

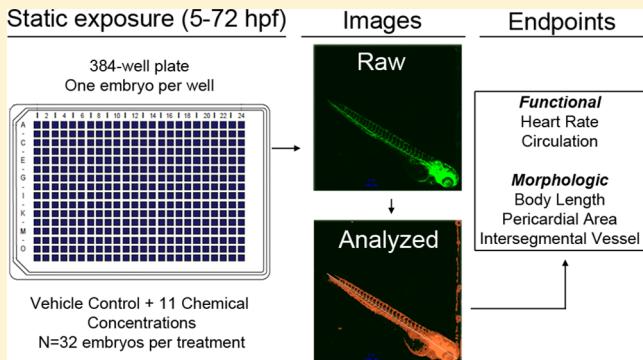
## High-Content Screening Assay for Identification of Chemicals Impacting Cardiovascular Function in Zebrafish Embryos

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 Supporting Information

**ABSTRACT:** Targeted assays are needed to better evaluate effects of chemicals on organogenesis and begin classification of chemicals by toxicologically relevant modes-of-action. Using transgenic zebrafish (*fli1:eGFP*) that stably express eGFP within vascular endothelial cells, we have developed and optimized a 384-well-based high-content screening (HCS) assay that enables us to screen and identify chemicals affecting cardiovascular function at sublethal, nonteratogenic concentrations. Following static exposure of one embryo per well from 5 to 72 h postfertilization (hpf), automated image acquisition procedures and custom image analysis protocols are used to quantify body length, circulation, heart rate, pericardial area (a biomarker for cardiac looping defects), and intersegmental vessel area within freshly hatched live embryos. After optimizing 72 hpf anesthetization procedures, we evaluated each end point across four independent control plates containing 384 initial embryos per plate. Survival and imaging success rates across these plates ranged from 93 to 99% and 42 to 74%, respectively. Criteria were then defined for assay success and analysis of treatments, and 10 chemicals were screened for targeted effects on cardiovascular function. Compared to existing zebrafish-based assays, this method provides a comprehensive discovery platform with (1) increased sample sizes; (2) broad concentration-response format; and (3) the ability to identify chemicals that target cardiovascular function at nonteratogenic concentrations.



### INTRODUCTION

Whole-organism toxicity test guidelines standardized and issued by the Organization for Economic Co-operation and Development (OECD) are used to support human health and ecological risk assessments around the world. While toxicity testing under the European Union's Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) Regulation ([http://ec.europa.eu/environment/chemicals/reach/reach\\_intro.htm](http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm)) is a relatively recent development for new and existing industrial chemicals, food/feed additives, and cosmetics, regulatory authorities have long required crop protection companies to submit a battery of guideline studies for pesticides prior to approval. In addition to pesticides regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (<http://www.ag.senate.gov/download/fifra>), potential amendments to the Toxic Substances Control Act (TSCA) (<http://www.epw.senate.gov/tscact.pdf>) within the United States could significantly increase the demand for generating toxicity data across multiple species and hundreds to thousands of registered and new chemicals in commerce. However, for both mammalian toxicity and ecotoxicity testing, these guideline studies are costly, animal-intensive, and provide minimal information about chemical mode of action (MOA) due to a principal focus on apical end points such as survival, development, and reproduction. Due to these limitations, resource-efficient alternatives

such as high-throughput screening assays have been proposed as key components of a future testing strategy for regulatory toxicology and ecotoxicology.<sup>1–4</sup>

Zebrafish offer one of the most promising alternative and cost-effective vertebrate models for predicting prenatal developmental toxicity<sup>5</sup> and fish early life-stage toxicity,<sup>6</sup> particularly since zebrafish embryos are nonprotected life stages and, as such, considered alternative testing models within the European Union and United States.<sup>7–10</sup> Unlike cell lines, zebrafish embryos offer an intact, multicellular system that models integrative physiological processes and, at the same time, is amenable to noninvasive, whole-animal imaging. Therefore, combined with rapid, ex utero development, high fecundity, transparency, and small size, zebrafish are ideal model systems for supporting chemical toxicity screening and prioritization efforts within the United States and abroad.

Due to these advantages, high-content screening (HCS) assays using zebrafish have been developed to evaluate locomotion,<sup>11,12</sup> nanoparticle and chemical exposures,<sup>5,13</sup> bacterial infections,<sup>14</sup> energy expenditure,<sup>15</sup> and drug-induced heart malformations

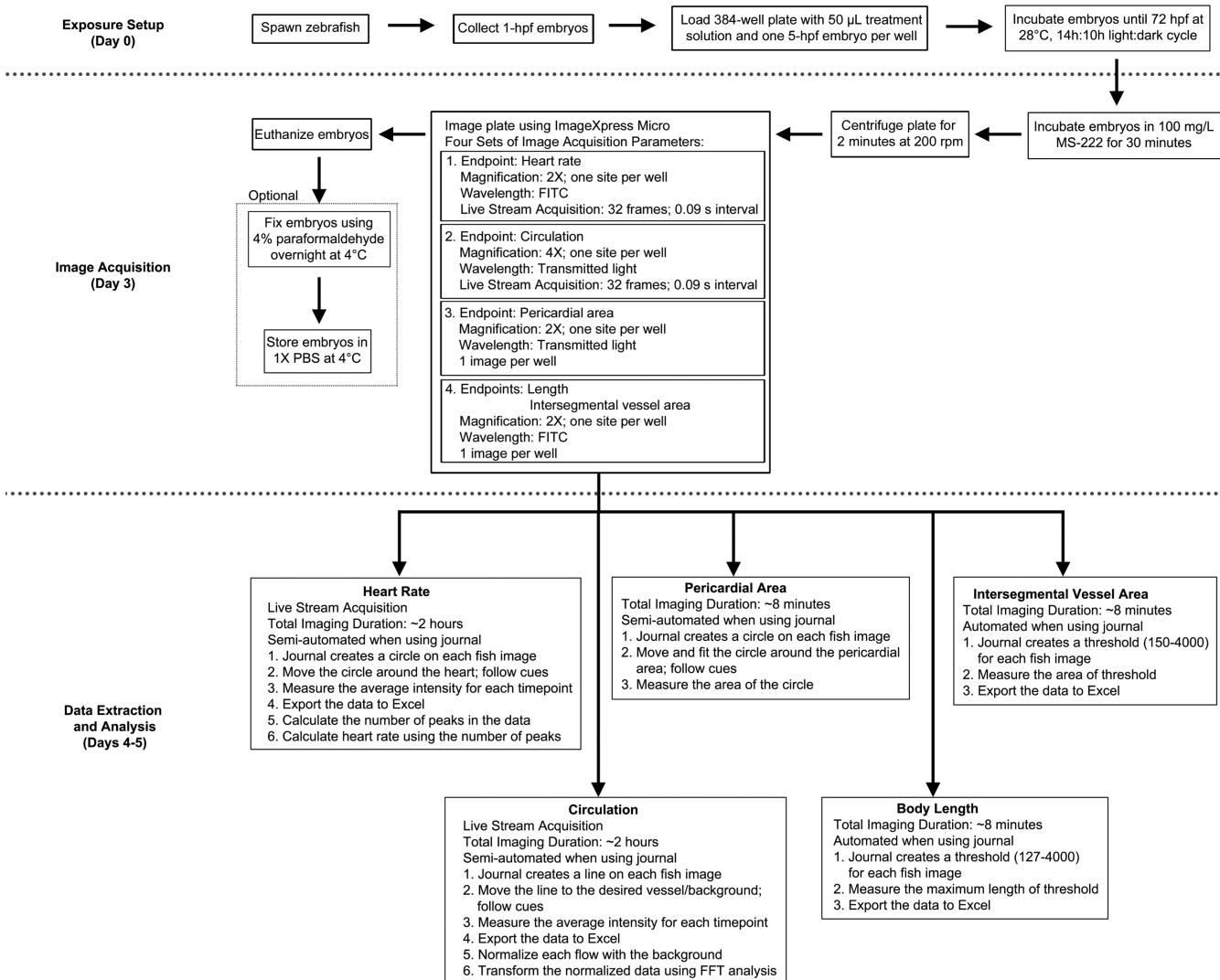
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**Figure 1.** Flow diagram summarizing the exposure setup, image acquisition, and data extraction/analysis protocols for the 384-well-based HCS assay. Using automated image acquisition protocols and parameters optimized for our ImageXpress Micro Widefield High-Content Screening System, each *fli1:egfp* embryo was imaged to analyze the following end points: heart rate; circulation; pericardial area; body length; and intersegmental vessel area.

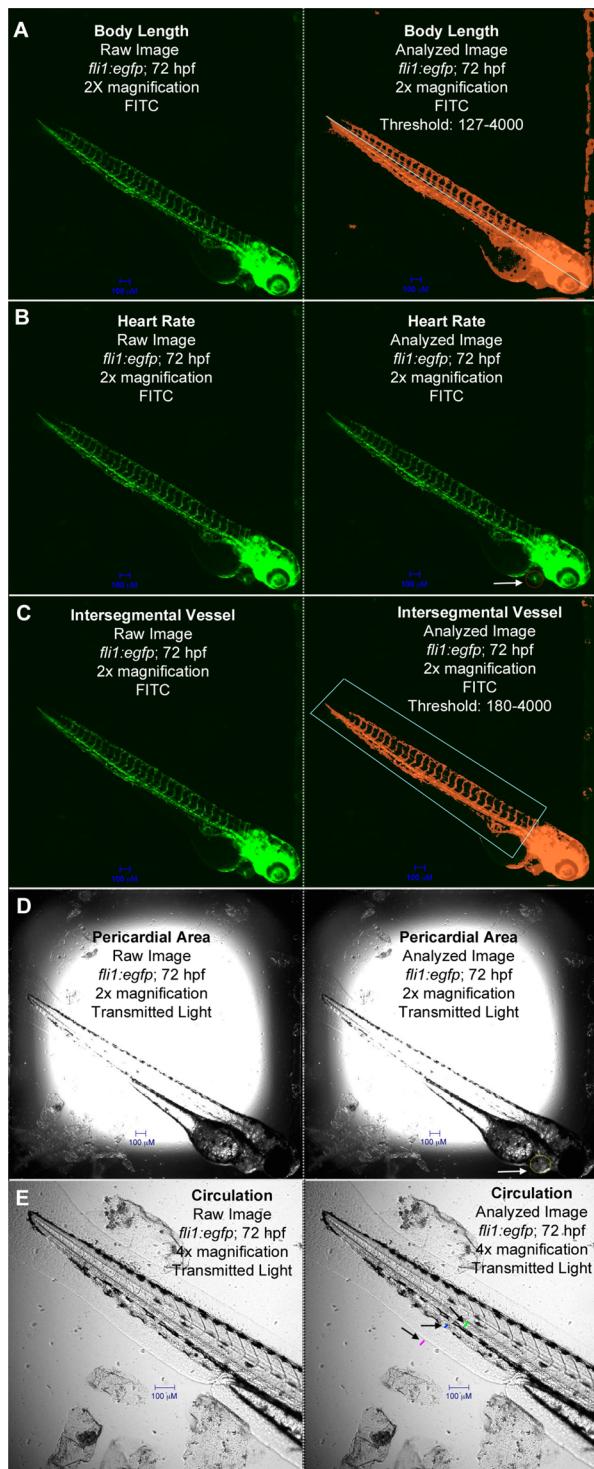
and impacts on cardiac function.<sup>16–19</sup> Despite significant advancements, targeted HCS assays using zebrafish are needed to better evaluate chemical effects on organogenesis and begin classification of chemicals by toxicologically relevant MOAs. Therefore, the objective of this study was to develop a HCS assay with sufficient power, replication, and exposure concentrations to screen and identify chemicals that affect cardiovascular function at sublethal, nonteratogenic concentrations.

## MATERIALS AND METHODS

**Animals.** For all assays described below, we relied on a robust line of transgenic zebrafish (*fli1:egfp*) that stably express enhanced green fluorescent protein (eGFP) within vascular endothelial cells.<sup>20</sup> Adult *fli1:egfp* zebrafish were maintained on a 14 h:10 h light:dark cycle within a Mini Mass Embryo Production System (mini-MEPS) (Aquatic Habitats, Inc., Apopka, FL) containing a photoperiod light cycle dome and recirculating conditioned reverse osmosis (RO) water (~27–28 °C). Adult females and males were bred directly within the mini-MEPS, or bred off-system within a light- and temperature-controlled incubator using breeding traps suspended within 1 L tanks. For all

experiments described below, newly fertilized eggs were staged according to previously described methods.<sup>21</sup> All fish were handled and treated in accordance with approved Institutional Animal Care and Use Committee (IACUC) protocols at the University of South Carolina—Columbia.

**Chemicals.** Chemicals were purchased from ChemService, Inc. (West Chester, PA), Tocris Bioscience (Bristol, UK), and Cambridge Isotope Laboratories, Inc. (Andover, MA); chemical names, chemical formulas, CAS registry numbers, and purities are provided within Supporting Information (SI) Table S1. Stock solutions of each chemical were prepared by dissolving chemicals in HPLC-grade dimethyl sulfoxide (DMSO), and then performing 2-fold serial dilutions into DMSO to create stock solutions for each working solution. All stock solutions were stored at room temperature within 2 mL amber glass vials containing polytetrafluoroethylene (PTFE)-lined caps. For each assay, working solutions of tricaine methanesulfonate (MS-222) (Western Chemical, Inc., Ferndale, WA) were freshly prepared by dissolving MS-222 into embryo media (EM) (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl<sub>2</sub>, 0.33 mM MgSO<sub>4</sub>), and working solutions of all treatments were freshly prepared by spiking



**Figure 2.** Representative raw (left) and analyzed (right) images of vehicle-treated zebrafish embryos used to determine (A) body length; (B) heart rate; (C) intersegmental vessel area; (D) pericardial area; and (E) circulation. Lines, circles, and boxes within analyzed images denote areas of measurement for each end point. For circulation, the green, blue, and purple lines (black arrows) within the analyzed image denote lines used to quantify arterial circulation, venous circulation, and background intensity, respectively.

stock solutions into EM, resulting in 0.1% DMSO within all vehicle control and treatment groups.

**High-Content Screening (HCS) Assay. Exposure Setup.** Black 384-well microplates containing 0.17 mm glass-bottom

**Table 1. Image Success Rates for Analysis of Heart Rate within Control 72 hpf Zebrafish Embryos Following a 30 Minute Immersion Within 100–300 mg/L MS-222<sup>a</sup>**

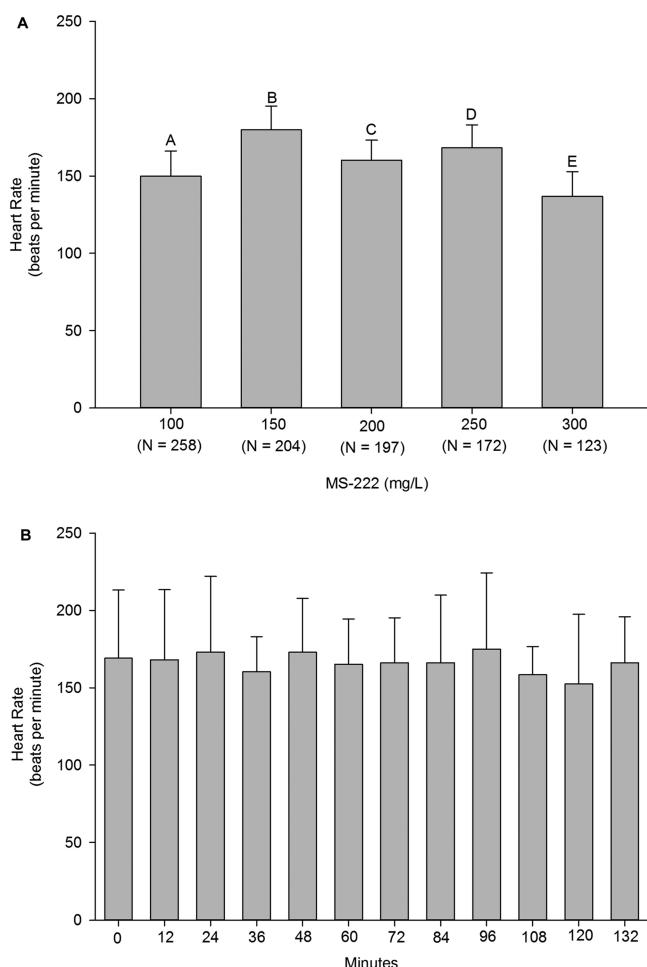
MS-222 (mg/L)	live + hatched		heart rate	
	total (no.)	total (%)	analyzed (no.)	analyzed (%)
100	352	91.7	258	73.3
150	319	83.1	204	63.9
200	370	96.4	197	53.2
250	332	86.5	172	51.8
300	315	82.0	123	39.0

<sup>a</sup>The percentage of embryos that were hatched and alive was relative to an initial sample size of 384 embryos per MS-222 concentration, whereas the percentage of embryos that were analyzed was relative to the number of hatched and alive embryos.

wells (Matrical Bioscience, Spokane, WA) were used for all HCS assays. Immediately following spawning, newly fertilized eggs were collected and placed in groups of approximately 50 per glass Petri dish within a light- and temperature-controlled incubator until 5 h postfertilization (hpf). For each HCS assay, 384 viable *fli1:egfp* embryos were arrayed at 5 hpf into a 384-well plate (one embryo per well) containing 50 µL per well of vehicle (0.1% DMSO) or treatment solution, and then incubated at 28 °C under a 14 h:10 h light:dark cycle and static conditions until 72 hpf.

**Image Acquisition.** At 72 hpf, the plate was removed from the incubator, and zebrafish embryos were anesthetized with 100 mg/L MS-222 by adding 25 µL of 300 mg/L MS-222 to 50 µL of vehicle or treatment solution. The plate was then centrifuged at 200 rpm for 2 min to help orient hatched embryos into right or left lateral recumbency. Using automated image acquisition protocols (Figure 1) and parameters (SI Tables S2–S5) optimized for our ImageXpress Micro (IXM) Widefield High-Content Screening System (Molecular Devices, Sunnyvale, CA), each embryo was imaged to analyze the following end points: heart rate; circulation; pericardial area; body length; and intersegmental vessel area. During the entire image acquisition period, internal temperature within the IXM system was maintained between 25 and 27 °C by removing panels on both sides of the IXM system and blowing air from left to right through the IXM with a portable fan; internal temperature was monitored and recorded at initiation and termination of each imaging protocol. In accordance with National Institutes of Health (NIH) guidelines,<sup>22</sup> 72 hpf embryos were then euthanized by placing the plate at 4 °C for 30 min.

**Data Extraction.** Custom journal scripts for extraction of heart rate, circulation, pericardial area, body length, and intersegmental vessel area data were developed using MetaXpress 4.0.0.24 software (Molecular Devices, Sunnyvale, CA). Prior to data extraction, stream acquisitions within each well were inspected within MetaXpress to assess embryo orientation and survival. Coagulated embryos or developed embryos lacking a heartbeat were considered dead. Using these survival criteria, only hatched and alive embryos positioned in right or left lateral recumbency were analyzed. Interactive semiautomated journal scripts were used to isolate regions of interest and quantify heart rate, circulation, pericardial area, and intersegmental vessel area, whereas a fully automated journal script was used to quantify body length. Examples of raw and analyzed images of control zebrafish for each end point are



**Figure 3.** Mean heart rate ( $\pm$ SD) of 72 hpf *fl1:egfp* zebrafish embryos following a 30 min immersion within 100–300 mg/L MS-222 (A) or every 12 min over a 132 min image acquisition period following initial immersion within 100 mg/L MS-222 ( $N = 22$ ) for 30 min (B). In panel A, treatments that do not share the same letter are significantly different ( $p < 0.05$ ) from each other. In panel B, no significant differences were detected across all time points.

provided in Figure 2. Additional details of the data extraction and analysis process are provided in Figure 1.

**Statistical Analyses.** All statistical procedures were performed using SPSS Statistics 19.0 (Chicago, IL). A general linear model (GLM) analysis of variance (ANOVA) ( $\alpha = 0.05$ ) was used for all data, as these data did not meet the equal variance assumption for non-GLM ANOVAs. Pair-wise Tukey-based

multiple comparisons of least-squares means were performed to identify significant treatment-related effects.

## RESULTS

**Optimization of MS-222-Based Anesthesia.** As high MS-222 concentrations are known to adversely impact cardiac function within zebrafish embryos,<sup>17</sup> we first determined the lowest MS-222 concentration that fully anesthetized hatched 72 hpf embryos in the absence of significant effects on heart rate. We exposed 72 hpf zebrafish to 50–300 mg/L MS-222 for 30 min, and then acquired and analyzed images for heart rate as described in Figure 1. As 50 mg/L MS-222 was insufficient to fully anesthetize hatched 72 hpf embryos, MS-222-induced effects on heart rate were only analyzed within 100–300 mg/L MS-222 treatment groups. Although percent survival was similar across all MS-222 concentrations (82–96%), there was a concentration-dependent decrease in sample size (Figure 3A) and percent of successfully imaged embryos used for analysis of heart rate (Table 1)—an effect that was largely a result of a concentration-dependent increase in the percentage of embryos with orientation in dorsal recumbency.

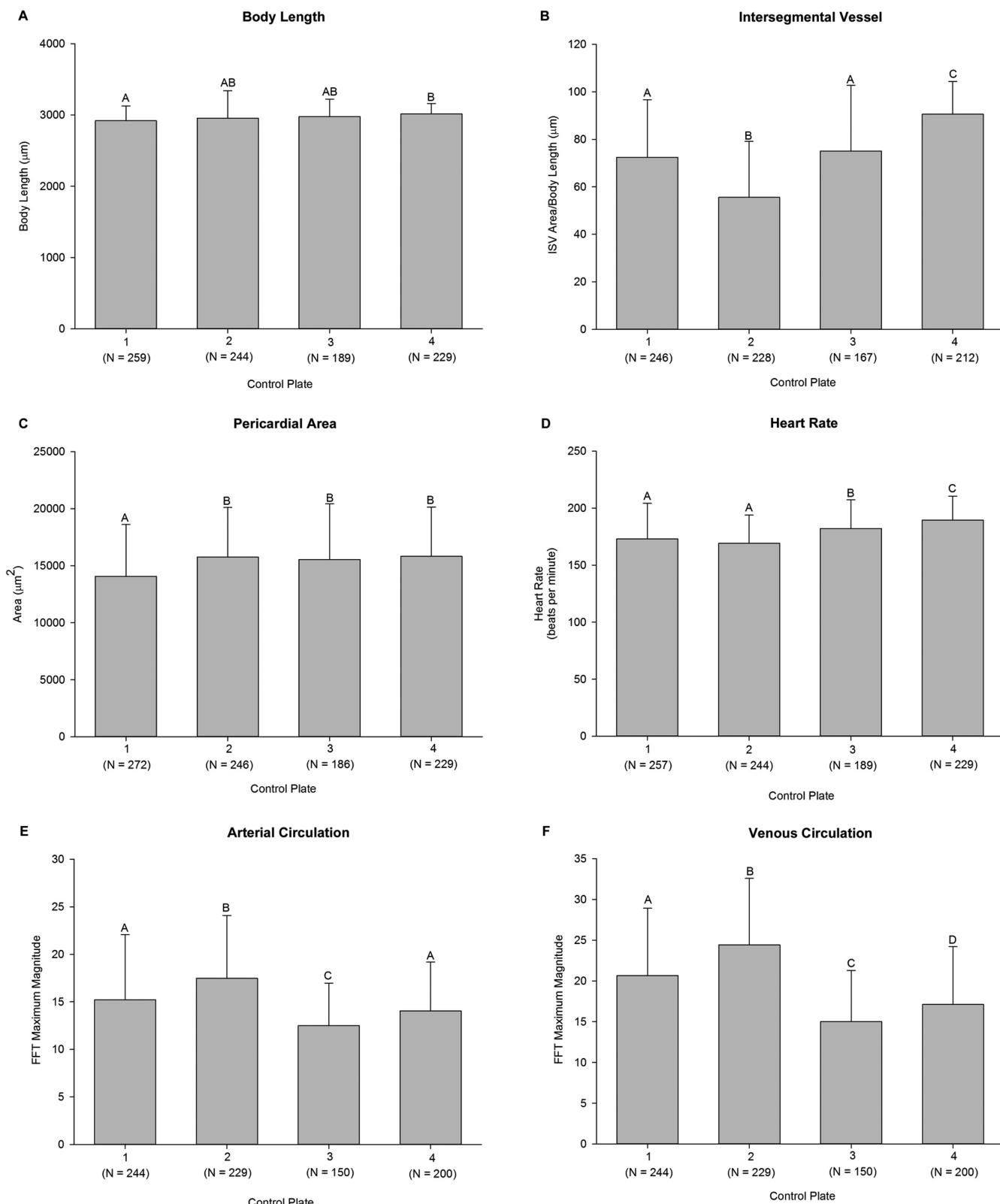
While mean heart rates were significantly different across all concentrations tested (Figure 3A), these minor differences were a result of high sample sizes, minimal variation, and as a result, high statistical power. Although mean heart rates across all MS-222 concentrations tested were similar to heart rates of nonanesthetized 72 hpf zebrafish analyzed at 28 °C,<sup>17</sup> we selected 100 mg/L MS-222 for all subsequent HCS assays since this concentration (1) represented the lowest concentration necessary to fully anesthetize hatched 72 hpf embryos and (2) yielded the highest percent of successfully imaged embryos (Table 1). Using this MS-222 concentration, we then assessed heart rate every 12 min over a 132 min image acquisition period (following an initial immersion for 30 min), but found no significant effects on heart rate across all time-points analyzed (Figure 3B). Therefore, these data demonstrated that prolonged immersion of hatched 72 hpf embryos in 100 mg/L MS-222 provided full anesthetization and high imaging success rates in the absence of significant effects on heart rate over time.

**Assessment of Background Variability.** To evaluate background variability, we imaged four control plates containing embryos incubated in EM from 5 to 72 hpf, and then immersed in 100 mg/L MS-222 for 30 min prior to imaging. Heart rate, circulation, pericardial area, body length, and intersegmental vessel area were analyzed for each control plate using the parameters described in Figure 1 and SI Tables S2–S5. Percent survival and image success of hatched embryos ranged from 93 to 99% and 42–74%, respectively (Table 2).

**Table 2. Image Success Rates for Analysis of All End Points within Control 72 hpf Zebrafish Embryos Across Four Independent Plates Containing an Initial Sample Size of 384 Embryos Per Plate<sup>a</sup>**

control plate	live + hatched		length		heart rate		pericardial area		intersegmental vessel area		circulation	
	total (no.)	total (%)	analyzed (no.)	analyzed (%)	analyzed (no.)	analyzed (%)	analyzed (no.)	analyzed (%)	analyzed (no.)	analyzed (%)	analyzed (no.)	analyzed (%)
1	369	96.1	259	70.2	257	69.6	272	73.7	253	68.6	244	66.1
2	357	93.0	244	68.3	244	68.3	246	68.9	228	63.9	229	64.1
3	361	94.0	189	52.4	189	52.4	186	51.5	167	46.3	150	41.6
4	380	99.0	229	60.3	229	60.3	229	60.3	212	55.8	200	52.6

<sup>a</sup>The percentage of embryos that were hatched and alive was relative to an initial sample size of 384 embryos per MS-222 concentration, whereas the percentage of embryos that were analyzed was relative to the number of hatched and alive embryos. Across all five end points, variation in the percentage of embryos analyzed was primarily due to hatched embryos with orientation in dorsal rather than lateral recumbency.

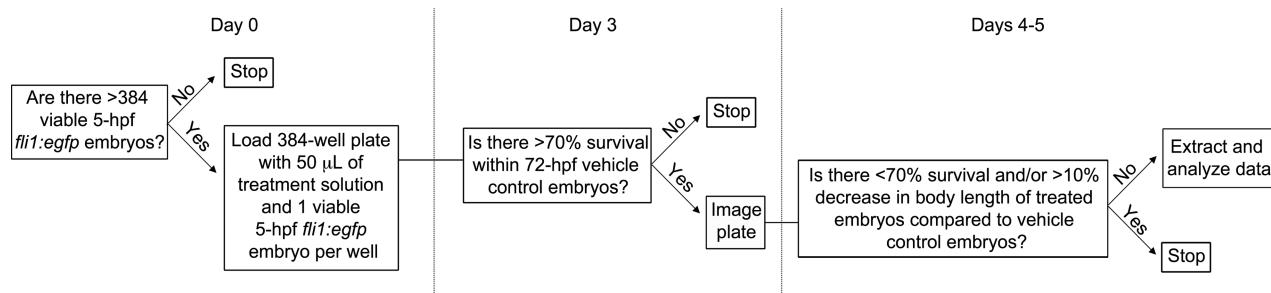


**Figure 4.** Mean body length ( $\pm\text{SD}$ ) (A), intersegmental vessel area ( $\pm\text{SD}$ ) (B), pericardial area ( $\pm\text{SD}$ ) (C), heart rate ( $\pm\text{SD}$ ) (D), arterial circulation ( $\pm\text{SD}$ ) (E), and venous circulation ( $\pm\text{SD}$ ) (F) across four independent control plates consisting of 384 initial embryos per plate. N = final number of embryos analyzed per plate. Treatments that do not share the same letter are significantly different ( $p < 0.05$ ) from each other.

Intersegmental vessel area and circulation data were variable; body length, pericardial area, and heart rates were relatively consistent within and across plates (Figure 4). Based on these data, we concluded that control performance within this HCS

assay was acceptable based on sample sizes ranging from 150 to 272 imaged embryos per plate.

**Chemical Screening.** Using protocols described in Figure 1, 10 chemicals (SI Table S1) were screened to evaluate the



**Figure 5.** Decision tree for evaluation of assay performance and validity during exposure setup (Day 0), image acquisition (Day 3), and data extraction/analysis (Days 4–5). Criteria for survival (>70% survival) and body length (<10% decrease relative to vehicle controls) were developed to (1) minimize Type I and II errors and reliably identify treatment-related effects and (2) focus our analyses on targeted impacts on the cardiovascular system in the absence of significant effects on embryonic growth.

potential for targeted effects on the cardiovascular system; raw data for all chemical screening assays are provided as Microsoft Excel spreadsheets within SI supplemental file 2. This subset of chemicals was selected in order to represent a range of chemical classes and mechanisms of action. Based on a published list of known classes of cardiovascular toxicants,<sup>23,24</sup> nicotine, pyridaben, CL-4AS-1, 17 $\beta$ -estradiol, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and genistein were hypothesized to be ‘positive’ within our assay, whereas endosulfan, paraoxon, cypermethrin, and imidacloprid were hypothesized to be ‘negative’ within our assay. Decision criteria for survival (>70% survival) and body length (<10% decrease relative to vehicle controls) were developed based on performance within our control plates (Table 2 and Figure 4) as well as existing OECD test guidelines ([http://www.oecd-ilibrary.org/content/package/chem\\_guide\\_pkg-en](http://www.oecd-ilibrary.org/content/package/chem_guide_pkg-en)) for fish early life-stage toxicity (OECD TG 210) and rodent chronic toxicity (OECD TG 452) to (1) minimize Type I and II errors and reliably identify treatment-related effects and (2) focus our analyses on targeted impacts on the cardiovascular system in the absence of significant effects on embryonic growth (Figure 5).

Based on the highest chemical concentration meeting both criteria, we then performed a cluster analysis using QC Canvas 1.2 (<http://compbio.sookmyung.ac.kr/~qcanvas/>) (Figure 6A). Pyridaben was the most potent chemical to affect survival, as 0.78 μM was the highest concentration analyzed due to 0% survival at higher concentrations (SI Figure S9). In addition, TCDD exposure resulted in the most potent effects on body length even though survival was >70% at all concentrations screened ( $1.51 \times 10^{-4}$ –0.155 μM) (SI Figure S10). Importantly, 0.155 μM TCDD was the highest concentration tested since we were limited by the concentration of DMSO stock solution purchased from Cambridge Isotope Laboratories; therefore, exposure to TCDD concentrations >0.155 μM would likely have resulted in <70% survival. On the contrary, imidacloprid and nicotine were the least potent chemicals and did not affect body length nor survival across all concentrations tested (0.05–50 μM) (SI Figures S6 and S7).

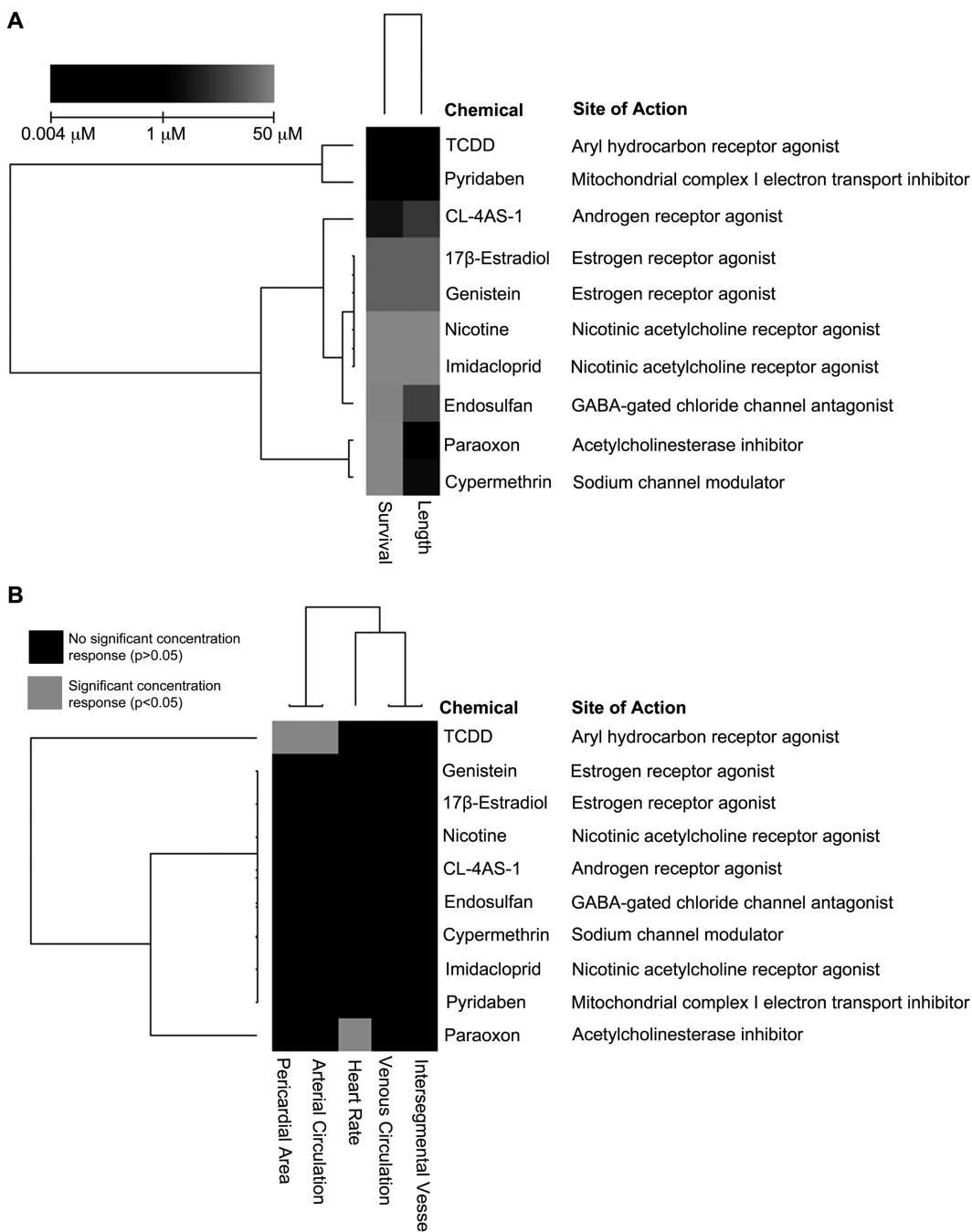
To determine targeted effects on the cardiovascular system, heart rate, circulation, pericardial area, and intersegmental vessel area were assessed within treatment groups with >70% survival and/or <10% decrease in body length relative to vehicle controls. Out of 10 chemicals screened, TCDD and paraoxon were the only chemicals to result in a significant concentration-dependent effect on one or more end points analyzed within treatment groups meeting both analysis criteria (Figure 6B). Mean body length was >10% lower than vehicle controls for paraoxon and TCDD concentrations higher than

0.78 and 0.004 μM, respectively (SI Figures S8 and S10). Therefore, these concentrations were the highest concentrations analyzed for all end points. TCDD significantly decreased ( $p < 0.05$ ) arterial circulation at the highest concentration analyzed (0.004 μM), and significantly increased ( $p < 0.05$ ) pericardial area at all concentrations analyzed ( $1.51 \times 10^{-4}$ –0.004 μM) without significantly affecting venous circulation, heart rate, and intersegmental vessel area (SI Figure S10). On the other hand, paraoxon significantly decreased heart rate ( $p < 0.05$ ) at all concentrations tested (0.05–50 μM) in the absence of effects on any other end points (SI Figure S8).

## DISCUSSION

Although previous HCS assays using zebrafish have examined drug-induced heart malformations and impacts on cardiac function,<sup>5,16–19</sup> to our knowledge this is the first HCS assay to provide a more comprehensive platform for quantifying body length, heart rate, pericardial area, circulation, and intersegmental vessel area within the same embryo—an advance that will, in the long term, decrease the amount of time, zebrafish embryos, and high-purity reference material needed for screening and identification of chemicals impacting cardiovascular function. Using 384-well format, we were also able to increase sample size (32 per treatment) and examine a broad concentration–response (vehicle control and 11 chemical concentrations per plate). While we are not the first to use 384-well plates, the majority of HCS assays using zebrafish rely on 96-well plates or low sample sizes per treatment if using 384-well plates.<sup>5,16,19</sup> To reduce the potential for adverse side effects of anesthetizing with MS-222, we attempted to immobilize hatched embryos by pre-exposing to light as suggested in Burns et al. (2005);<sup>17</sup> however, these attempts were unsuccessful, precluding the possibility of eliminating MS-222-based anesthetization. Nonetheless, anesthetization of embryos using MS-222 did not affect heart rate over time (Figure 3), and heart rates in this study were similar to heart rates of nonanesthetized, 72-hpf zebrafish embryos incubated at the same temperature.<sup>17</sup> After analysis of control plates demonstrated the assay was reliable, we then screened 10 chemicals and determined that only TCDD and paraoxon exhibited a concentration-dependent effect on cardiovascular function in the absence of effects on body length.

Using the highest concentration meeting body length and survival criteria (Figure 6A), we found that chemicals were clustered by site of action, suggesting that, within 384-well plates used for this assay, the magnitude of toxicity for chemicals with a common site of action is similar within zebrafish embryos.



**Figure 6.** Hierarchical cluster of chemical screening data based on the highest concentration meeting survival and body length criteria (A) and presence or absence of a concentration-dependent response on the cardiovascular system at nonteratogenic concentrations (B). SI supporting figures and raw data for all chemical screening assays are provided in SI supplemental files 1 and 2, respectively.

However, when we clustered chemicals based on targeted effects to the cardiovascular system (Figure 6B), we found that only two out of 10 chemicals—TCDD and paraoxon—affected the cardiovascular system in a concentration-dependent manner, suggesting that, despite differences in potency based on gross malformations, this assay has the potential to identify cardiovascular toxicants.

Eight of the 10 chemicals screened did not adversely impact the cardiovascular system using this assay, including five chemicals we initially hypothesized to be “positive”. For each chemical tested, a range of 11 concentrations based on 2-fold serial dilutions was used to increase the probability of capturing

concentration-response curves for survival, body length, and cardiovascular-specific end points. With the exception of nicotine and imidacloprid (both of which were nontoxic up to 50  $\mu$ M, the highest concentration tested), all chemicals screened either decreased survival and/or body length, suggesting that a negative finding was not a result of minimal uptake into developing embryos due to high rates of chemical absorption to microplate wells or low rates of chemical transport across the chorion. Rather, our negative findings for chemicals hypothesized to be positive for cardiovascular toxicity may be due to differences in life stages (embryos vs adults), experimental systems (whole animals vs cell lines), taxa (fish vs rodents),

exposure routes (aqueous vs nonaqueous exposure), and/or absence of chemical-specific sites of action within zebrafish. Since exposure to nicotine and imidacloprid did not affect any of the end points evaluated within our assay, these negative findings may have been due to limited embryonic uptake and/or low sensitivity to acetylcholine receptor (AChR) activation during this stage of development. Nonetheless, these negative results further support the conclusion that, despite chemical potency based on apical end points such as survival and body length, this assay has the potential to identify chemicals that target cardiovascular function.

Within zebrafish embryos, TCDD is known to cause severe heart malformations such as altered looping and reduced blood flow by activating the aryl hydrocarbon receptor (AHR).<sup>25–27</sup> Similarly, in our HCS assay TCDD significantly decreased arterial circulation and increased pericardial area—an end point associated with incomplete cardiac looping—without significantly affecting venous circulation, heart rate, and intersegmental vessel area (SI Figure S10). While paraoxon is a known acetylcholinesterase (AChE) inhibitor and is often associated with developmental neurotoxicity,<sup>28,29</sup> in our HCS assay paraoxon significantly decreased heart rate at all concentrations tested in the absence of effects on any other end points (SI Figure S8). Therefore, this paraoxon-induced effect on heart rate may have been undetected within cell-based systems, suggesting that targeted HCS assays using intact embryos provide an opportunity to reveal unexpected chemical MOAs.

In summary, compared to existing zebrafish-based assays, this HCS assay provides a comprehensive discovery platform with sufficient power, replication, and exposure concentrations to screen and identify potential cardiovascular toxicants. While mechanisms of toxicity were not investigated within this study, in the future this HCS assay can be readily coupled with reverse genetics approaches to identify the potential role of xenobiotic receptors in chemically induced effects on cardiovascular development within zebrafish embryos. Therefore, over the long-term, we envision that this HCS assay will significantly increase the rate of screening and prioritizing chemicals for hypothesis-driven MOA research as well as uncover mechanisms of cardiovascular toxicity for understudied high-production volume chemicals currently regulated under REACH and TSCA.

## ASSOCIATED CONTENT

### Supporting Information

Chemical names, chemical formulas, CAS registry numbers, and purities (Table S1), as well as imaging parameters (Tables S2–S5) and supporting figures (Figures S1–S10) for chemical screening results are provided within Supplemental File 1. Microsoft Excel spreadsheets containing raw data for all chemical screening assays are provided within Supplemental File 2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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