bioassay/concentration in environment), could be achieved with a PSD configuration that has a greater surface area-to-volume ratio than the LDPE tubes used in this work.

Both studies presented here started with qualitatively screening samples for thousands of chemicals to inform researchers about mixture compositions. Qualititative screening was followed by quantitative analyses. The chemical screen is a compromise between time-efficient targeted quantitation of a small number of analytes and time-consuming non-targeted analysis for a wide range of chemicals. Future work should focus on converting the qualitative chemical screen to a quantitative screen. This would allow more refined assessment of exposure or effects without performing multiple quantitative analyses to identify target compounds. Alternatively, exposures are sometimes binned into broad ranges or percentiles,[5] so achieving supreme precision may not be necessary. Non-target analysis is still necessary to identify unknown components and should continue to be included in EDA, especially with advances workflows to increase sample throughput.[6]

Using the zebrafish developmental assay we were able to tentatively identify multiple toxicants with different modes of action on a single platform. We used static exposures and morphological endpoints in the zebrafish assay to screen for developmental hazards in environmental samples. Complex sorption kinetics of HOCs between water, plastic test chambers, headspace, and organisms can complicate

comparisons of effect concentrations. Future studies that couple PSD extracts and bioassays would benefit from methods that maintain aqueous concentrations of HOCs. High sample volume requirements may prohibit flow-through designs, but passive dosing is a promising technique for testing PSD extracts.[7] Passive dosing would lower detection limits and cover critical windows of development in the zebrafish embryo test. Many endpoints besides malformations are available with the zebrafish early life stage test. Such additional endpoints, including behavioral and genetic measurements, are also relatively rapid and could be used in EDA to target more specific mechanisms of action.

We used a combination of univariate and multivariate analyses to identify patterns in chemicals sequestered by wristbands, and to compare effect concentrations in the zebrafish bioassay. The improvements to chemical and bioanalytical measurements discussed above would also improve the mathematical analyses available for exposure and effect assessments. With quantitative data for hundreds of chemicals, multivariate analyses would be more effective for identification of typical chemical mixtures and the lifestyle factors that contribute to these mixtures. In hazard identification, more reliable effect concentrations would allow comparison of mixture toxicity to databases of single chemicals and application of the toxic equivalency approach.

In total, this dissertation demonstrates that complex environmental mixtures are not unsolvable. Through the strategic and innovative implementation of passive sampling and compatible tools, this work identified hazards in, and exposures to, chemical mixtures as a necessary step in assessing the costs of environmental contamination.

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APPENDICES

Appendix A. Supplement to Chapter 2.

Information about stationary environmental passive sampling in the Alto Mayo

To get an overview of what pesticides and other compounds might be present in the environment in the Alto Mayo, we conducted an initial survey of bioavailable contaminants in the surface water of the Alto Mayo in August 2013.

The LDPE passive sampling devices were prepared as described in Anderson *et al.* [1] LDPE tubing (2.5 cm wide by 70 um thick, Brentwood Plastic, Inc. St Louis, MO) were cut to 110 cm (4.82 ± 0.08 g).[2] The LDPE were placed in amber glass jars and conditioned to remove impurities with three rounds of soaking in hexane with gentle agitation for 48 hours. Clean LDPE was dried under vacuum for 48 hours, placed in pre-cleaned air-tight metal cans and stored at -20°C until deployment preparation. To prepare the LDPE strips for deployment, a loop was heat-sealed at one end of the strip, performance reference compounds (PCB65-d5, PCB115-d3, PCB156-d3) were added to the inter-membrane space, air removed, and the open end sealed with a loop to a final length of approximately 100 cm. Prepared LDPE were sealed in PTFE bags and stored at -20°C until transport to the field. LDPE were deployed in water on steel frames, five of which were housed in a steel cage (Environmental Sampling Technologies; St. Joseph, MO).

LDPE strips were deployed at six locations in the Alto Mayo region of Peru (Figure 1) for 4-6 days in August 2013. Sites were selected for a wide spatial distribution, variety in water body types, and potential contaminant sources. Aguas Verdes was the farthest upstream location, targeted for being in a region of low human impact. The samples at Alto Michuco were collected from an irrigation channel that drains rice fields and flows next to residences. La Victoria samplers were in an aquaculture pond that houses farmed tilapia in a small village. Tioyacu is a river downstream of agriculture and a major regional cement factory. Rio Indoche is near the town of Yantaló, downstream of various agriculture fields. Another sample was collected from the Rio Mayo, downstream of the locations mentioned above and downstream of Moyobamba. Triplicate sets of PSDs were placed at Alto Michuco and Rio Indoche. An additional deployment was repeated at Alto Michuco for 42 days beginning immediately after the triplicate samplers were retrieved. Recovered LDPE were sealed in PTFE bags and returned to Oregon State University for analysis. The LDPE had little visible biofouling. To remove any periphyton or particulates, the LDPE were cleaned in the laboratory by light scrubbing and sequential dousing in 1 N HCl, 18 MΩ*cm water, and two iterations of isopropanol. Dry LDPE were stored in amber glass jars at -20°C until extraction. Surrogate standards (TCMX, PCB-100, and PCB-209) were pipetted onto the LDPE strips immediately before extraction with 2 rounds, 12 hours followed by 2 hours, of 200 mL n-

hexane. Extracts were reduced to approximately 1 mL with TurboVap closed cell concentrator (Biotage, Uppsala, Sweden), quantitatively transferred to conical centrifuge tubes, and reduced again to 1 mL.

GC-MS screen

LDPE extracts were screened with GC-MS for 1,182 compounds following methods described in Anderson *et al.*[3]

GC-ECD

Pesticides in LDPE extracts were analyzed with GC-μECD as described previously [3]. The columns were 17MS and XLB, at 30 m by 0.25 mm by 0.25 μm (Agilent). Inlets were operated at 250˚C, with 2 μL injection volume and helium carrier gas. The oven program started at 110˚C for 1 min, then 4˚C/min to 300˚C, held for 10 min. Detectors at 320˚C with makeup gas held at 60 mL/min.

LDPE vs. wristband analysis

Collection and analysis of LDPE water samples preceded the wristband components by several months. Method upgrades during that time resulted in some differences in operating parameters and number of target analytes between the sets of samples. Four compounds were therefore detected in wristbands that were not among target compounds for water deployed LDPE: fipronil sulfide, fipronil sulfone, deltamethrin, and galaxolide.

Surface water concentrations

Water concentrations of pesticides measured quantitatively with GC-μECD were calculated using *in situ* calibration with performance reference compounds and methods described previously [3].

Chemicals detected in surface water

Pesticides, personal care products, and PAHs were detected in LDPE samplers deployed in surface water of the Alto Mayo (Table A1). No compound except for benz[a]anthracene, a PAH, was above detection limits at Aguas Verdes. Chlorpyrifos was measured at all of the other five locations and ranged from 0.14 to 1.9 ng/L (Figure A1). Chlorpyrifos was at very similar concentrations in sequential measurements at Alto Michuco (Figure A1) suggesting that chlorpyrifos may be relatively constant over time although more sampling would be needed to confirm. Other compounds included the pesticides cyclafuramid and butachlor and the fragrances tonalide and cashmeran.

Equation S1.

The proportion of chemicals detected in wristbands from each demographic group are shown in Figure 2.2 and Figure A6. The proportions were compared for each class according to the following equation:

$$Proportion\ detected = \frac{x_{CG}}{n_C \times n_G}$$

Where x_{CG} is the number of compounds detected for each class (*C*) in each demographic group (*G*), n_C is the number of total compounds of each class that were detected in any sample, and n_G is the total number of participants in each demographic group.

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Table A1. Chemicals detected in water of Alto Mayo.

location	compound	method[1]	concentration (ng/L)
Aguas Verdes	PAHs[2]	DRS	
Alto Michuco	chlorpyrifos	ECD + DRS	0.87
	tonalide	DRS	
	p,p'-DDT[3]	ECD	0.0068
	p,p'-DDE[3]	ECD + DRS	0.0056
	p,p'-DDD[3]	ECD	0.0053
	cypermethrin[3]	ECD + DRS	0.092
	λ-cyhalothrin[3]	ECD	0.032
	cyclafuramid[3]	DRS	
	biphenyl[3]	DRS	
	PAHs[2]	DRS	
Rio Indoche[4]	chlorpyrifos	ECD	0.33
	PAHs[2]	DRS	
Tioyacu	chlorpyrifos	ECD	0.14
	tonalide	DRS	
	sulfur	DRS	
	cashmeran	DRS	
	PAHs[2]	DRS	
La Victoria	chlorpyrifos	ECD + DRS	1.9
	PAHs[2]	DRS	
Rio Mayo	chlorpyrifos	ECD	0.20
	tonalide	DRS	
	butachlor	DRS	
	PAHs[2]	DRS	

[1] Detection using gas chromatography with electron capture detection (ECD) or gas chromatography with mass spectrometry and deconvolution reporting software (DRS) or both.
[2] Various PAHs were detected at every site.
[3] This compound was only measured in the 42 day deployment at Alto Michuco.
[4] Sample exposed to air during deployment so the calculated value does not reflect true water concentration.

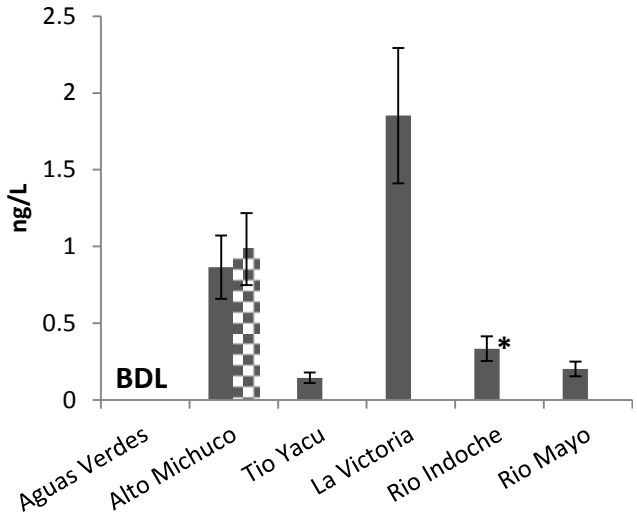

Figure A1. Chlorpyrifos measured in surface water of the Alto Mayo using LDPE deployed for 4 days. Error bars represent 1 standard deviation from the mean as measured for replicates at Alto Michuco and Rio Indoche and estimated for the others based on the average relative standard deviation for the replicated sites. The checkered bar represents LDPE deployed at Alto Michuco for 42 days. Sites are arranged by their approximate order of upstream to downstream in the watershed. *Water concentrations at Rio Indoche samplers are rough estimates because samplers were not submerged for the whole deployment period. BDL: Below detection limits.

Figure A2. Hierarchical clustering of GC-MS screen results. Distance between observations was determined using Ward's method (JMP Pro 12). Gender: red = male, white = female. Occupation: Red = Other, white = Farm worker. The clusters are colored in the community identifiers on the right axis and separated with solid black likes. The number of clusters was determined by analyzing the scree plot (bottom left of figure) for the natural break in the distance bridged by forming clusters. 2D clusters (black ovals) were labeled as the general class of compounds that make up the majority of the clusters. PCPP: personal care product and pharmaceutical. PAH: polycyclic aromatic hydrocarbon. DDTs: p,p'-dichlorodiphenyltrichloroethane and its metabolites.

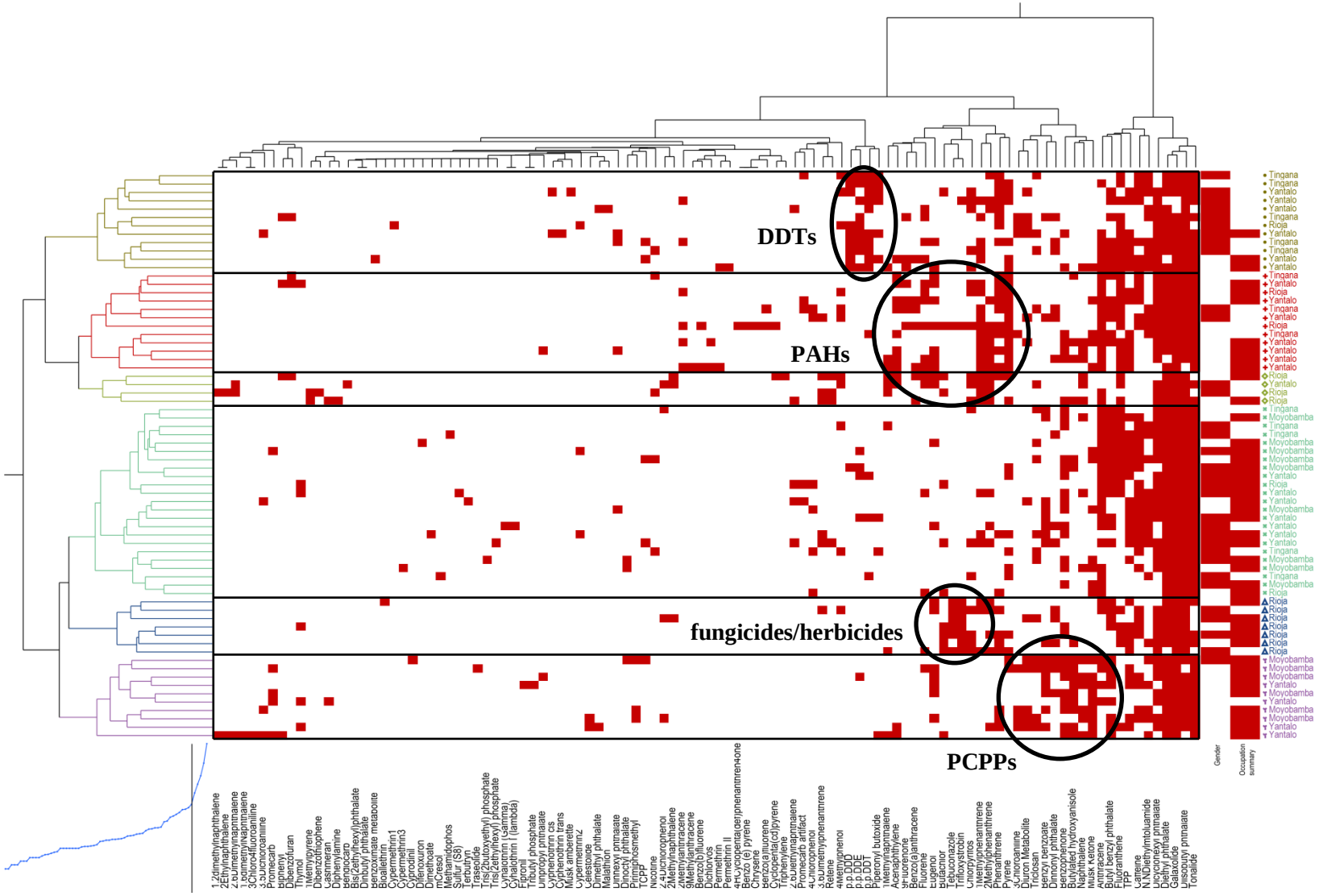
DDTs
PAHs
fungicides/herbicides
PCPPs
Gender
Occupation summary

Figure A2.

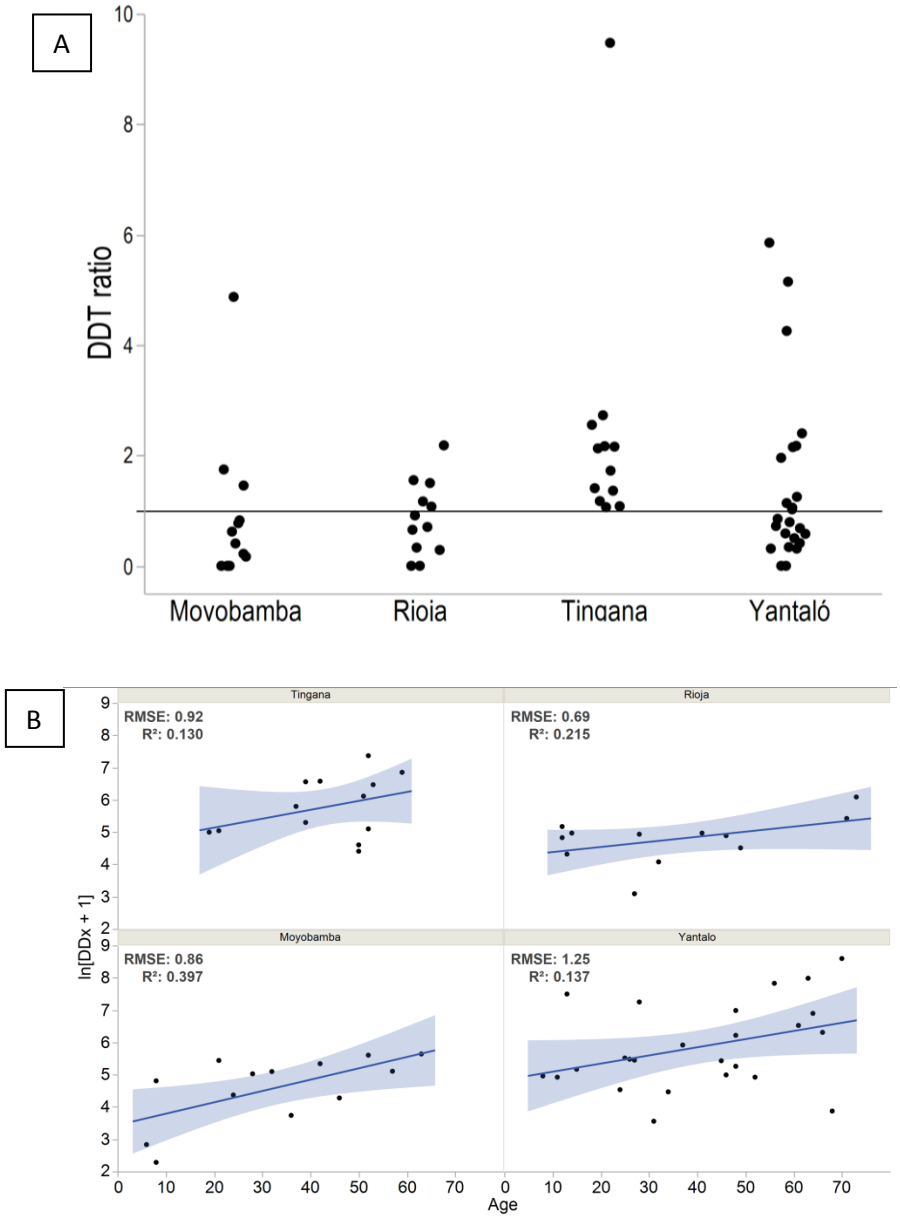

Figure A3. DDT and its metabolites in four communities of the Alto Mayo. (A) DDT ratio (DDT/[DDE + DDD]) greater than one indicates a relatively recent application of DDT. The solid horizontal line is a reference at DDT ratio = 1. (B) Correlations of DDx concentrations with age in each of the four communities. Shaded areas are the 95% confidence intervals. The concentration of DDx in wristbands from Tingana had the worst correlation with age of any community (B). In contrast, wristbands from the capital city, Moyobamba, contained the lowest DDT ratios and the greatest correlation with age. Rioja and Yantaló followed the same trend that high DDT ratios were associated with low correlations of wristband DDx concentrations with age. Together, these analyses support that an association with age indicates legacy contamination.

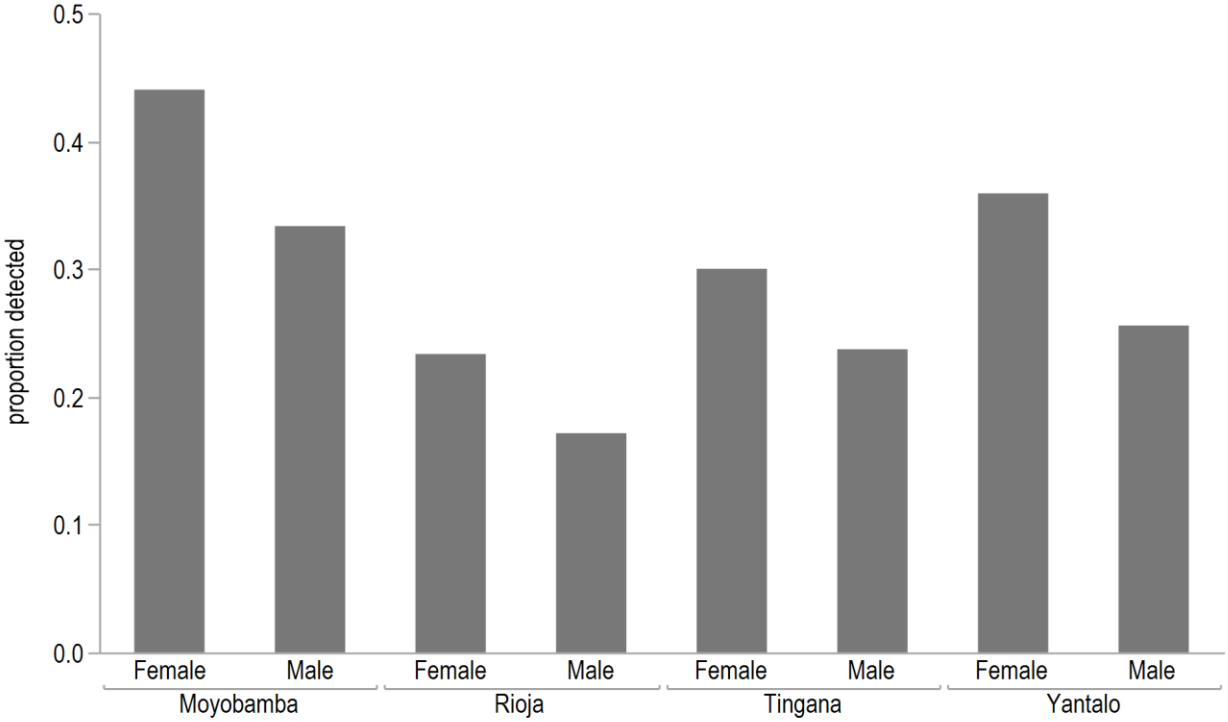

Figure A4. PCPP detections by gender and community. More PCPPs were detected in females' wristbands than males' in every community.

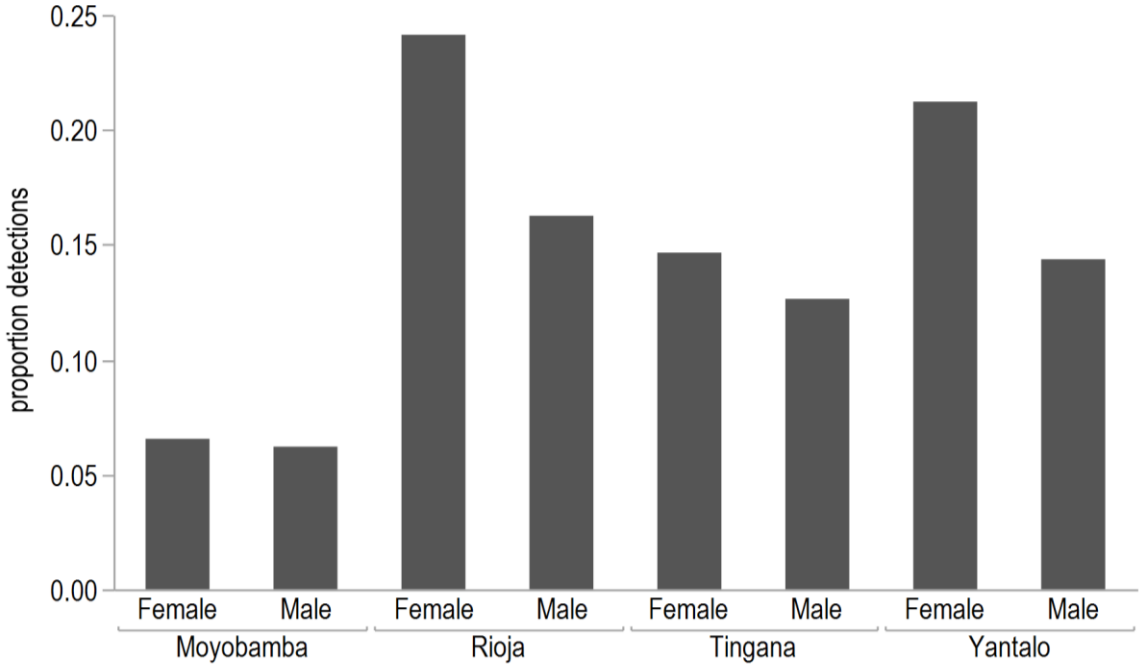

Figure A5. PAH detections by gender and community. More PAHs were detected in females' wristbands than males, especially in Rioja and Yantaló.

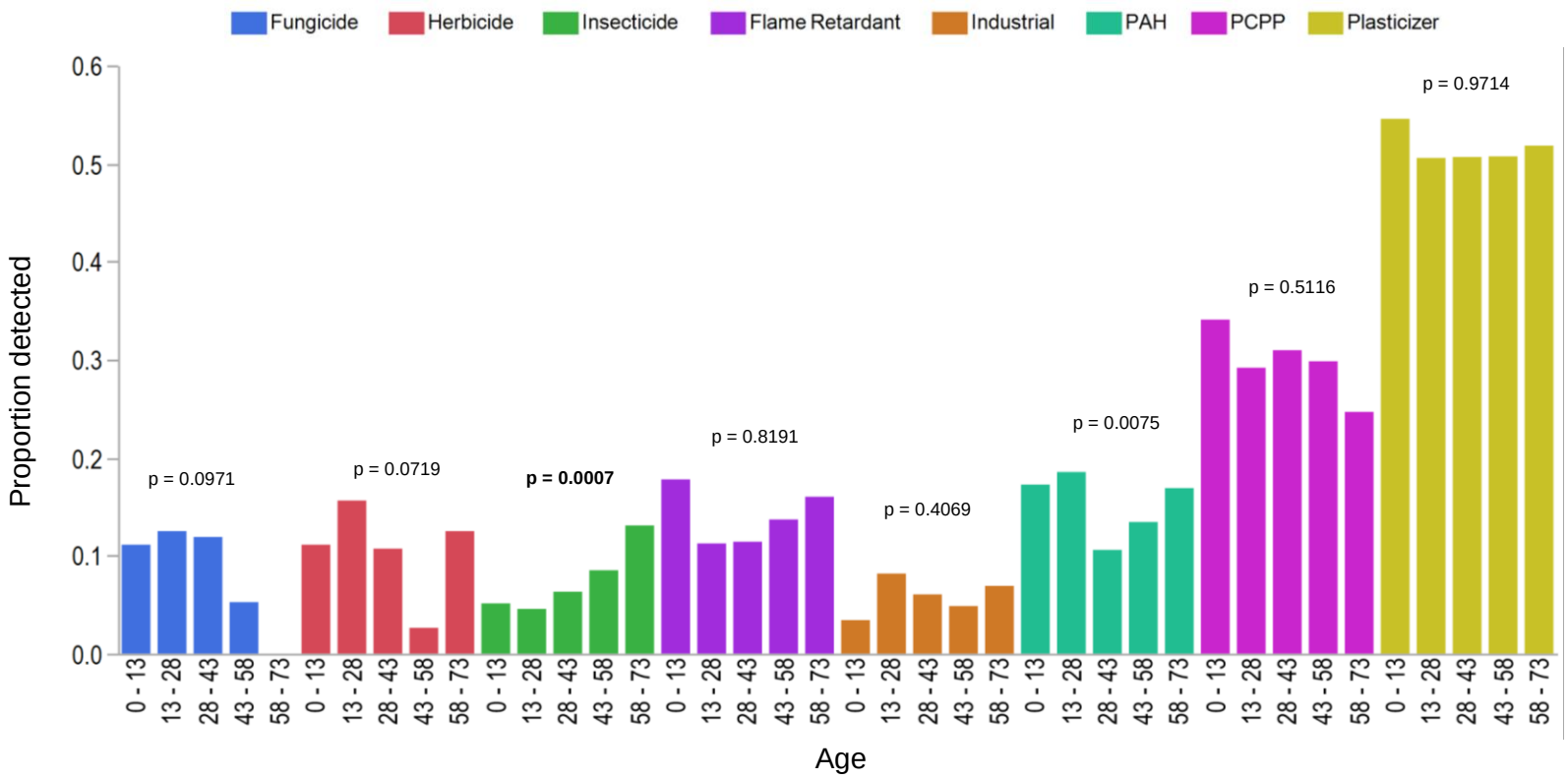

Figure A6. Effect of Age on the detection rate of eight chemical classes in wristbands worn by residents of the Alto Mayo. Age groups were compared with a Chi-squared likelihood ratio test.

Table A2. Statistical results of the Chi-square likelihood ratio test comparing community for the *ln*(concentration + 1) of the top three pesticides as seen in Figure 2.4.

Compound	Community 1	vs	Community 2	Score Mean Difference	Standard Error	Z	p-Value
chlorpyrifos	Rioja		Yantaló	6.35	3.80	1.67	0.095
	Rioja		Tingana	4.52	3.06	1.48	0.139
	Tingana		Yantaló	2.69	3.80	0.71	0.479
	Moyobamba		Yantaló	-9.47	3.79	-2.50	0.013
	Moyobamba		Tingana	-9.54	3.00	-3.18	0.002
	Moyobamba		Rioja	-9.64	3.05	-3.16	0.002
cypermethrin	Moyobamba		Rioja	0.00	2.88	0.00	1.000
	Moyobamba		Tingana	-2.31	2.94	-0.79	0.432
	Rioja	vs	Tingana	-3.56	3.02	-1.18	0.238
	Tingana		Yantaló	-6.49	3.79	-1.71	0.087
	Moyobamba		Yantaló	-8.54	3.75	-2.27	0.023
	Rioja		Yantaló	-9.25	3.77	-2.45	0.014
DDx	Moyobamba		Rioja	2.15	3.06	0.70	0.482
	Tingana		Yantaló	-0.12	3.80	-0.03	0.976
	Moyobamba		Tingana	-7.08	3.00	-2.36	0.018
	Rioja		Tingana	-9.12	3.06	-2.99	0.003
	Moyobamba		Yantaló	-9.35	3.80	-2.46	0.014
	Rioja		Yantaló	-11.76	3.81	-3.09	0.002

Table A3. Alternative multiple linear regression results. Non-detects substituted with LOD/sqrt(2).

	Parameter	**Estimate**	**SE**	**p**	**r^2**
ln(chlorpyrifos)	Intercept	5.29	0.24	**<0.0001**	0.20
	Community				
	Yantaló-Moyobamba	0.00	0.35	0.9967	
	Tingana-Moyobamba	0.43	0.43	0.3249	
	Rioja-Moyobamba	1.11	0.42	**0.0098**	
ln(cypermethrin)	Intercept	3.02	0.66	**<0.0001**	0.20
	Community				
	Yantaló-Moyobamba	1.08	0.44	**0.0164**	
	Tingana-Moyobamba	0.31	0.54	0.5740	
	Rioja-Moyobamba	-0.58	0.52	0.2696	
	Age	0.03	0.02	**0.0418**	
ln(DDx)	Intercept	4.17	0.34	**<0.0001**	0.40
	Community				
	Yantaló-Moyobamba	0.66	0.22	**0.0041**	
	Tingana-Moyobamba	0.42	0.28	0.1372	
	Rioja-Moyobamba	-0.76	0.26	**0.0053**	
	Gender (male)	0.20	0.15	0.1836	
	Age	0.03	0.01	**0.0025**	

Appendix B. Supplement to Chapter 3.

PRC spiking, dissipation, and calculating water concentrations

Each LDPE strip was heat-sealed at one end and 100 µL of a PRC solution containing 373, 15.7, and 15.7 µg/mL of fluorene-d10, pyrene-d10, and benzo[b]fluoranthene-d12, respectively, was pipetted into each LDPE tube prior to closing (heat-sealing) the second side. Five LDPE were co-deployed per replicate and extracted into 1 mL. Initial PRC concentrations and recovery per 1 mL are shown in Table B4. Water concentrations of target PAHs were calculated using the dissipation rates of PRCs and equations B1 through B6.[1]

$LogK_{sw}$ was predicted from a relationship with $logK_{ow}$.

$$\boldsymbol{Eq\ B1}.\ logK_{SW} = -2.61 + (2.321 * logK_{OW}) - (0.1618 * logK_{OW}{}^2)$$

The depuration rates (k_e, day^{-1}) of PRCs were calculated assuming first order kinetics where PRC_i and PRC_t are the initial concentrations and the amount remaining at time (t, days), respectively.

$$\boldsymbol{Eq\ B2}.\ k_e = \frac{-ln\left(\frac{PRC_t}{PRC_i}\right)}{t}$$

The PRC sampling rates (R_{sPRC}, L/day) were calculated where V_s is the volume (cm^3) of the LDPE samplers.

$$\boldsymbol{Eq\ B3}.\ R_{sPRC} = k_e * K_{sw} * V_s/1000$$

The sampling rates (R_s, L/day) for target PAHs were derived from a relationship with the PRC sampling rate,

$$\boldsymbol{Eq\ B4}.\ R_s = R_{sPRC} * \frac{\alpha_{analyte}}{\alpha_{PRC}}$$

that uses a compound-specific adjustment (α, unitless) determined from the following relationship with $logK_{ow}$.

$$\boldsymbol{Eq\ B5}.\ log\ \alpha = (0.013 * logK_{ow}{}^3) - (0.3173 * logK_{ow}{}^2) + (2.244 * logK_{ow})$$

Finally, water concentrations (C_w, ng/L) were determined with equation S6, where $N_{analyte}$ is the mass (ng) of target compound in the LDPE extract.

$$\boldsymbol{Eq\ B6}.\ C_w = \frac{N_{analyte}}{V_s K_{sw}(1 - \exp\left(-\frac{R_s t}{V_s K_{sw}}\right))}$$

Toxicity modeling and EC_{20} calculations

Using JMP Pro 12, counts of the binary responses for mortality, pericardial edema, and notochord distortions were fit to a generalized linear model using a logit link function and binomial distribution. Where x is concentration and π is the response in proportion, the model is:

$$logit(\pi) = \beta_0 + \beta_1 \times ln(x + 1)$$

The inverse prediction at an effect proportion of 0.2 and the corresponding 95% confidence interval were calculated from the model fit.

Additional fatty acid discussion

Quantitation of FAs

The fatty acids PA and SA were identified in the GC-MS chromatograms using MSD Chemstation comparison to NIST08 mass spectral library (match factor = 99%). The retention times and mass spectra of PA and SA were confirmed with analytical standards. Concentrations of PA and SA were determined by creating an external calibration on the GC-MS method described for the 1408 analyte screen. The full-scan TIC was extracted for the molecular mass to charge ratio for PA (256 m/z) and SA (284 m/z). The relative standard deviation (n=5) of the peak area was 2.13% for PA and 4.48% for SA. Free fatty acids are not ideal for GC analysis as apparent in the non-Gaussian peak shape and high detection limits. However, the external calibration yielded R^2 values of 0.995 and 0.991 for PA and SA, respectively, for concentrations between approximately 10 and 1000 μg/mL.

PA and SA were tested for developmental toxicity as a binary mixture in the zebrafish embryos assay in a fixed ratio of 2:1 SA to PA based on early estimates of relative concentration in the LDPE extracts. The ratio of SA to PA in LDPE extracts was later determined to be closer to 1:1. This is a surrogate mixture, which assumes a sufficiently similar mechanism of action for represented compounds. Under this assumption, we report the results in terms of total FA

Spatial variation of fatty acids

The greatest concentrations of fatty acids were in samples from outside the superfund, at RM 18.5 and RM 1NW. These locations are notably just upstream of the confluence with small urban waterways. RM 18.5 is near the mouths of Johnson and Kellogg creeks which drain southern Portland and beyond. RM 1NW is just upstream of the Columbia Slough which cuts through northern Portland. In particular, the water quality of Johnson creek has been characterized as 'Very Poor' including indicators of eutrophication.[2] The lower Willamette River is tidally influenced so dissolved organic matter entering the river from a eutrophic tributary could encounter the LDPE sitting just upstream (~0.25 miles).

Toxicity and plausible mechanism of fatty acids

The results of fatty acid toxicity are expressed in terms of total fatty acid (PA + SA) concentrations, ignoring the relative amount of individual fatty acids. This assumes that both PA and SA have the same toxic mechanism of action which is supported by their structural similarity. As surfactants, it is possible that the toxicity occurs through disruption of lipid membranes, *i.e.* narcosis, as hypothesized for oleic acid mechanism of toxicity to *V. fisherii*,[3] and as has been suggested as the molecular initiated event for sulfonated surfactants in various fish species.[4,5]

Fatty acids are also natural ligands for the peroxisome proliferator activated receptor (PPAR-gamma).[6] Fang *et al.* [7] determined that PA and SA induced PPAR-gamma to a similar degree in an *in vitro* assay but were relatively weak inducers of PPAR-gamma compared to myristic acid and the unsaturated FA, oleic acid. PA and SA were the most abundant fatty acids in the LDPE extracts but other free fatty acids, including palmitoleic and oleic acids, were identified in RM18.5. It is unclear whether PPAR induction is responsible for fatty acid induced zebrafish mortality but it highlights that although the fatty acids are likely acting with the same mechanism, they might have different potencies which contribute to uncertainty when using only PA and SA in a surrogate mixture.

Additional PCA discussion

The 33 PAHs common to the present study (sample year: 2014) and Allan *et al.*[8] (sample years 2009 and 2010) were compared in a principal components analysis. The samples collected in 2014 cluster away from 2009 and 2010 along principal component 3. This is driven largely by 9-methylanthracene and 6-methylchrysene (vectors p15 and p25 in figure B9c). These compounds were identified in every sample in

2014 but rarely detected by Allan *et al.* The current study measured PAHs with a more sensitive GC-MS/MS method than the GC-MS method of Allan *et al.* and could explain the increased frequency of detections for these compounds. In 2014, 9-methylanthracene and 6-methylchrysene made up an average of 0.54% (± 0.42%) and 0.15% (± 0.047%), respectively, of the total PAH concentration by mass.

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Table B1. Compounds used in toxicant confirmation experiments.

Compound	CAS RN	Method of detection	company	purity (%)
4,4'-DDD	72-54-8	DRS	Accustandard (New Haven CT, USA)	99.3
benzanthrone	82-05-3	DRS	Sigma Aldrich (Darmstadt, Germany)	99
benzophenone	119-61-9	DRS	Sigma Aldrich	99
bis(2-ethylhexyl) phthalate	117-81-7	DRS	Sigma Aldrich	99.6
diethyl phthalate	84-66-2	DRS	Accustandard	97
diisobutyl phthalate	84-69-5	DRS	Accustandard	100
di-n-butyl phthalate	84-74-2	DRS	Sigma Aldrich	99
ethiolate	2941-55-1	DRS	Accustandard	99.8
galaxolide	1222-05-5	DRS	Sigma Aldrich	88.4
tonalide	1506-02-1	DRS	Santa Cruz Biotechnology (Dallas, TX, USA)	97
palmitic acid	57-10-3	TIC	Nu-Chek Prep (Elysian, MN, USA)	99
stearic acid	57-11-4	TIC	Nu-Chek Prep	99

Table B2. Deployment and site characteristics for PSD samples.

Deployment start	**River mile (nominal)**	**Duration (days)**	**Average temperature (SD) (˚C)** [a]	**Average flow (m^3 s^{-1})** [b]
August 27 2014	1NW	21	21.3 (1.1)	276 for Sep 2014
	3.5W		21.7 (1.1)	
	11E		21.1 (1.2)	
	18.5		20.7 (1.3)	

[a]From co-deployed temperature logger (Hoboware) series statistics, omitting any time points that were collected out of the water. SD = standard deviation.

[b]Historical monthly average of discharge for Willamette River at Portland (9,749 ft^3 s^{-1}). http://nwis.waterdata.usgs.gov/or/nwis/monthly?referred_module=sw&site_no=14211720&por_14211720_2=546878,00060,2,1972-10,2014-10&format=html_table&date_format=YYYY-MM-DD&rdb_compression=file&submitted_form=parameter_selection_list

Table B3. Additional AMDIS parameters for GC-MS screening method.

RESOLUTION=1

PEAKSHAPE=1

PEAKWIDTH=12

OMITMZ=1

OMITEDMZ=73 207 281

ANALMODE=1

CURRENT_RI_NUMBER=0

MULTIDEN=0

SHOWSTD=0

ONLYREVERSE=0

MINMF=60

CLASSIDEN=0

RILEVEL=5

RIMINABS=30

RIMINPCT=0

RIMAX=60

RINOLIB=10

RTLEVEL=5

RTWINDOW=0.2

RTMAX=20

RTNOLIB=10

MXMASS=910

LOMASS=40

SCANDIR=-1

Table B4. PRC spiking and dissipation information.

Compound	CAS	River Mile	PRC initial mass [pg/uL]	PRC final mass [pg/uL (sd)]	PRC dissipation [% remaining (sd)]
fluorene-d10	81103-79-9	1NW 3.5W 11E 18.5	1,870,000	7.56 389 (333) 8.34 2.26	0.000404 0.0208 (0.0178) 0.000446 0.000121
pyrene-d10	1718-52-1	1NW 3.5W 11E 18.5	7850	909 2600 (315) 1750 2160	11.6 33.2 (4.01) 22.3 27.5
benzo[b] fluoranthene-d12	205-99-2	1NW 3.5W 11E 18.5	7850	5670 6230 (119) 6060 5920	72.2 79.3 (1.51) 77.2 75.4

Table B5. Recovery of ΣPAHs in gel permeation chromatography (GPC) and normal phase (NP) chromatography fractionation. WE: whole extract.

	Σ_{PAH} in WE (μM)	GPC recovery (%)	% of recovered PAHs in:			NP recovery (%)	% of recovered PAHs in:			
			F1	F2	F3		F2.1	F2.2	F2.3	F2.4
1NW	102	88.7	1.0	98.4	0.6	115.0	61.7	33.1	4.9	0.4
3.5W	267	104.0	2.2	96.4	1.4	124.0	67.5	29.3	3.0	0.2
11E	36.5	96.2	1.6	97.8	0.7	122.0	81.6	16.2	2.1	0.1
18.5	26.3	87.6	3.3	95.9	0.8	118.0	83.7	12.8	2.9	0.5
	average	94.1	2.0	97.1	0.9	120.0	73.6	22.9	3.2	0.3

Table B6. Estimated concentrations (ppb) of compounds identified in toxic fractions and the toxicity of surrogate mixtures.

			Edema and notochord					Mortality						Compounds tested as surrogate mixture	
			F1				F1.6	F2.3		F2.4					
Compound	CASRN	Method of detection[b]	1NW	3.5W	11E	18.5	3.5W	1NW	18.5	1NW	3.5W	11E	18.5	maximum conc. in mixture (ppb)	Zebrafish results
4,4'-DDD	72-54-8	DRS	---	---	---	---	---	100	---	---	---	---	---	2,000	
benzanthrone	82-05-3	DRS	---	---	---	---	---	40	100	---	---	---	---	2,000	No effect
benzophenone	119-61-9	DRS	---	---	---	---	---	80	100	---	---	---	---	2,000	
bis(2-ethylhexyl) phthalate	117-81-7	DRS	7000	6000	9000	9000	4000	600	200	100	500	100	70	20,000	
diethyl phthalate	84-66-2	DRS	---	400	100	100	80	---	---	---	200	---	---	200	
diisobutyl phthalate	84-69-5	DRS	500	---	300	400	300	---	---	---	---	---	---	200	
di-n-butyl phthalate	84-74-2	DRS	500	10000	2000	2000	6000	---	---	---	---	---	---	2,000	
ethiolate	2941-55-1	DRS	---	---	200	100	100	---	---	---	---	---	---	200	No effect
galaxolide	1222-05-5	DRS	6000	20000	10000	10000	6000	---	70	---	---	---	---	20,000	
methoxychlor	72-43-5	DRS	---	---	60	---	---	---	---	---	---	---	---	NT[c]	
tonalide	1506-02-1	DRS	300	600	700	800	400	---	---	---	---	---	---	2,000	
tri-p-tolyl phosphate	1330-78-5	DRS	---	30	---	---	---	---	---	---	---	---	---	NT[c]	
palmitic acid	57-10-3	TIC	---	---	---	---	---	---	270	160	95	150	190	400,000	Mortality
stearic acid	57-11-4	TIC	---	---	---	---	---	---	200	200	110	180	110	200,000	

[a]Concentrations are pg/uL of LDPE extract.
[b]Compounds measured with AMDIS and deconvolution reporting software (DRS) or identified by manual examination of the total ion chromatogram followed by retention time confirmation (TIC).
[c]Not Tested

Figure B1. Sampling locations and site map. Lower Willamette River at the confluence with Columbia River. Portland Harbor Superfund Mega-site (PHSM) is the stretch of river between the red hatch marks. Green circles are locations of sampling sites (River miles 1NW, 3.5W, 11E, and 18.5).

Figure B2. Deployment configuration of LDPE passive samplers as they were lowered into the river. PRC-containing LDPE were housed in a stainless steel cage, samplers for bioassays (no PRCs) were cage-free.

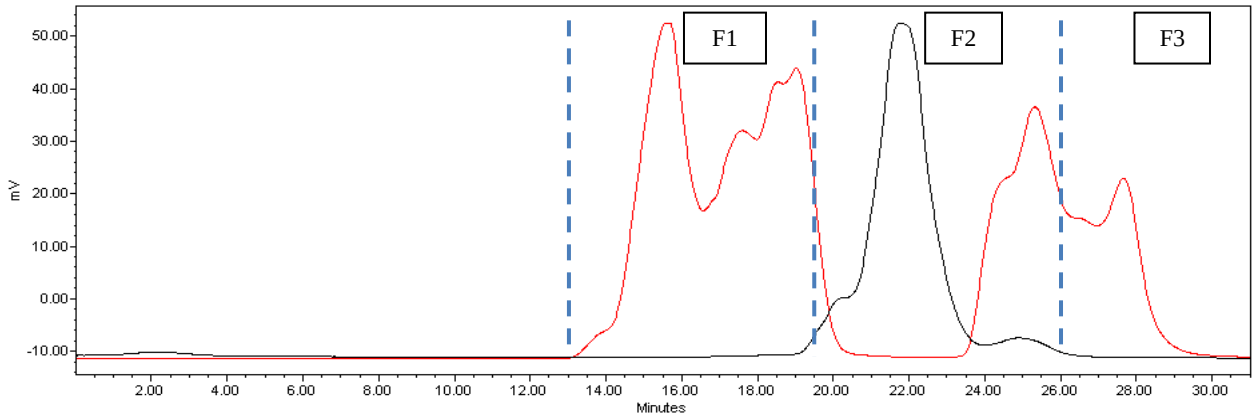

Figure B3. Overlaid chromatograms of GPC calibration solutions that were used to determine fraction collection times. GPC calibration mixture (red) containing corn oil, methoxychlor, phthalates, perylene, and elemental sulfur. PAH solution of 16 EPA priority pollutants

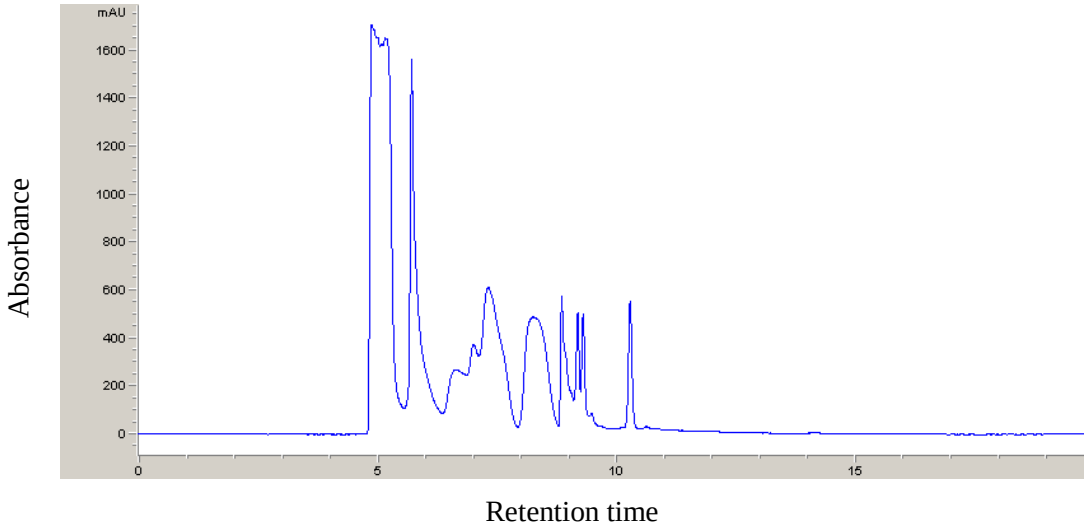

Figure B4. Normal phase separation of standard solution of 10 PAHs. Retention time is in minutes, response is absorbance (mAU) at 254 nm.

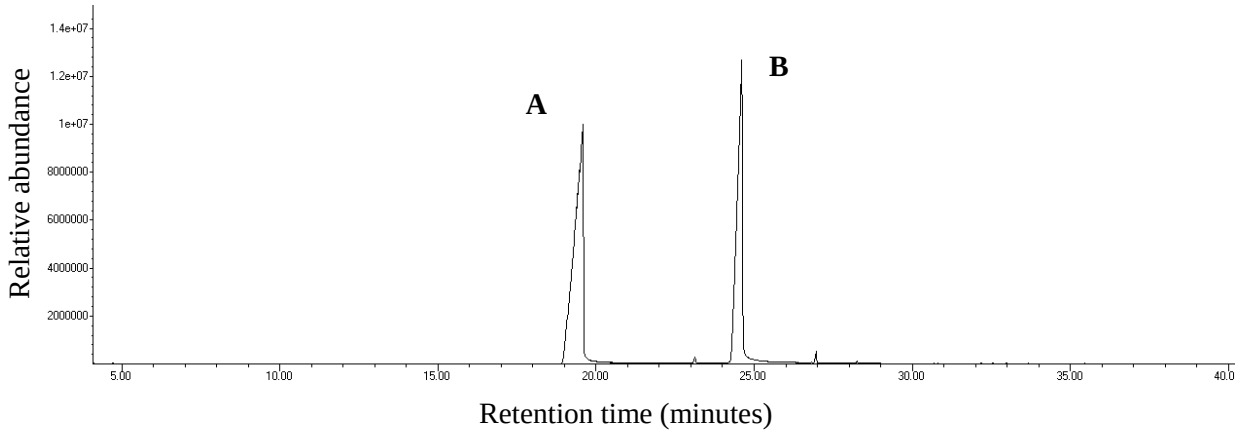

Figure B5. GC-MS total ion chromatogram of surrogate fatty acids. Palmitic acid (A) and stearic acid (B).

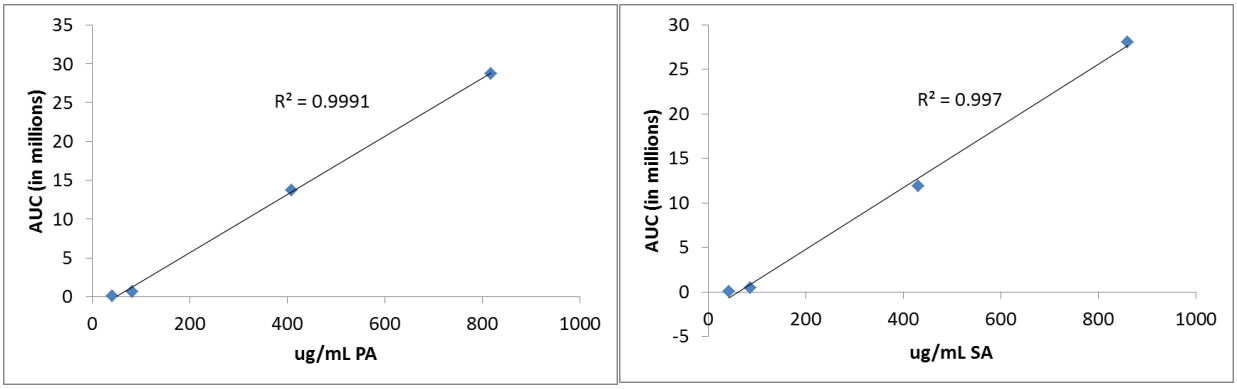

Figure B6. External calibration of palmitic acid (PA) and stearic acid (SA). AUC: area under the curve – integrated with Chemstation for extracted ions 256 and 284 for PA and SA, respectively. The relative standard deviation (n=5) of the peak area was 2.13% for PA and 4.48% for SA.

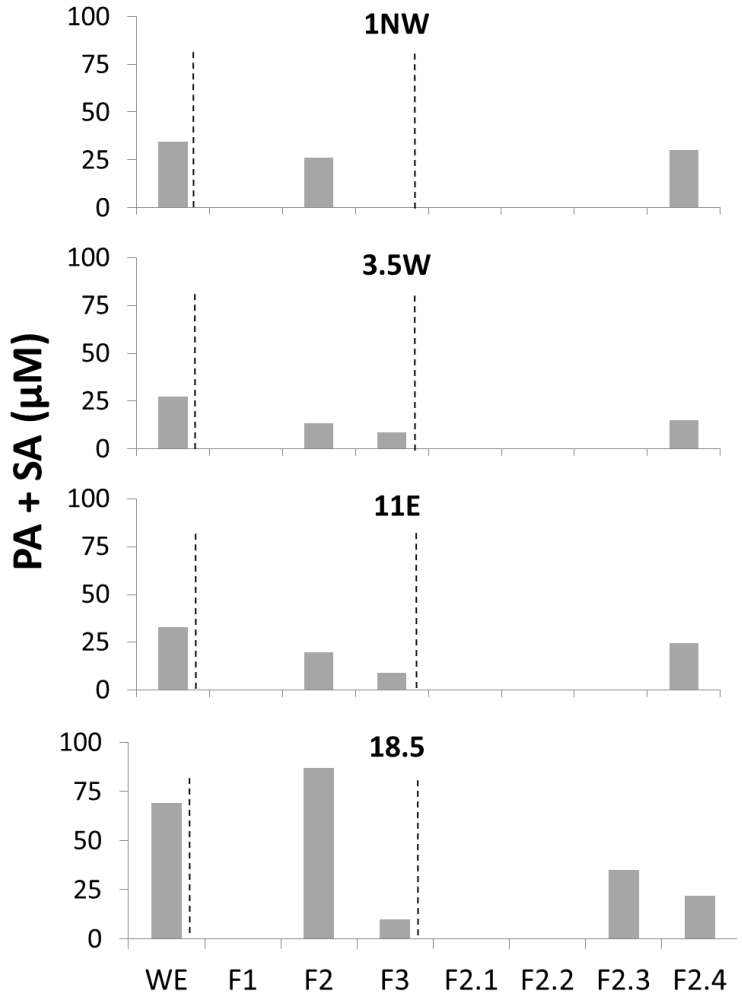

Figure B7. Maximum total fatty acid (PA + SA) nominal concentrations in bioassay exposure solutions after being measured in whole LDPE extracts (WE) and fractions with an external calibration. Dashed lines separate tiers of fractionation.

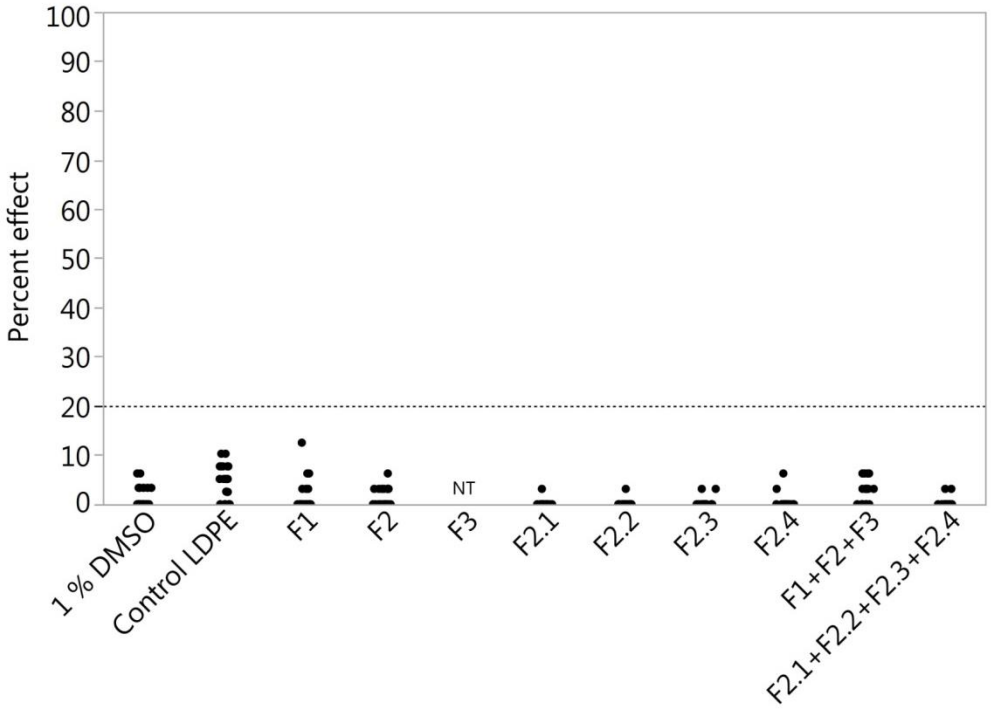

Figure B8. Toxicity data for QC samples corresponding to each sample processing event. 1 % DMSO is the vehicle, Control LDPE is non-deployed LDPE extract (240mg/mL), fractions are collected from blank injections of n-hexane on either GPC (F1, F2) or NP-HPLC (F2.1-F2.4) and those fractions recombined (F1+F2+F3 and F2.1+F2.2+F2.3+F2.4). Each point represents the response for one endpoint (mortality or a sub-lethal response). Dotted line indicates data quality objectives that negative controls elicit less than 20% effect. NT: Not tested.

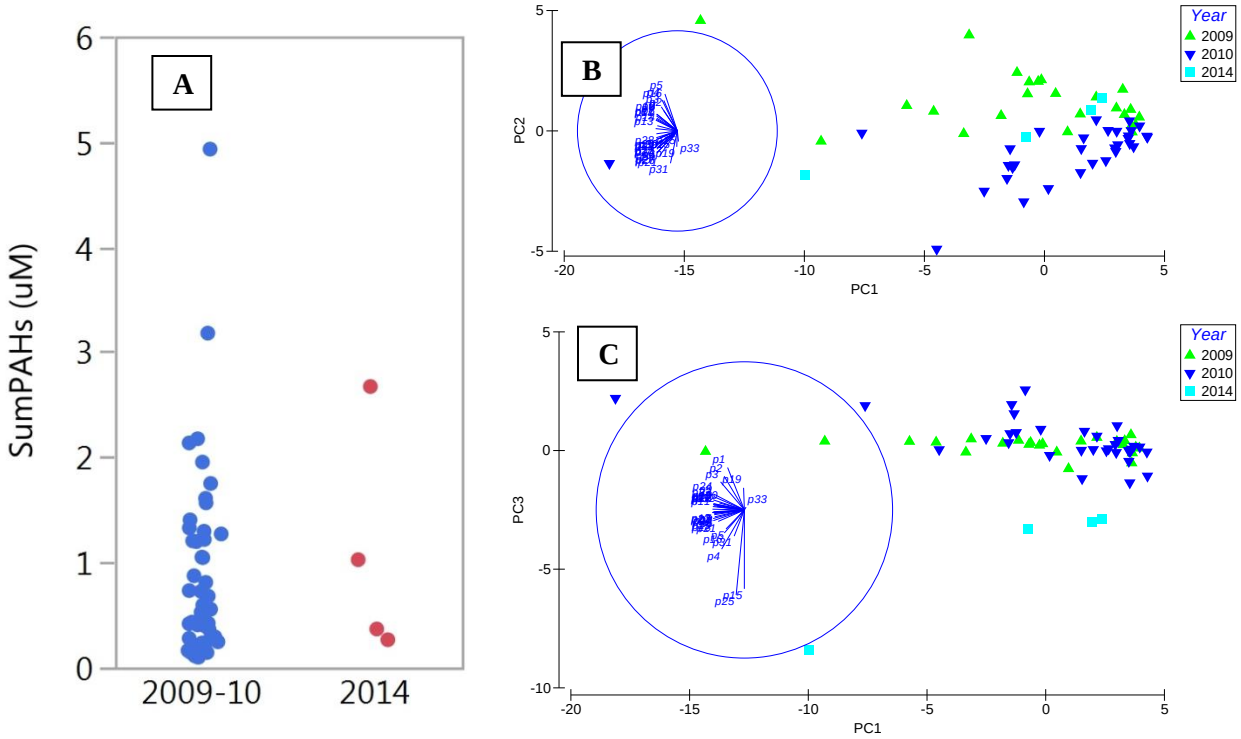

Figure B9. Comparison of PAH concentrations between 2014 samples (n=4, this paper) and samples collected in 2009-2010 (n=50) for Allan *et al.* (2012). Only the 33 PAHs common to both studies were included. PCA performed with Primer. (A) The nominal concentrations of sum 33PAHs in the exposure solutions of the whole extracts for samples from Allan *et al.* (2012) (blue) and the present study (red). (B) PC1 vs. PC2 and (C) PC1 vs. PC3 from the principal components analysis of individual PAH concentrations. PC 1, 2, and 3 explain 55.7, 9.5, and 6.7% of variability, respectively.

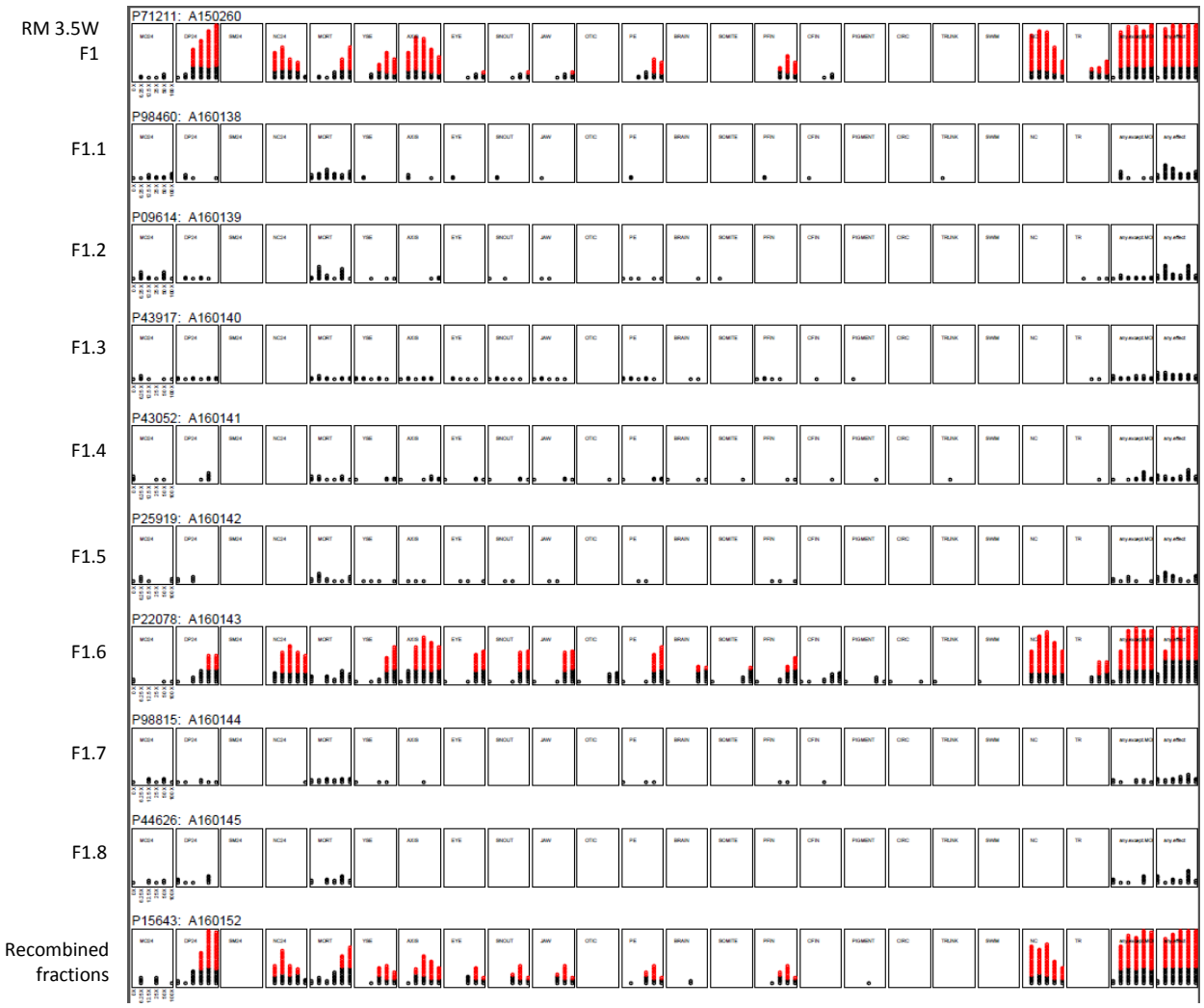

Figure B10. F1.6 is the only toxic sub fraction of F1 of RM 3.5W. From left to right, the boxes show recorded effects to zebrafish embryos: The first four are mortality, delayed developmental progression, spontaneous movement, and notochord distortion at 24 hours; the remaining are mortality at 120 hours and malformations of the yolk sac, axis, eye, snout, jaw, otic nerve, pericardium, brain, somites, pectoral fin, caudal fin, pigmentation, circulation, trunk, swim bladder, notochord, and lastly whether the fish respond to a touch stimulus. The two boxes on the far right show the number of fish with at least one of the described effects either excluding or including mortality.

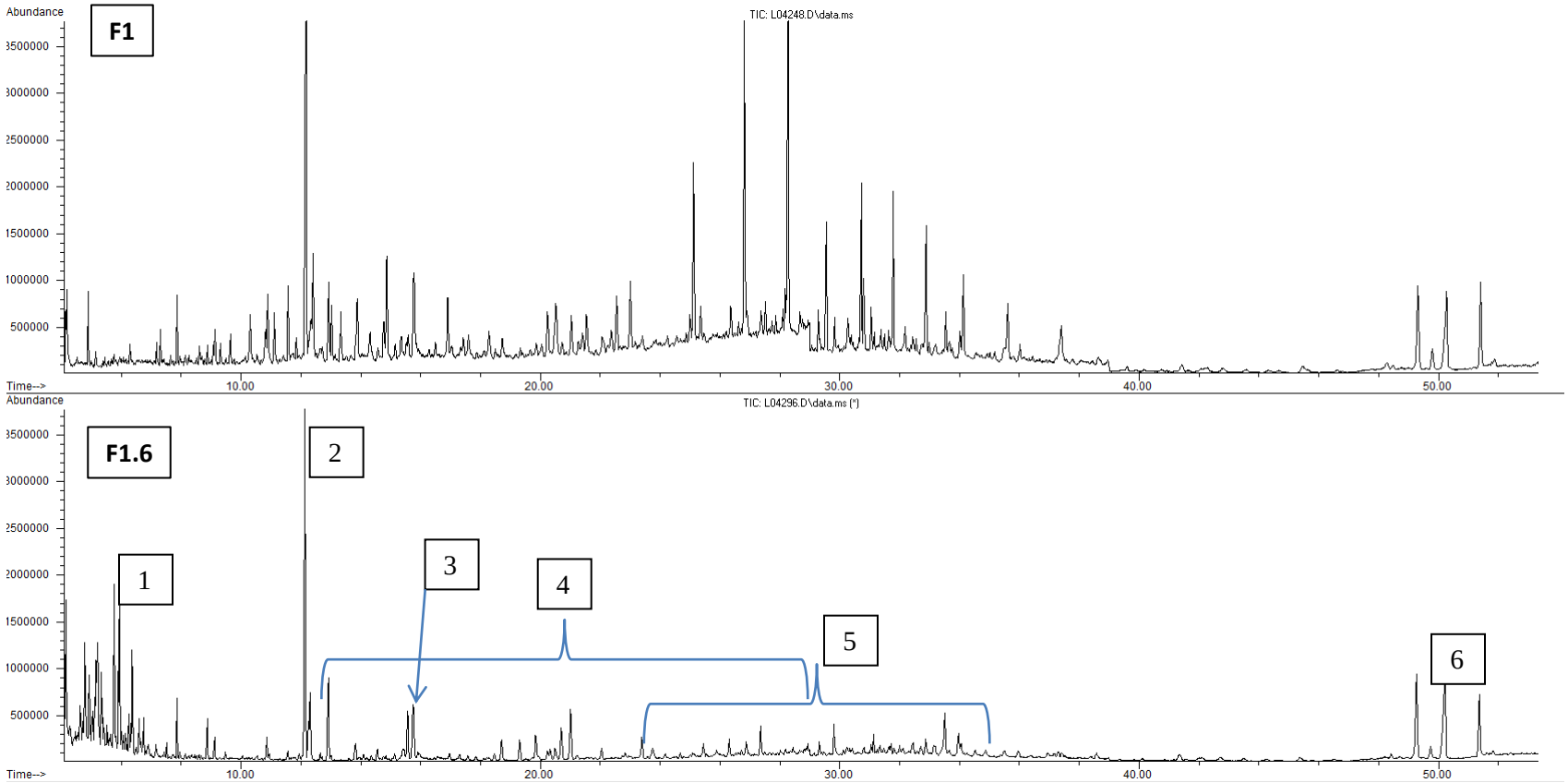

Figure B11. Fractionation with NP-HPLC reduced the chromatographic background in RM 3.5W F1. F1.6 was the only toxic sub-fraction of F1, closely resembling F1 toxicity. 1) NP-HPLC background. Not toxic in control fractions. 2) Propenoic acid pentadecyl ester. (chemstation MF = 90) 3) Galaxolide. 4) Several compounds that hint at dithiocarbamates (see Figure B12). 5) Several phthalates. 6) Undetermined compounds.

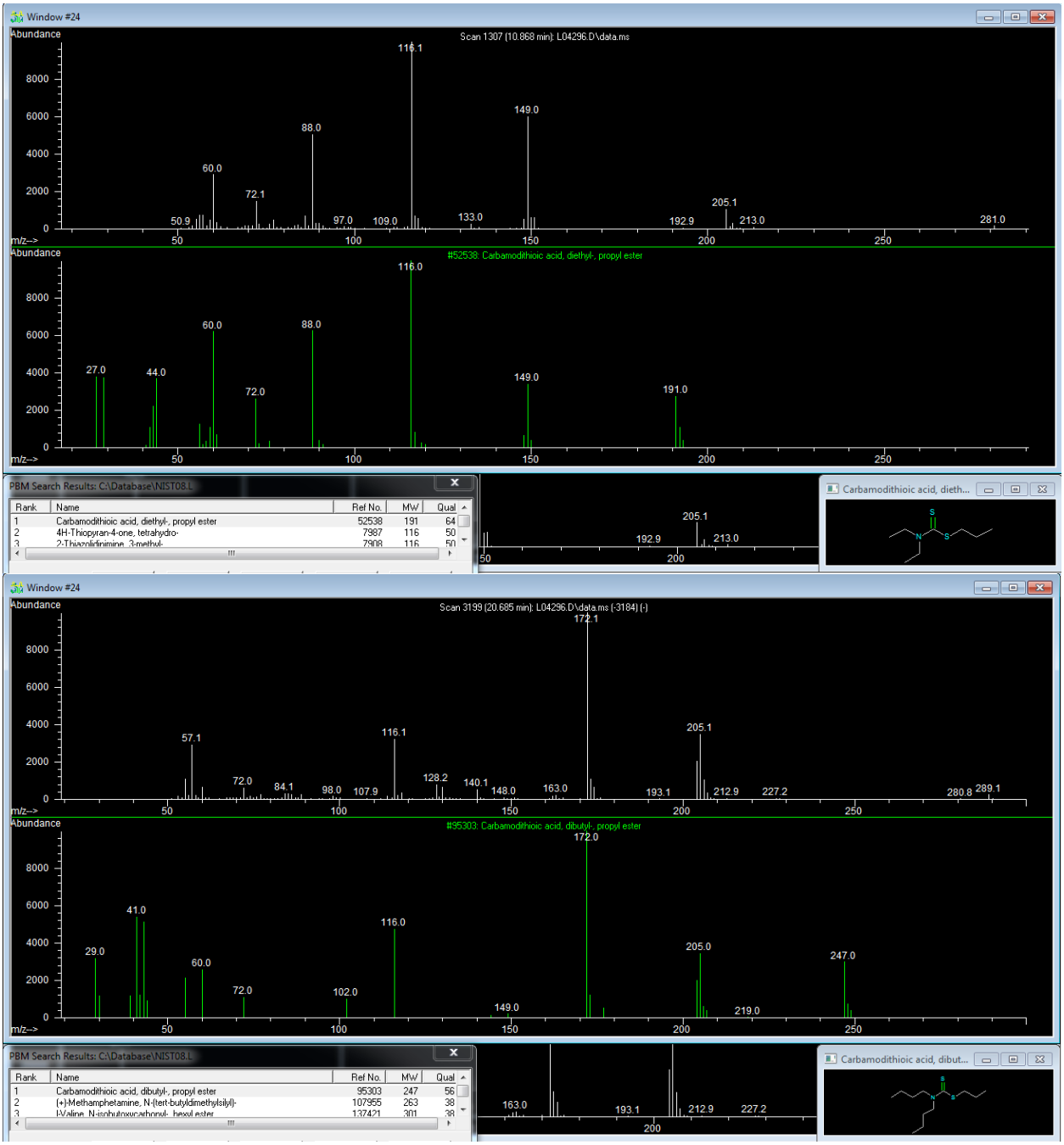

Figure B12. Screenshots of possible DTC spectra. Two representative spectra (white) from the chromatogram of F1.6 (shown in Figure B11) and the best corresponding NIST08 mass spectral library matches (green).

Appendix C. List of 1397 target analytes in GC-MS Screen.

(2,3-Dibromopropyl) (2,4,6-tribromophenyl) ether

1,2,3,4,6,7,8,9-Octachlorodibenzofuran

1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin

1,2,3,4,6,7,8-Heptachlorodibenzofuran

1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin

1,2,3,4,6,7,9-Heptachlorodibenzo-p-dioxin

1,2,3,4,6,7-Hexachlorodibenzo-p-dioxin

1,2,3,4,7,8-Hexachlorodibenzofuran

1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin

1,2,3,4,7-Pentachlorodibenzo-p-dioxin

1,2,3,4-Tetrachlorodibenzofuran

1,2,3,4-Tetrachlorodibenzo-p-dioxin

1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin

1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin

1,2,3,7,8-Pentachlorodibenzofuran

1,2,3,7,8-Pentachlorodibenzo-p-dioxin

1,2,3,8,9-Pentachlorodibenzo-p-dioxin

1,2,3-Trichlorodibenzo-p-dioxin

1,2,4,6,7,9/1,2,4,6,8,9-Hexachlorodibenzo-p-dioxin

1,2,4,6,8/1,2,4,7,9-Pentachlorodibenzo-p-dioxin

1,2,4,7,8-Pentachlorodibenzo-p-dioxin

1,2,4-Trichlorobenzene

1,2,4-Trichlorodibenzo-p-dioxin

1,2,5,6,9,10-Hexabromocyclododecane

1,2,6,7-Tetrachlorodibenzo-p-dioxin

1,2,6,8-Tetrachlorodibenzo-p-dioxin

1,2,7,8-Tetrachlorodibenzo-p-dioxin

1,2,8,9-Tetrachlorodibenzo-p-dioxin

1,2-Bis(2,4,6-tribromophenoxy)ethane

1,2-Dibromo-3-chloropropane

1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane

1,2-Dichlorodibenzo-p-dioxin

1,2-dimethylnaphthalene

1,3,5-Tribromobenzene

1,3,6,8-Tetrachlorodibenzofuran

1,3,6,8-Tetrachlorodibenzo-p-dioxin

1,3,7,8-Tetrachlorodibenzo-p-dioxin

1,3,7,9-Tetrachlorodibenzo-p-dioxin

1,3-Dichlorobenzene

1,3-Dichlorodibenzo-p-dioxin

1,3-Dinitropyrene

1,4-Anthraquinone

1,4-Dichlorodibenzo-p-dioxin

1,4-dimethylnaphthalene

1,4-Dioxino(2,3,b,5,6,b')dipyridine

1,5-dimethylnaphthalene

1,6-Benzo(a)pyrene-quinone

1,6-Dichlorodibenzo-p-dioxin

1,6-dimethylnaphthalene

1,6-Dinitropyrene

1,7,8-Trichlorodibenzo-p-dioxin

1,8-dimethylnaphthalene

1,8-Dinitropyrene

17a-Ethynylestradiol

1-Chlorodibenzo-p-dioxin

1-Hydroxynaphthalene

1-methylnaphthalene

1-methylphenanthrene

1-methylpyrene

1-Nitronaphthalene

1-Nitropyrene

2-(1-naphthyl)acetamide

2-(2-Butoxyethoxy)ethyl thiocyanate

2-(3-Chlorophenoxy)propionamide

2-(Octylthio)ethanol

2,2',3,3',4,4'-Hexabromodiphenyl ether

2,2',3,3',4,5',6,6'-Octabromodiphenyl ether

2,2',3,4,5,5',6-Heptabromodiphenyl ether

2,2',3,4,5,6-Hexabromodiphenyl ether

2,2',3,4,5',6-Hexabromodiphenyl ether

2,2',3,4,6'-Pentabromodiphenyl ether

2,2',3,4,6-Pentabromodiphenyl ether

2,2',4,5',6-Pentabromodiphenyl ether

2,2',4,6'-Tetrabromodiphenyl ether

2,2',4,6-Tetrabromodiphenyl ether

2,2',5-Tribromobiphenyl

2,2',6-Tribromodiphenyl ether

2,2'-Dibromobiphenyl

2,2'-Dibromodiphenyl ether

2,3,3',4,5,6-Hexabromodiphenyl ether

2,3,3',4,5'-Pentabromodiphenyl ether

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

2,3,4,4',6-Pentabromodiphenyl ether

2,3,4,5-Tertrachloronitrobenzene

2,3,4,5-Tetrachlorophenol

2,3,4,6-Tetrabromodiphenyl ether

2,3',4,6-Tetrabromodiphenyl ether

2,3,4,6-Tetrachlorophenol

2,3,4,7,8-PeCDF

2,3,4-Tribromodiphenyl ether

2,3,4-Tribromophenol

2,3,4-Trichlorophenyl-4-nitrophenyl ether

2,3,5,6-Tetrachlorophenol

2,3,5,6-Tetrachloro-p-terphenyl

2,3',5-Tribromobiphenyl

2,3',5-Tribromodiphenyl ether

2,3,5-Trichlorophenol

2,3,5-Trichlorophenyl-4-nitrophenyl ether

2,3,5-Trimethacarb

2,3',6-Tribromodiphenyl ether

2,3,6-Trichloroanisole

2,3,6-Trichlorophenyl-4-nitrophenyl ether

2,3,7,8-TCDD

2,3,7,8-Tetrabromodibenzo-p-dioxin

2,3,7,8-Tetrachlorodibenzofuran

2,3,7-Trichlorodibenzo-p-dioxin

2,3-Dibromoanisole

2,3'-Dibromodiphenyl ether

2,3-Dibromophenol

2,3-Dichlorodibenzo-p-dioxin

2,3-Dichlorophenyl-4-nitrophenyl ether

2,3-dimethylanthracene

2,4,5-T methyl ester

2,4,5-Tribromoanisole

2,4',5-Tribromodiphenyl ether

2,4,5-Trichloroaniline

2,4,5-Trichlorophenol

2,4,5-Trichlorophenyl-4-nitrophenyl ether

2,4,5-Trichloro-p-terphenyl

2,4,5-Trimethylaniline

2,4,6-Tribromoanisole

2,4,6-Tribromophenol

2,4,6-Tribromophenyl allyl ether

2,4,6-Trichloroanisole

2,4,6-Trichlorophenol

2,4,8-Trichlorodibenzofuran

2,4-D methyl ester

2,4-D sec-butyl ester

2,4-DB methyl ester

2,4'-DDD

2,4'-DDE

2,4'-DDT

2,4-Dibromoanisole

2,4-Dibromobiphenyl

2,4-Dibromophenol

2,4-Dibromophenyl-4-nitrophenyl ether

2,4'-Dichlorobenzophenone (2,4'-Dicofol decomposition product)

2,4-Dichlorophenol

2,4-Dichlorophenyl benzenesulfonate

2,4-Dimethylaniline

2,4-Dimethylphenol

2,5-Dibromoanisole

2,5-Dibromobiphenyl

2,5-Dibromodiphenyl ether

2,5-Dibromophenol

2,5-Dichlorophenyl-4-nitrophenyl ether

2,6-Dibromoanisole

2,6-Dibromobiphenyl

2,6-Dibromophenol

2,6-Dichlorobenzamide

2,6-Dichlorobenzonitrile

2,6-Dichlorophenol

2,6-Dichlorophenyl-4-nitrophenyl ether

2,6-Dichlorosyringaldehyde

2,6-diethylnaphthalene

2,6-Dimethylaniline

2,6-dimethylnaphthalene

2,7-Dichlorodibenzo-p-dioxin

2,8-Dichlorodibenzofuran

2,8-Dichlorodibenzo-p-dioxin

2-Bromoanisole

2-Bromobiphenyl

2-chlorodibenzofuran

2-Chlorodibenzo-p-dioxin

2-Chlorophenol

2-Chlorophenyl-4-nitrophenyl ether

2-Chlorosyringaldehyde

2-Ethyl-1,3-hexanediol

2-ethyl-6-methylaniline

2-ethylnaphthalene

2'-Hydroxy-2,4,4'-tribromodiphenyl ether

2'-Hydroxy-4-monobromodiphenyl ether

2-Hydroxyestradiol

2'-Methoxy-2,4,4'-tribromodiphenyl ether

2-methylanthracene

2-methylnaphthalene

2-methylphenanthrene

2-Methylphenol

2-Nitroanthracene

2-Nitrobiphenyl

2-Nitrofluorene

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

2-Nitronaphthalene
2-Nitrophenol
2-Nitropyrene
2-Phenoxypropionic acid
3,3',4,5,5'-Pentabromodiphenyl ether
3,3'-Dimethoxybenzidine
3,4,5-Trichlorophenyl-4-nitrophenyl ether
3,4,5-Trimethacarb
3,4,6-Trichloroguaiacol
3,4-Dichloroaniline
3,4-Dichlorocatechol
3,4-Dichloroguaiacol
3,4-Dichlorophenyl-4-nitrophenyl ether
3,5-Dibromoanisole
3,5-Dibromophenol
3,5-Dichloroaniline
3,5-Dichlorophenyl-4-nitrophenyl ether
3,6-dimethylphenanthrene
3-Aminophenol
3-Bromoanisole
3-Bromobiphenyl
3-Bromophenol
3-Bromostyrene
3-Chloro-4-fluoroaniline
3-Chloro-4-methoxyaniline
3-Chloroaniline
3-Chlorophenyl-4-nitrophenyl ether
3-Hydroxycarbofuran
3-Indolylacetonitrile
3-Methoxy-2,2',4,4',6-pentabromodiphenyl ether
3-Nitrobenzanthrone
3-Nitrobiphenyl
3-nitrodibenzofuran
3-Nitrofluoranthene
3-Nitrophenanthrene
3-Trifluormethylaniline
4,4'-DDD
4,4'-DDE
4,4'-DDT
4,4'-Dibromobiphenyl
4,4'-Dichlorobenzophenone
4,4'-Oxydianiline
4,5-Dichloroguaiacol
4,6-Dichloroguaiacol
4,6-Dinitro-o-cresol (DNOC)
4-Aminobiphenyl
4-Bromoaniline
4-Bromoanisole
4-Bromobiphenyl
4-Bromophenol
4-Bromostyrene
4-Chloro-2-methylaniline
4-Chloro-3-methylphenol
4-Chloroaniline
4-Chlorocatechol
4-Chlorodibenzofuran
4-Chloroguaiacol
4-Chlorophenol
4-Chlorophenyl isocyanate
4-Chlorophenyl phenyl ether
4-Chlorophenyl-4-nitrophenyl ether
4H-Cyclopenta(def)phenanthren-4-one
4-Isopropylaniline
4-Methylphenol
4-Nitrobiphenyl
4-Nitrophenol
4-Nitrophenyl phenyl ether
4-Nonylphenol
5,12-Naphthacene-quinone
5,6-Dichlorovanillin
5,7-Dihydroxy-4'-methoxyisoflavone
5-Chlorovanillin
5-methylchrysene
5-Nitroacenaphthene
6-Chlorovanillin
6-methylchrysene
6-Nitrobenzo(a)pyrene
6-Nitrochrysene
7,12-dimethylbenz[a]anthracene
7-Nitrobenz(a)anthracene
9,10-Anthraquinone
9,10-dimethylanthracene
9,10-Phenanthrenequinone
9-Fluorenone
9-methylanthracene
9-Nitroanthracene
9-Nitrophenanthrene
acenaphthene
Acenaphthenequinone
acenaphthylene
Acephate
Acequinocyl
acetamiprid
Acetochlor
Acifluorfen methyl ester
Aclonifen
Acrinathrin
Alachlor
Aldrin
Allidochlor
alpha, alpha-Dibromo-m-xylene
alpha-BHC
alpha-Chlordane
Ametryne
Amidithion
Aminocarb

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Amitraz

Amitraz metabolite [Methanimidamide, N-(2,4-dimethylphenyl)-N'-methyl-]

Ancymidol

Anilazine

Aniline

Anilofos

anthanthrene

anthracene

Aramite

Aramite II

Atraton

Atrazine

Atrazine-desethyl

Azaconazole

Azamethiphos

Azibenzolar-S-methyl

Azinphos-ethyl

Azinphos-methyl

Aziprotryn metabolite [2-Amino-4-isopropylamino-6-methylthio-1,3,5-triazine]

Aziprotryne

Azobenzene

Azoxybenzene

Azoxystrobin

Barban

Beflubutamid

Benalaxyl

Benazolin-ethyl

Bendiocarb

Benfluralin

Benfuracarb

Benfuresate

Benodanil

Benoxacor

Bentazone

Bentazone methyl derivative

Benthiocarb

Benz(a)anthracene-7,12-dione

benz[a]anthracene

benz[j]and[e]aceanthrylene

Benzanthrone

Benzenesulfonamide

Benzidine

Benzo(a)fluoren-11-one

Benzo(a)pyrene-7,8-dione

Benzo(c)phenanthrene(1,4)quinone

benzo[a]chrysene

benzo[a]fluorene

benzo[a]pyrene

benzo[b]fluoranthene

benzo[b]fluorene

benzo[b]perylene

benzo[c]fluorene

benzo[e]pyrene

benzo[ghi]perylene

benzo[j]fluoranthene

benzo[k]fluoranthene

Benzophenone

Benzoximate metabolite

Benzoylprop ethyl

Benzyl benzoate

b-Estradiol

beta-BHC

BHC epsilon isomer

Bifenazate metabolite (5-Phenyl-o-anisidine)

Bifenox

Bifenthrin

Binapacryl

Bioallethrin

Bioallethrin S-cyclopentenyl isomer

Bioresmethrin

Biphenyl

Bis(2,3,3,3-tetrachloropropyl) ether

Bis(2-butoxyethyl) phthalate

Bis(2-ethylhexyl)phthalate

Bisphenol A

Bitertanol I

Bitertanol II

Boscalid (Nicobifen)

Bromacil

Bromfenvinphos-(E)

Bromfenvinphos-(Z)

Bromobutide

Bromocyclen

Bromophos

Bromophos-ethyl

Bromopropylate

Bromoxynil

Bromoxynil octanoic acid ester

Bromuconazole I

Bromuconazole II

Bufencarb

Bupirimate

Buprofezin

Butachlor

Butafenacil

Butamifos

Butoxycarboxim

Butralin

Butyl benzyl phthalate

Butylate

Butylated hydroxyanisole

Cadusafos

Cafenstrole

Caffeine

Captafol

Captan

Carbaryl

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Carbetamide
Carbofuran
Carbofuran-3-keto
Carbofuran-7-phenol
Carbophenothion
Carbosulfan
Carboxin
Carfentrazone-ethyl
Carpropamid
Carvone
Cashmeran
Cekafix
Celestolide
Chinomethionat
Chloramben methyl ester
Chloranocryl
Chlorbenside
Chlorbenside sulfone
Chlorbicyclen
Chlorbromuron
Chlorbufam
Chlordene, trans-
Chlordimeform
Chlorethoxyfos
Chlorfenapyr
Chlorfenethol
Chlorfenprop-methyl
Chlorfenson
Chlorfenvinphos
Chlorfenvinphos, cis-
Chlorfenvinphos, trans-
Chlorflurecol-methyl ester
Chlormefos
Chlornitrofen
Chlorobenzilate
Chloroneb
Chloropropylate
Chlorothalonil
Chlorotoluron
Chlorpropham
Chlorpyrifos
Chlorpyrifos Methyl
Chlorthiamid
Chlorthion
Chlorthiophos
Chlorthiophos sulfone
Chlorthiophos sulfoxide
Chlozolinate
chrysene
Cinerin I
Cinerin II
Cinidon-ethyl
cis-Nonachlor
Clodinafop-propargyl
Clomazone
Cloquintocet-mexyl
coronene
Coumaphos
Crimidine
Crotoxyphos
Crufomate
Cyanazine
Cyanofenphos
Cyanophos
Cyclafuramid
Cycloate
cyclopenta[cd]pyrene
Cyclopentadecanone
Cycluron
Cyflufenamid
Cyfluthrin I
Cyfluthrin II
Cyfluthrin III
Cyfluthrin IV
Cyhalofop-butyl
Cyhalothrin (Gamma)
Cyhalothrin I (lambda)
Cymiazole
Cymoxanil
Cypermethrin-1
Cypermethrin-2
Cypermethrin-3
Cypermethrin-4
Cyphenothrin cis-
Cyphenothrin trans-
Cyprazine
Cyproconazole
Cyprodinil
Cyprofuram
Cyromazine
d-(cis-trans)-Phenothrin-I
d-(cis-trans)-Phenothrin-II
Dacthal
Dazomet
DDMU [1-Chloro-2,2-bis(4'-chlorophenyl)ethylene]
delta-BHC
Deltamethrin
Demephion
Demeton-s
Demeton-S-methyl
Demeton-S-methylsulfon
Desbromo-bromobutide
Desmedipham
Desmetryn
Dialifos
Diallate I
Diallate II
Diamyl phthalate
Diazinon
Diazinon-oxon
dibenzo[a,e]fluoranthene

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

dibenzo[a,e]pyrene
dibenzo[a,h]anthracene
dibenzo[a,h]pyrene
dibenzo[a,i]pyrene
dibenzo[a,l]pyrene
dibenzo[e,l]pyrene
Dibenzofuran
Dibenzo-p-dioxin
dibenzothiophene
Dicamba
Dicamba methyl ester
Dicapthon
Dichlofenthion
Dichlofluanid
Dichlofluanid metabolite (DMSA)
Dichlone
Dichloran
Dichlormid
Dichlorophen
Dichlorprop
Dichlorprop methyl ester
Dichlorvos
Diclobutrazol
Diclocymet I
Diclocymet II
Diclofop methyl
Dicrotophos
Dicyclohexyl phthalate
Dicyclopentadiene
Dieldrin
Diethatyl ethyl
Diethofencarb
Diethyl dithiobis(thionoformate) (EXD)
Diethyl phthalate
Diethylene glycol
Diethylstilbestrol
Difenoconazol I
Difenoconazol II
Difenoxuron
Diflufenican
Diisobutyl phthalate
Dimefox
Dimepiperate
Dimethachlor
Dimethametryn
Dimethenamid
Dimethipin
Dimethoate
Dimethomorph-(E)
Dimethomorph-(Z)
Dimethyl phthalate
Dimethylvinphos(E)
Dimethylvinphos(Z)
Dimetilan
Dimoxystrobin
Di-n-butyl phthalate
Di-n-hexyl phthalate
Diniconazole
Dinitramine
Di-n-nonyl phthalate
Dinobuton
Dinocap
Dinocap II
Dinocap III
Dinocap IV
Di-n-octyl phthalate
Dinoseb
Dinoseb acetate
Dinoseb methyl ether
Dinoterb
Dinoterb acetate
Di-n-propyl phthalate
Diofenolan I
Diofenolan II
Dioxabenzofos
Dioxacarb
Dioxathion
Diphacinone
Diphenamid
Diphenyl phthalate
Diphenylamine
Dipropetryn
Dipropyl isocinchomeronate
Disulfoton
Disulfoton sulfone
Ditalimfos
Dithiopyr
Diuron
Diuron Metabolite [3,4-Dichlorophenyl isocyanate]
Dodemorph I
Dodemorph II
Drazoxolon
Edifenphos
Empenthrin I
Empenthrin II
Empenthrin III
Empenthrin IV
Empenthrin V
Endosulfan ether
Endosulfan I
Endosulfan II
Endosulfan lactone
Endosulfan sulfate
Endrin
Endrin aldehyde
Endrin ketone
EPN
Epoxiconazole
EPTC
Erbon
Esfenvalerate

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Esprocarb
Etaconazole
Ethalfluralin
Ethidimuron
Ethiofencarb
Ethiolate
Ethion
Ethofenprox
Ethofumesate
Ethofumesate, 2-Keto
Ethoprophos
Ethoxyfen-ethyl
Ethoxyquin
Ethylenethiourea
Etoxazole
Etridiazole, deschloro- (5-ethoxy-3-dichloromethyl-1,2,4-thiadiazole)
Etrimfos
Eugenol
Exaltolide [15-Pentadecanolide]
Famoxadon
Famphur
Fenamidone
Fenamiphos
Fenamiphos sulfoxide
Fenamiphos-sulfone
Fenarimol
Fenazaflor
Fenazaflor metabolite
Fenazaquin
Fenbuconazole
Fenchlorazole-ethyl
Fenchlorphos
Fenchlorphos-oxon
Fenclorim
Fenfuram
Fenhexamid
Fenitrothion
Fenitrothion-oxon
Fenobucarb
Fenoprop
Fenoprop methyl ester
Fenothiocarb
Fenoxanil
Fenoxaprop-ethyl
Fenoxycarb
Fenpiclonil
Fenpropathrin
Fenpropidin
Fenson
Fensulfothion
Fensulfothion-oxon
Fensulfothion-oxon -sulfone
fensulfothion-sulfone
Fenthion
Fenthion sulfoxide
Fenthion-sulfone
Fenuron
Fenvalerate
Fenvalerate II
Fepropimorph
Fipronil
Fipronil, Desulfinyl-
Fipronil-sulfide
Fipronil-sulfone
Flamprop-isopropyl
Flamprop-methyl
Fluacrypyrim
Fluazifop-p-butyl
Fluazinam
Fluazolate
Flubenzimine
Fluchloralin
Flucythrinate I
Flucythrinate II
Fludioxonil
Flufenacet
Flumetralin
Flumiclorac-pentyl
Flumioxazin
Fluometuron
fluoranthene
fluorene
Fluorodifen
Fluoroglycofen-ethyl
Fluoroimide
Fluotrimazole
Fluoxastrobin cis-
Fluquinconazole
Flurenol-butyl ester
Flurenol-methylester
Fluridone
Flurochloridone I
Flurochloridone II
Flurochloridone, deschloro-
Fluroxypyr-1-methylheptyl ester
Flurprimidol
Flurtamone
Flusilazole
Fluthiacet-methyl
Flutolanil
Flutriafol
Fluvalinate-tau-I
Fluvalinate-tau-II
Folpet
Fonofos
Formothion
Fosthiazate I
Fosthiazate II
Fuberidazole
Furalaxyl

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Furathiocarb
Furilazole
Furmecyclox
Galaxolide
gamma-Chlordane
Halfenprox
Haloxyfop-methyl
Heptachlor
Heptachlor epoxide
Heptachlor epoxide isomer A
Heptenophos
Hexabromobenzene
Hexachlorobenzene
Hexachlorophene
Hexaconazole
Hexazinone
Hexestrol
Hydroprene
Imazalil
Imazamethabenz-methyl I
Imazamethabenz-methyl II
Imibenconazole
Imibenconazole-desbenzyl
Imidan
indeno[1,2,3-cd]pyrene
Indoxacarb and Dioxacarb decomposition product [Phenol, 2-(1,3-dioxolan-2-yl)-]
Ioxynil
Ioxynil octanoate
Ipconazole
Iprobenfos
Iprodione
Iprovalicarb I
Iprovalicarb II
Irgarol
Isazophos
Isobenzan
Isobornyl thiocyanoacetate
Isocarbamide
Isocarbophos
Isodrin
Isofenphos
Isofenphos-oxon
Isomethiozin
Isoprocarb
Isopropalin
Isoprothiolane
Isoproturon
Isoxaben
Isoxadifen-ethyl
Isoxaflutole
Isoxathion
Jasmolin I
Jasmolin II
Jodfenphos
Kepone
Kinoprene
Kresoxim-methyl
Lactofen
Lenacil
Leptophos
Leptophos oxon
Lindane
Linuron
Malathion
Malathion-o-analog
MCPA methyl ester
MCPA-butoxyethyl ester
MCPB methyl ester
m-Cresol
Mecarbam
Mecoprop methyl ester
Mefenacet
Mefenpyr-diethyl
Mefluidide
Menazon
Mepanipyrim
Mephosfolan
Mepronil
Metalaxyl
Metamitron
Metazachlor
Metconazole I
Metconazole II
Methabenzthiazuron [decomposition product]
Methacrifos
Methamidophos
Methfuroxam
Methidathion
Methiocarb
Methiocarb Sulfone
Methiocarb sulfoxide
Methomyl
Methoprene I
Methoprene II
Methoprotryne
Methoxychlor
Methoxychlor olefin
Methyl (2-naphthoxy)acetate
Methyl paraoxon
Methyl-1-naphthalene acetate
Methyldymron
Metobromuron
Metolachlor
Metolcarb
Metominostrobin (E)
Metominostrobin (Z)
Metrafenone
Metribuzin
Mevinphos
Mirex

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Molinate
Monalide
Monocrotophos
Monolinuron
Musk amberette
Musk Ketone
Musk Moskene
Musk Tibetene (Moschustibeten)
Musk xylene
Myclobutanil
N,N-Diethyl-m-toluamide
N-1-Naphthylacetamide
Naled
naphthalene
Naphthalic anhydride
Naphthanthrone
naphtho[1,2-b]fluoranthene
naphtho[2,3-a]pyrene
naphtho[2,3-e]pyrene
naphtho[2,3-j]fluoranthene
naphtho[2,3-k]fluoranthene
Naproanilide
Napropamide
Nickel dibutyldithiocarbamate
Nicotine
Nitralin
Nitrapyrin
Nitrofen
Nitrothal-isopropyl
N-Methyl-N-1-naphthyl acetamide
Norflurazon
Norflurazon, Desmethyl-
Nuarimol
Octachlorostyrene
Octamethyl pyrophosphoramide
o-Dichlorobenzene
Ofurace
Omethoate
o-Phenylphenol
Orbencarb
ortho-Aminoazotoluene
Oryzalin
Oxabetrinil
Oxadiazon
Oxadixyl
Oxamyl
Oxycarboxin
Oxychlordane
Oxydemeton-methyl
Oxyfluorfen
p,p'-DDM [bis(4-chlorophenyl)methane]
p,p'-Dibromobenzophenone
p,p'-Dicofol
Paclobutrazol
Paraoxon
Parathion-ethyl
Parathion-methyl
PBB 101
PBB 169 Hexabrombiphenyl
PBB 52 Tetrabrombiphenyl
PBB-101
PBB-103
PBB-114
PBB-137
PBB-141
PBB-153
PBB-155
PBB-156
PBB-159
PBB-169
PBB-180
PBB-189
PBB-200
PBB-29
PBB-30
PBB-31
PBB-49
PBB-53
PBB-77
PBB-80
PBDE 1
PBDE 10
PBDE 100
PBDE 11
PBDE 116
PBDE 118
PBDE 119
PBDE 12
PBDE 13
PBDE 138
PBDE 15
PBDE 153
PBDE 154
PBDE 155
PBDE 166
PBDE 17
PBDE 2
PBDE 25
PBDE 28
PBDE 3
PBDE 30
PBDE 32
PBDE 33
PBDE 35
PBDE 37
PBDE 47
PBDE 49
PBDE 66
PBDE 7
PBDE 71
PBDE 75

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

PBDE 77	PCB 13	PCB 163
PBDE 8	PCB 130	PCB 164
PBDE 85	PCB 131	PCB 165
PBDE 99	PCB 132	PCB 166
PCB 1	PCB 133	PCB 167
PCB 10	PCB 134	PCB 168
PCB 101	PCB 135	PCB 169
PCB 102	PCB 136	PCB 17
PCB 103	PCB 137	PCB 170
PCB 104	PCB 138	PCB 171
PCB 105	PCB 139	PCB 172
PCB 106	PCB 14	PCB 173
PCB 107	PCB 140	PCB 174
PCB 108	PCB 141	PCB 175
PCB 109	PCB 142	PCB 176
PCB 11	PCB 143	PCB 177
PCB 110	PCB 144	PCB 178
PCB 111	PCB 145	PCB 179
PCB 112	PCB 146	PCB 18
PCB 113	PCB 147	PCB 180
PCB 114	PCB 148	PCB 181
PCB 115	PCB 149	PCB 182
PCB 116	PCB 15	PCB 183
PCB 117	PCB 150	PCB 184
PCB 118	PCB 151	PCB 185
PCB 119	PCB 152	PCB 186
PCB 12	PCB 153	PCB 187
PCB 120	PCB 154	PCB 188
PCB 121	PCB 155	PCB 189
PCB 122	PCB 156	PCB 19
PCB 123	PCB 157	PCB 190
PCB 124	PCB 158	PCB 191
PCB 125	PCB 159	PCB 192
PCB 126	PCB 16	PCB 193
PCB 127	PCB 160	PCB 194
PCB 128	PCB 161	PCB 195
PCB 129	PCB 162	PCB 196

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

PCB 197
PCB 198
PCB 199
PCB 2
PCB 20
PCB 200
PCB 201
PCB 202
PCB 203
PCB 204
PCB 205
PCB 206
PCB 207
PCB 208
PCB 21
PCB 22
PCB 23
PCB 24
PCB 25
PCB 26
PCB 27
PCB 28
PCB 29
PCB 3
PCB 30
PCB 31
PCB 32
PCB 33
PCB 34
PCB 35
PCB 36
PCB 37
PCB 38
PCB 39
PCB 4
PCB 40
PCB 41
PCB 42
PCB 43
PCB 44
PCB 45
PCB 46
PCB 47
PCB 48
PCB 49
PCB 5
PCB 50
PCB 51
PCB 52
PCB 53
PCB 54
PCB 55
PCB 56
PCB 57
PCB 58
PCB 59
PCB 6
PCB 60
PCB 61
PCB 62
PCB 63
PCB 64
PCB 65
PCB 66
PCB 67
PCB 68
PCB 69
PCB 7
PCB 70
PCB 71
PCB 72
PCB 73
PCB 74
PCB 75
PCB 76
PCB 77
PCB 78
PCB 79
PCB 8
PCB 80
PCB 81
PCB 82
PCB 83
PCB 84
PCB 85
PCB 86
PCB 87
PCB 88
PCB 89
PCB 9
PCB 90
PCB 91
PCB 92
PCB 93
PCB 94
PCB 95
PCB 96
PCB 97
PCB 98
PCB 99
p-Dichlorobenzene
Pebulate
Penconazole
Pendimethalin
Pentabromoethylbenzene
Pentabromotoluene
Pentachloroaniline
Pentachloroanisole
Pentachlorobenzene
Pentachloronitrobenzene
Pentachlorophenol

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Pentanochlor
Permethrin
Permethrin II
Perthane
Phantolide
phenanthrene
Phenanthrene-1,4-dione
Phenkapton
Phenol
Phenothiazine
Phenothrin I
Phenothrin II
Phenoxyacetic acid
Phenthoate
Phorate
Phorate sulfone
Phorate sulfoxide
Phorate-oxon
Phosalone
Phosfolan
Phosphamidon
Phthalide
Phthalimide
Picloram methyl ester
Picolinafen
Picoxystrobin
Pindone
Piperalin
Piperonyl butoxide
Piperophos
Pirimicarb
Pirimiphos-ethyl
Pirimiphos-methyl
Plifenat
p-Nitrotoluene
Potasan
Prallethrin, cis-
Prallethrin, trans-
Pretilachlor
Probenazole
Prochloraz
Procymidone
Prodiamine
Profenofos
Profenofos metabolite (4-Bromo-2-chlorophenol)
Profluralin
Prohydrojasmon I
Prohydrojasmon II
Promecarb
Promecarb artifact [5-isopropyl-3-methylphenol]
Prometon
Prometryn
Propachlor
Propamocarb
Propanil
Propaphos
Propargite
Propargite metabolite [Cyclohexanol, 2-(4-tert-butylphenoxy)]
Propazine
Propetamphos
Propham
Propiconazole-II
Propisochlor
Propoxur
Propyzamide
Prosulfocarb
Prothioconazole-desthio
Prothiofos
Prothoate
Pyracarbolid
Pyraclofos
Pyraflufen-ethyl
Pyrazon
Pyrazophos
Pyrazoxyfen
pyrene
Pyrethrin I
Pyrethrin II
Pyributicarb
Pyridaben
Pyridaphenthion
Pyridate
Pyridinitril
Pyrifenox I
Pyrifenox II
Pyriftalid
Pyrimethanil
Pyrimidifen
Pyriminobac-methyl (E)
Pyriminobac-methyl (Z)
Pyriproxyfen
Pyroquilon
Quinalphos
Quinoclamine
Quinoxyfen
Quintozene metabolite (pentachlorophenyl methyl sulfide)
Quizalofop-ethyl
Rabenzazole
Resmethrin
Resmethrine II
retene
Rotenone
S,S,S-Tributylphosphorotrithioate
Sebuthylazine
Sebuthylazine-desethyl
Secbumeton
Silafluofen

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Silthiopham
Simazine
Simeconazole
Simetryn
Spirodiclofen
Spiromesifen
Spiroxamine I
Spiroxamine II
Spiroxamine metabolite (4-tert-butylcyclohexanone)
Sudan I
Sudan II
Sudan Red
Sulfallate
Sulfanilamide
Sulfentrazone
Sulfotep
Sulfur (S8)
Sulprofos
Swep
Tamoxifen
TCEP
TCMTB
TCPP
Tebuconazole
Tebufenpyrad
Tebupirimifos
Tebutam
Tebuthiuron
Tecnazene
Tefluthrin, cis-
Temephos
Terbacil
Terbucarb
Terbufos
Terbufos-oxon-sulfone
Terbufos-sulfone
Terbumeton
Terbuthylazine
Terbuthylazine-desethyl
Terbutryn
Terrazole
Tetrabromo-o-chlorotoluene
Tetrabromophthalate diol
Tetrachloroguaiacol
Tetrachlorvinphos
Tetraconazole
Tetradifon
Tetraethyl pyrophosphate
Tetrahydrophthalimide, cis-1,2,3,6-
Tetramethrin I
Tetramethrin II
Tetrapropyl thiodiphosphate
Tetrasul
Thenylchlor
Theobromine
Thiabendazole
Thiazopyr
Thifluzamide
Thiofanox
Thiometon
Thionazin
Thymol
Tilt
Tiocarbazil I
Tiocarbazil II
Tolclofos-methyl
Tolfenpyrad
Tolylfluanid
Tolylfluanid metabolite (DMST)
Tolyltriazole [1H-Benzotriazole, 4-methyl-]
Tolyltriazole [1H-Benzotriazole, 5-methyl-]
Tonalide
Toxaphene Parlar 26
Toxaphene Parlar 50
Toxaphene Parlar 62
TPP
Transfluthrin
trans-Nonachlor
Traseolide
Triadimefon
Triadimenol
Triallate
Triamiphos
Triapenthenol
Triazamate
Triazophos
Tribromoneopentyl alcohol
Tributyl phosphate
Trichlamide
Trichlorfon
Trichloronate
Trichlorosyringol
Triclopyr methyl ester
Triclosan
Triclosan-methyl
Tricresylphosphate, meta-
Tricresylphosphate, ortho-
Tricyclazole
Tridemorph , 4-tridecyl-
Tridiphane
Trietazine
Triethyl phosphate
Trifenmorph
Trifloxystrobin
Triflumizole
Trifluralin
triphenylene
Tri-p-tolyl phosphate
Tris(2-butoxyethyl) phosphate

Appendix C. List of 1397 target analytes in GC-MS Screen. Continued.

Tris(2-ethylhexyl) phosphate

Triticonazole

Tryclopyrbutoxyethyl

Tycor (SMY 1500)

Uniconizole-P

Vamidothion

Vernolate

Vinclozolin

XMC (3,4-Dimethylphenyl N-methylcarbamate)

XMC (3,5-Dimethylphenyl N-methylcarbamate)

Zinc diethyldithiocarbamate

Zoxamide

Zoxamide decomposition product