EPA Public Access

Author manuscript

Cardiovasc Toxicol. Author manuscript; available in PMC 2020 June 19.

About author manuscripts

Submit a manuscript

Published in final edited form as:

Cardiovasc Toxicol. 2018 December ; 18(6): 569–578. doi:10.1007/s12012-018-9469-8.

Inhalation of Simulated Smog Atmospheres Affects Cardiac Function in Mice

Haiyan Tong¹, Jonathan D. Krug², Q. Todd Krantz¹, Charly King¹, Marie. M. Hargrove³, M. Ian Gilmour¹, Stephen H. Gavett¹

¹Environmental Public Health Division, National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, North Carolina, USA

²Exposure Methods and Measurements Division, National Exposure Research Laboratory, US Environmental Protection Agency, Research Triangle Park, North Carolina, USA

³Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

Abstract

The health effects of individual criteria air pollutants have been well investigated. However, little is known about the health effects of air pollutant mixtures that more realistically represent environmental exposures. The present study was designed to evaluate the cardiac effects of inhaled simulated smog atmospheres (SA) generated from the photochemistry of either gasoline and isoprene (SA-G) or isoprene (SA-Is) in mice. Four-month-old female mice were exposed for 4 h to filtered air (FA), SA-G, or SA-Is. Immediately and 20 h after exposure, cardiac responses were assessed with a Langendorff preparation using a protocol consisting of 20 min of global ischemia followed by 2 h of reperfusion. Cardiac function was measured by index of left-ventricular developed pressure (LVDP) and cardiac contractility (dP/dt) before ischemia. Pre-ischemic LVDP was lower in mice immediately after SA-Is exposure (52.2 ± 5.7 cm H₂O compared to 83.9 ± 7.4 cm H₂O after FA exposure; $p=0.008$) and 20 h after SA-G exposure (54.0 ± 12.7 cm H₂O compared to 79.3 ± 7.4 cm H₂O after FA exposure; $p=0.047$). Pre-ischemic left ventricular contraction dP/dt_{max} was lower in mice immediately after SA-Is exposure (2025 ± 169 cm H₂O/sec compared to 3044 ± 219 cm H₂O/sec after FA exposure; $p<0.05$) and 20 h after SA-G exposure (1864 ± 328 cm H₂O/sec compared to 2650 ± 258 cm H₂O/sec after FA exposure; $p=0.05$). In addition, SA-G reduced the coronary artery flow rate 20 h after exposure compared to the FA control. This study demonstrates that acute SA-G and SA-Is exposures decrease LVDP and cardiac contractility in mice, indicating that photochemically-altered atmospheres affect the cardiovascular system.

Corresponding Author: Haiyan Tong, MD, PhD, Environmental Public Health Division, National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, 109 TW. Alexander Dr., Research Triangle Park, North Carolina, 27711, Phone: (919) 966-4993, Fax: (919) 966-6271, Tong.haiyan@epa.gov.

DECLARATION

Competing interests: The authors declare that they have no actual or competing interests.

Disclaimer: The research described in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. EPA, and approved for publication. The contents of this article should not be construed to represent Agency policy nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Keywords

cardiac function; cardiac contractility; simulated atmospheres; photochemical reaction; mice

BACKGROUND

Epidemiological studies have linked acute and chronic ambient air pollution exposure with cardiovascular morbidity and mortality[1]. Research on health effects from air pollution exposure has been largely focused on individual “criteria pollutants”. However, “real world” air pollution is far more complex than exposure to individual pollutants, and contains freshly emitted primary pollutants as well as photochemically aged secondary pollutants formed in the atmosphere during the oxidation of gas-phase precursors[2]. Therefore, a multi-pollutant experimental approach is needed to understand the effects of ambient air pollutant mixtures on human health[3]. Furthermore, data on the mechanisms by which complex air pollutant mixtures impact key target organ systems associated with morbidity and mortality are sparse. A health-based integrated air quality index (AQHI) developed by Environment Canada predicts the health impact of multiple air pollutants based on the combined 3-hour average concentrations of ozone (O₃), nitrogen dioxide (NO₂), and fine particulate matter (PM_{2.5})[4].

In recent years, epidemiological studies have suggested that traffic-derived air pollution may drive greater cardiovascular effects than individual air pollutants, such as particulate matter (PM)[5, 6]. Motor vehicle emissions from gasoline combustion are major contributors to urban air pollution. Volatile organic compound (VOCs) are among these motor vehicle emissions and may pose a health risk. Controlled human exposure studies have demonstrated the respiratory inflammatory effects of VOCs[7, 8]. A recent epidemiologic study showed that hydrocarbon groups of VOCs, alkenes and alkynes, were associated with emergency department visits for cardiovascular diseases and ketone groups were associated with emergency department visits for asthma[9]. In addition, VOCs of diesel exhaust have been shown to cause enhanced constriction and reduced relaxation in blood vessels[10]. Emissions of primary organic aerosol (POA) and gas-phase secondary organic aerosol (SOA) precursors are major contributors to urban ambient organic aerosol[11]. SOA accounts for a significant portion of urban ambient aerosol and has major impact on human health[12]. A recent study showed that photochemically-derived secondary organic aerosols (POA + SOA) at ambient traffic levels of PM_{2.5} decreased heart rate variability in animals[13]. In the present study, we have developed a precursor formula to generate test simulated smog atmospheres (SA) from irradiation of gasoline headspace and isoprene with nitric oxide (NO). Isoprene (2-methyl-1,3-butadiene) is emitted from many types of plants, especially broadleaf tropical and temperate trees, and is estimated to comprise half (535 Tg/yr) of total biogenic VOC emissions worldwide [14, 15]. In combination with moderate to high levels of NO, photo-oxidation of isoprene contributes to formation of O₃ and NO₂ and other reaction products that contribute to formation of SOAs [16, 17].

Experimental studies have demonstrated the ability of individual air pollutants to cause cardiopulmonary toxicity in animals. For example, acute exposure to PM can increase

pulmonary and systemic inflammation and lung injury, cause vascular dysfunction, alter heart rate variability, induce arrhythmia, and enhance cardiac ischemic injury[1]. We[18, 19] and others[20] have previously shown that PM exposure enhanced cardiac ischemia/reperfusion injury in animals. We have also reported that particle free multipollutant mixtures generated in a photochemical reaction chamber under sunlight depressed cardiac function in mice[21]. In the present study, we examined whether photochemically-reacted atmospheres generated from mixtures of gasoline and/or isoprene could affect the cardiovascular system in animals. We used a large scale environmental irradiation chamber to generate simulated atmospheres under UV light from gasoline and isoprene with NO, which produces high levels of ozone (SA-G), or SOA from isoprene (SA-Is). The SAs were administered as complete effluent containing remaining parent hydrocarbons (HCs) and secondary reaction products including SOA, OVOCs, aldehydes, O₃, and NO₂. We tested the hypothesis that inhalation of simulated atmospheres SA-G and SA-Is results in impairment of cardiac function in isolated mouse hearts. We report here that the cardiac effects were apparent immediately after SA-Is exposure and 20 h after SA-G exposure including decreased left ventricular developed pressure and contractility, suggesting the possibility of photochemical reaction products affecting the cardiac responses.

METHODS AND MATERIALS

Experimental Animals

All experimental procedures were performed in compliance with protocols approved by our institutional IACUC according to NIH guidelines. All animals were treated humanely and with regard for alleviation of suffering. Mice were maintained at 22°C and 50% relative humidity in an AAALAC-approved facility with a 12-h light/dark cycle and free access to food (Prolab RHM 3000; PMI Nutrition International, St. Louis, MO) and water. Female C57Bl/6 mice (4 month old, average weight 20.6±0.2 gram) were purchased from Jackson Laboratory (Bar Harbor, ME) and acclimated for two weeks before they were used.

Generation of simulated atmospheres containing gasoline and isoprene

A 14.27 m³ photochemical reaction chamber that uses UV light to approximate the NO₂ photolysis rate of natural sunlight was used in a dynamic mode such that a constant output of effluent could be maintained[22]. Details on the generation of SAs and measurements methods used are described in Krug et al. [22]. The SAs originated from the continuous injection of hydrocarbons (HC; from gasoline and/or isoprene) and NO from high-pressure cylinders, and a nebulized (NH₄)₂SO₄ seed which served as a nucleation base for SOA formation. Concentrations of the 20 most abundant hydrocarbons in the chamber prior to photochemical reactions are shown in Table 1. The reactants were initially introduced until the concentrations reached steady state. The reaction was initiated by turning on UV lights and until a steady-state mixture of reactants and reaction products was attained. The SA-G used in this study corresponded to a subset of the MR059 atmosphere described in Krug et al.[22]. In brief, SA-G was generated by injection of a mixture of 7.21 ppmC gasoline vapor, 5.23 ppmC isoprene for a total HC concentration of 12.44 ppmC, and 794 ppb NO followed by irradiation with UVB until equilibrium was established. The reactant mixture was diluted with filtered air at a total flow rate of 60 L/min and the mean photochemical residence time

for SA-G and SA-Is was 4 hours. At least three residence times (12 h) of irradiation were allowed before exposure were initiated. The reaction consumed 6.13 ppmC of the parent HCs (1.37 ppmC gasoline, 4.76 ppmC isoprene), and yielded criteria pollutant concentrations of 659 ppb NO₂, 447 ppb O₃, and 52.3 µg/m³ SOA in the photochemical reaction chamber. Final conditions of the atmosphere in the exposure chamber consisted of 584 ppb NO₂, 360 ppb O₃, 304 µg/m³ SOA (in size range of PM_{2.5}), and an estimated 2,959 µgC/m³ of gas-phase reaction products (Table 2).

SA-Is was generated by injection of 22.85 ppmC isoprene and approximately 1,660 ppb NO followed by an identical irradiation procedure. The reaction consumed 18.38 ppmC isoprene and yielded criteria pollutant concentrations of 1,283 ppb NO₂, 395 ppb O₃, and 287 µg/m³ SOA in the photochemical reaction chamber. Final conditions in the exposure chamber consisted of 509 ppb NO₂, 246 ppb O₃, 160 µg/m³ SOA, and an estimated 5,069 µgC/m³ of gas-phase reaction products (Table 2).

Animal Exposure

Two separate experiments were conducted to examine the health effects of SA exposures. In the first study, we examined the cardiovascular effects in mice of exposure to SA-G or FA for 4 h. In the second study, we examined the effects of exposure to SA-Is or FA for 4 h. Mice were exposed in 180 L Hinners-type whole-body chambers, in which animals were housed in stainless-steel individual compartments allowing freedom of movement.

Cardiac Function

As described previously[23], immediately and 20 h after exposure, mice were anesthetized with an i.p. injection of sodium pentobarbital (80 mg/kg). After intravenous heparin (100 units) injection the hearts were rapidly excised and placed in ice-cold Krebs-Henseleit buffer. The aortas were cannulated and perfused retrograde at constant pressure of 100 cm H₂O. The non-recirculating perfusate was a Krebs-Henseleit buffer containing (in mmol/L) 120 NaCl, 5.9 KCl, 1.2 MgSO₄, 1.75 CaCl₂, 25 NaHCO₃, and 11 glucose. The buffer was aerated with 95% O₂ and 5% CO₂, and maintained at pH 7.4 and 37°C.

For assessment of contractile function, a latex balloon on the tip of a polyethylene catheter was inserted through the left atrium into the left ventricle. The catheter was connected to a pressure transducer (Argon Medical Devices, Athens, TX) at the same height as the heart. The pressure of the left ventricular balloon was inflated to 0–5 cm H₂O. A PowerLab system was used to collect and process the heart rate, left ventricular developed pressure (LVDP=LV peak minus end-diastolic pressure (LVEDP)), and contractility (dP/dt; maximum rate of rise of the left ventricular isovolumetric pressure) data (AD Instruments, Milford, MA). Coronary flow rate was measured by collecting the effluent draining from the coronary sinus and pulmonary artery. All hearts were perfused for 20 min when the baseline pre-ischemic measurements were taken prior to initiating 20 min of global no-flow ischemia followed by 2 h of reperfusion. Onset of ischemic contracture was detected when the left ventricular pressure began to increase during ischemia. Recovery of LVDP, expressed as a percentage of the initial pre-ischemic LVDP was measured at 60 min of reperfusion after 20 min of ischemia.

Statistical Analysis

Data are expressed as means $\pm$ SE. Comparisons among SA-G or SA-Is and FA control group immediately and 20 h post exposure were performed by two-way ANOVA with exposure (FA vs. SA) and time (0 h vs. 20 h) as two variables followed by Student-Newman-Keuls multiple comparison test. The statistical analysis was performed using SigmaPlot Version 13. The statistical significance level was set at $p = 0.05$.

RESULTS

Composition of Photochemically Generated Simulated Smog Mixtures

During the photochemical reaction, average chamber NO and parent HC levels decreased and levels of secondary chemical products, such as O₃, NO₂, OVOCs, and aldehyde levels increased. Table 2 shows the final PM mass, O₃, NO₂, and estimated gas-phase reaction product concentrations in the chambers during the 4 h of exposure. The SA-G atmosphere had 90% higher PM, 46% higher O₃, and 15% higher NO₂ than the SA-Is atmosphere, while estimated organic gas-phase products were 71% higher in SA-Is compared with SA-G. Calculated AQHI was 99.7 for the SA-G system and 75.0 for the SA-Is system (Table 2). The concentrations of aldehydes and peroxyacetic nitric anhydride (PAN) analogues are shown in Table 3. Among these propionaldehyde, methacrolein, peroxyacetic nitric anhydride (PAN), peroxypropionic nitric anhydride (PPN), and peroxyethacrylic nitric anhydride (MPAN) concentrations were higher in the SA-Is chamber. A more detailed analysis of the composition of SA-G is presented in Krug et al. [22].

Cardiac Effect

Simulated smog generated from gasoline (SA-G)—There was no statistically significant difference of pre-ischemic heart rate in mice immediately and 20 h after exposure to either FA or SA-G (Figure 1A and Table 4). However, one mouse had marked elevation of heart rate and depression of LVDP and dP/dT at 20 h after FA exposure. Further analysis demonstrated that there was negative correlation between heart rate and dP/dT_{max} ($p=0.001$) and positive correlation between LVDP and coronary flow rate ($p=0.007$) in this group.

Pre-ischemic LVDP was significantly different among exposure groups ($p=0.024$) but not at different times. At 20 h post-exposure SA-G hearts had significantly lower pre-ischemic LVDP ($p=0.047$) compared with the FA hearts (Figure 1B). There was not a significant interaction between exposure and time in pre-ischemic LVDP. Pre-ischemic coronary artery flow rate was also significantly different among exposure ($p=0.007$) but not at different time. Coronary flow rate at 20 h after exposure was significantly reduced in the SA-G hearts ($p=0.008$) compared to FA hearts (Figure 1C). There was not a statistically significant interaction between exposure and time in coronary artery flow rate. Exposure to SA-G also decreased pre-ischemic left ventricular contractility (Figure 2). The difference in dP/dT_{max} among exposure ($p=0.033$) and time ($p=0.036$) was significantly different. However, there was no significant interaction between exposure and time. Pre-ischemic rate of left ventricular contraction (dP/dt_{max}) was significantly reduced 20 h post SA-G exposure ($p=0.05$) compared with the FA hearts (Figure 2A). There was a trend of reduced dP/dt_{max}

20 h post exposure compared to the immediately post exposure in SA-G hearts ($p=0.053$). Pre-ischemic rate of left ventricular relaxation ($dP/dt_{\min}$) was not significantly different among the exposure groups (Figure 2B) at either time points.

Time to ischemic contracture during 20 min of ischemia was not significantly affected by SA-G immediately post (9.0 ± 0.7 min) and 20 h post-exposure (12.8 ± 2.6 min) compared with the FA hearts immediately post (8.8 ± 1.5 min) and 20 h post exposure (8.3 ± 1.1 min). Post-ischemic recovery of LVDP (% LVDP) at 60 min after reperfusion was not significantly different among the exposure groups (Table 4).

Simulated smog generated from isoprene (SA-Is)—There were no significant difference of pre-ischemic heart rate and coronary artery flow rate among the exposure groups at either immediately and 20 h time points (Table 4 & Figure 3A&C). Pre-ischemic LVDP immediately post exposure was significantly lower in the SA-Is-exposed hearts ($p=0.0008$) compared with the FA hearts (Figure 3B). Pre-ischemic LVDP at 20 h after exposure was significantly lower ($p=0.039$) compared to the immediately post exposure in the FA hearts (Figure 3B). There was a significant interaction between exposure and time ($p=0.047$). Exposure to SA-Is also decreased pre-ischemic left ventricular contractility (Figure 4). Pre-ischemic rate of left ventricular contraction ($dP/dt_{\max}$) immediately post exposure was significantly reduced in the SA-Is-exposed hearts ($p=0.021$) compared with the FA hearts (Figure 4A). There was not a statistically significant interaction between exposure and time. Pre-ischemic rate of left ventricular relaxation ($dP/dt_{\min}$) was not significantly different among the different exposure and time (Figure 4B). Time to ischemic contracture was not different during ischemia immediately post (6.5 ± 0.5 min for the FA and 10.1 ± 1.5 min for the SA-Is) and 20 h post exposure (6.8 ± 0.9 min for the FA and 11.0 ± 3.1 min for the SA-Is). Post-ischemic % LVDP at 60 min after reperfusion was not significantly different among the exposure groups (Table 4).

DISCUSSION

In the last several decades, research studies on the health effects of air pollution have focused on assessing the effects of single pollutants. However, humans are exposed to multi-pollutant mixtures and the challenge facing current air pollution health research is how to address these mixtures. We used an environmental irradiation reaction chamber to simulate the mixture of gases and particles present in an urban ambient environment. Our previous study demonstrated that inhalation of particle-free photochemically-derived urban mixtures decreased cardiac contractile function with minimal effects on the lung in mice[21]. The present study was designed to evaluate the cardiac responses to inhalation of “real world” smog containing particles and gaseous compounds generated photochemically from gasoline and isoprene. We show that short-term inhalation to simulated atmosphere (SA) depressed cardiac function and cardiac contractility in isolated perfused hearts. Furthermore, we demonstrate that SAs generated from different sources resulted in different cardiac responses.

Epidemiological studies have linked exposure to ambient pollutants with cardiovascular mortality, including myocardial ischemia, atherosclerosis, and autonomic system

dysfunction[1]. A tool introduced by Environment Canada, air quality health index (AQHI), is used to inform the general public about the health risks of multi-pollutants from a combination of O₃, NO₂, and PM_{2.5}[4]. AQHI assumes that the health impacts from these three air pollutants are additive and are thought to produce a more conservative approach to public health warnings. However, because real world smog atmospheres contain a mixture of gaseous and particulate components such as the SA in this study, AQHI will not accurately reflect the health risks of the atmosphere. Findings from the present study on these two SAs suggest that health impact from air pollution exposure may not solely depend on the criteria pollutants, as aldehydes and other reaction products may exert significant toxicity.

In the present study, we have found that simulated smog generated from isoprene (SA-Is) containing less PM_{2.5} and more gas-phase reaction products caused cardiac effects immediately after exposure, while the cardiac responses were only apparent 20 h after exposure to SA-G. The different cardiac responses could be due to the photochemical reaction products generated in the chambers. Some evidence suggests that gaseous components in ambient air pollutants drive the cardiovascular effects[21], [24–28]. In this study, O₃ concentration was higher in the SA-G exposure than that in the SA-Is exposure, suggesting that O₃ does not act alone. Consistent with this observation our previous study showed that gaseous irritants other than O₃ elicited immediate cardiac effects[28]. In addition, our other study also demonstrated that the magnitude of the cardiovascular effect from the particle-free multi-pollutant-mixtures was greater than the effect of exposure to the same concentration of O₃ alone[21].

In urban ambient environments in the United States, formaldehyde and acetaldehyde are the dominant aldehyde compounds, with levels in the range of 1.5–7.4 ppb for formaldehyde and 0.8–2.7 ppb for acetaldehyde[29]. Formaldehyde can decrease cardiac function. It was demonstrated that intravenous infusion of formaldehyde in rats decreased left ventricular end-systolic pressure, heart rate, and cardiac output by impairing Ca²⁺ handling in cardiac excitation-contraction coupling[30]. Inhalation of 10 ppm of formaldehyde results in decreased heart rate, suggesting that sympathetic nervous activity was inhibited[31]. The levels of formaldehyde and acetaldehyde in the chambers were much higher in this study than ambient levels and the level of formaldehyde (1192.7 ppb) and propionaldehyde in SA-Is was two times higher than that in SA-G, which may have accounted for the rapid cardiac effects after SA-Is exposure. Our previous study also observed higher concentrations of formaldehyde and acetaldehyde in the photochemical reaction chamber which may have contributed to the cardiac toxicity[21]. In addition, propionaldehyde, methacrolein (270.8 ppb), PAN, PPN, and MPAN levels were also higher in SA-Is chamber than the SA-G chamber, which may have also contributed to rapid cardiac effects. There are very limited studies evaluating the health effects of these smog components. For instance, PAN was found to affect the oxygen uptake of human subjects during exercise[32]. Exposure to 3 ppm acrolein significantly increased heart rate variability and arrhythmia during exposure and increased LVDP 24 h after exposure[28]. More research is needed to evaluate the cardiovascular effects of exposure to these air contaminants.

The mechanistic basis for the cardiac effects of inhalation to simulated atmospheres is not explored in this study. However, there are several pathways by which air pollution mixtures

could affect the cardiovascular system[1]. Oxidative stress plays an important role in mediating the cardiovascular effects of air pollution[33]. Activation of pulmonary receptors could initiate a neurocardiogenic effect producing an intracardiac response affecting cardiac function[34]. It is also hypothesized that some components of air pollutants might translocate into the circulation with direct effects on the heart and vasculature[35, 36]. The decreased LVDP and cardiac contractility consequent to the exposure to inhaled mixtures could indicate altered intracellular Ca^{2+} regulation in the myocardium. The pro-redox components of secondary smog mixtures could modulate cardiac myocyte Ca^{2+} control by reducing intracellular Ca^{2+} , or by a change in the sensitivity of contractile proteins to Ca^{2+} , resulting in decreased cardiac contractility. Future studies are needed to better understand the role of intracellular Ca^{2+} regulation in mediating cardiovascular physiological effects from air pollution exposure.

In this study, a single exposure of SAs may not represent “real world” human exposure. However, the levels of criteria pollutants (PM, O_3 , and NO_2) in SAs were close to high ambient levels of outdoor pollution in some places in the world. For example, O_3 concentrations (361 ppb for SA-G and 246 ppb for SA-Is) would be close to ambient levels found in China in 2015 where daily maximum 8 h average O_3 concentrations ranged up to 365 ppb [37]. The aldehyde and PAN-analogue compounds levels in this study were certainly higher than ambient levels. Yet toxicological studies such as ours by necessity often have to use high concentrations of air pollution in order to investigate mechanisms underlying air pollution-induced cardiovascular effects with small numbers of animals.

CONCLUSIONS

The present study shows that inhalation of simulated atmospheres depressed cardiac function and cardiac contractility in isolated mouse hearts, with photochemical mixtures generated from isoprene (SA-Is) causing immediate cardiac effects after exposure, while photochemical reaction mixtures generated from gasoline and isoprene (SA-G) causing delayed cardiac effects. These data suggest the possibility that photochemical reaction products affect the cardiovascular system. Future studies are needed to identify the active components of photochemical reaction products responsible for effects on myocardial mechanical performance, and the mechanisms that underlie cardiac effects.

Acknowledgements:

The authors thank Drs. Mehdi Hazari and Daiwen Kang for careful review of the manuscript.

Funding: The U.S. Environmental Protection Agency Intramural Research Program supported this research.

LIST OF ABBREVIATIONS

AQHI	health-based integrated air quality index
FA	filtered air
HC	hydrocarbons
LVDP	left-ventricular developed pressure

MPAN	peroxymethacrylic nitric anhydride
NO₂	nitrogen dioxide
O₃	ozone
PAN	peroxyacetic nitric anhydride
PM	particulate matter
POA	primary organic aerosol
PPN	peroxypropionic nitric anhydride
SA	simulated smog atmospheres
SOA	secondary organic aerosol
VOC	Volatile organic compound

REFERENCES

- [1]. Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC Jr., Whitsett L, Kaufman JD, American Heart Association Council on, C.o.t.K.i.C.D. Prevention, P.A. Council on Nutrition, Metabolism, Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association, *Circulation*, 121 (2010) 2331–2378. [PubMed: 20458016]
- [2]. Kanakidou M, Seinfeld JH, Pandis SN, Barnes I, Dentener FJ, Facchini MC, Van Dingenen R, Ervens B, Nenes A, Nielsen CJ, Swietlicki E, Putaud JP, Balkanski Y, Fuzzi S, Horth J, Moortgat GK, Winterhalter R, Myhre CEL, Tsigaridis K, Vignati E, Stephanou EG, Wilson J, Organic aerosol and global climate modelling: a review, *Atmos Chem Phys*, 5 (2005) 1053–1123.
- [3]. Dominici F, Peng RD, Barr CD, Bell ML, Protecting human health from air pollution: shifting from a single-pollutant to a multipollutant approach, *Epidemiology*, 21 (2010) 187–194. [PubMed: 20160561]
- [4]. Stieb DM, Burnett RT, Smith-Doiron M, Brion O, Shin HH, Economou V, A new multipollutant, no-threshold air quality health index based on short-term associations observed in daily time-series analyses, *J Air Waste Manag Assoc*, 58 (2008) 435–450. [PubMed: 18376646]
- [5]. Hoffmann B, Moebus S, Mohlenkamp S, Stang A, Lehmann N, Dragano N, Schmermund A, Memmesheimer M, Mann K, Erbel R, Jockel KH, Heinz G Nixdorf Recall Study Investigative, Residential exposure to traffic is associated with coronary atherosclerosis, *Circulation*, 116 (2007) 489–496. [PubMed: 17638927]
- [6]. Adar SD, Kaufman JD, Cardiovascular disease and air pollutants: evaluating and improving epidemiological data implicating traffic exposure, *Inhalation toxicology*, 19 Suppl 1 (2007) 135–149. [PubMed: 17886061]
- [7]. Koren HS, Graham DE, Devlin RB, Exposure of humans to a volatile organic mixture. III. Inflammatory response, *Arch Environ Health*, 47 (1992) 39–44. [PubMed: 1540001]
- [8]. Pappas GP, Herbert RJ, Henderson W, Koenig J, Stover B, Barnhart S, The respiratory effects of volatile organic compounds, *Int J Occup Environ Health*, 6 (2000) 1–8. [PubMed: 10637531]
- [9]. Ye D, Klein M, Chang HH, Sarnat JA, Mulholland JA, Edgerton ES, Winquist A, Tolbert PE, Sarnat SE, Estimating Acute Cardiorespiratory Effects of Ambient Volatile Organic Compounds, *Epidemiology*, 28 (2017) 197–206. [PubMed: 27984424]
- [10]. Campen MJ, Babu NS, Helms GA, Pett S, Wernly J, Mehran R, McDonald JD, Nonparticulate components of diesel exhaust promote constriction in coronary arteries from ApoE^{-/-} mice,

Toxicological sciences : an official journal of the Society of Toxicology, 88 (2005) 95–102. [PubMed: 16093524]

- [11]. Gentner DR, Jathar SH, Gordon TD, Bahreini R, Day DA, El Haddad I, Hayes PL, Pieber SM, Platt SM, de Gouw J, Goldstein AH, Harley RA, Jimenez JL, Prevot AS, Robinson AL, Review of Urban Secondary Organic Aerosol Formation from Gasoline and Diesel Motor Vehicle Emissions, *Environmental science & technology*, 51 (2017) 1074–1093. [PubMed: 28000440]
- [12]. Hallquist M, Wenger JC, Baltensperger U, Rudich Y, Simpson D, Claeys M, Dommen J, Donahue NM, George C, Goldstein AH, Hamilton JF, Herrmann H, Hoffmann T, Iinuma Y, Jang M, Jenkin ME, Jimenez JL, Kiendler-Scharr A, Maenhaut W, McFiggans G, Mentel TF, Monod A, Prevot ASH, Seinfeld JH, Surratt JD, Szmigielski R, Wildt J, The formation, properties and impact of secondary organic aerosol: current and emerging issues, *Atmos Chem Phys*, 9 (2009) 5155–5236.
- [13]. Carll AP, Crespo SM, Filho MS, Zati DH, Coull BA, Diaz EA, Raimundo RD, Jaeger TNG, Ricci-Vitor AL, Papapostolou V, Lawrence JE, Garner DM, Perry BS, Harkema JR, Godleski JJ, Inhaled ambient-level traffic-derived particulates decrease cardiac vagal influence and baroreflexes and increase arrhythmia in a rat model of metabolic syndrome, *Particle and fibre toxicology*, 14 (2017) 16. [PubMed: 28545487]
- [14]. Guenther AB, Jiang X, Heald CL, Sakulyanontvittaya T, Duhl T, Emmons LK, Wang X, The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions, *Geosci Model Dev*, 5 (2012) 1471–1492.
- [15]. Sharkey TD, Monson RK, The future of isoprene emission from leaves, canopies and landscapes, *Plant Cell Environ*, 37 (2014) 1727–1740. [PubMed: 24471530]
- [16]. Williams J, Roberts JM, Fehsenfeld FC, Bertman SB, Buhr MP, Goldan PD, Hubler G, Kuster WC, Ryerson TB, Trainer M, Young V, Regional ozone from biogenic hydrocarbons deduced from airborne measurements of PAN, PPN, and MPAN, *Geophys Res Lett*, 24 (1997) 1099–1102.
- [17]. Claeys M, Graham B, Vas G, Wang W, Vermeylen R, Pashynska V, Cafmeyer J, Guyon P, Andreae MO, Artaxo P, Maenhaut W, Formation of secondary organic aerosols through photooxidation of isoprene, *Science*, 303 (2004) 1173–1176. [PubMed: 14976309]
- [18]. Cho SH, Tong H, McGee JK, Baldauf RW, Krantz QT, Gilmour MI, Comparative toxicity of size-fractionated airborne particulate matter collected at different distances from an urban highway, *Environmental health perspectives*, 117 (2009) 1682–1689. [PubMed: 20049117]
- [19]. Tong H, Cheng WY, Samet JM, Gilmour MI, Devlin RB, Differential cardiopulmonary effects of size-fractionated ambient particulate matter in mice, *Cardiovascular toxicology*, 10 (2010) 259–267. [PubMed: 20602262]
- [20]. Cozzi E, Hazarika S, Stallings HW 3rd, Cascio WE, Devlin RB, Lust RM, Wingard CJ, Van Scott MR, Ultrafine particulate matter exposure augments ischemia-reperfusion injury in mice, *American journal of physiology. Heart and circulatory physiology*, 291 (2006) H894–903. [PubMed: 16582015]
- [21]. McIntosh-Kastrinsky R, Diaz-Sanchez D, Sexton KG, Jania CM, Zavala J, Tilley SL, Jaspers I, Gilmour MI, Devlin RB, Cascio WE, Tong H, Photochemically altered air pollution mixtures and contractile parameters in isolated murine hearts before and after ischemia, *Environmental health perspectives*, 121 (2013) 1344–1348. [PubMed: 24148996]
- [22]. Krug JD, Lewandowski M, Offenberg JH, Turlington JM, Lonneman WA, Modak N, Krantz QT, King C, Gavett SH, Gilmour MI, DeMarini DM, Kleindienst TE, Photochemical Conversion of Surrogate Emissions for Use in Toxicological Studies: Role of Particulate- and Gas-Phase Products, *Environmental science & technology*, 52 (2018) 3037–3044. [PubMed: 29381868]
- [23]. Tong H, McGee JK, Saxena RK, Kodavanti UP, Devlin RB, Gilmour MI, Influence of acid functionalization on the cardiopulmonary toxicity of carbon nanotubes and carbon black particles in mice, *Toxicology and applied pharmacology*, 239 (2009) 224–232. [PubMed: 19481103]
- [24]. Campen M, Robertson S, Lund A, Lucero J, McDonald J, Engine exhaust particulate and gas phase contributions to vascular toxicity, *Inhalation toxicology*, 26 (2014) 353–360. [PubMed: 24730681]

- [25]. Carll AP, Hazari MS, Perez CM, Krantz QT, King CJ, Winsett DW, Costa DL, Farraj AK, Whole and particle-free diesel exhausts differentially affect cardiac electrophysiology, blood pressure, and autonomic balance in heart failure-prone rats, *Toxicological sciences : an official journal of the Society of Toxicology*, 128 (2012) 490–499. [PubMed: 22543275]
- [26]. Kurhanewicz N, McIntosh-Kastrinsky R, Tong H, Walsh L, Farraj AK, Hazari MS, Ozone co-exposure modifies cardiac responses to fine and ultrafine ambient particulate matter in mice: concordance of electrocardiogram and mechanical responses, *Particle and fibre toxicology*, 11 (2014) 54. [PubMed: 25318591]
- [27]. Hazari MS, Haykal-Coates N, Winsett DW, Costa DL, Farraj AK, A single exposure to particulate or gaseous air pollution increases the risk of aconitine-induced cardiac arrhythmia in hypertensive rats, *Toxicological sciences : an official journal of the Society of Toxicology*, 112 (2009) 532–542. [PubMed: 19748997]
- [28]. Kurhanewicz N, McIntosh-Kastrinsky R, Tong H, Ledbetter A, Walsh L, Farraj A, Hazari M, TRPA1 mediates changes in heart rate variability and cardiac mechanical function in mice exposed to acrolein, *Toxicology and applied pharmacology*, 324 (2017) 51–60. [PubMed: 27746315]
- [29]. Mohamed MF, Kang D, Aneja VP, Volatile organic compounds in some urban locations in United States, *Chemosphere*, 47 (2002) 863–882. [PubMed: 12079081]
- [30]. Takeshita D, Nakajima-Takenaka C, Shimizu J, Hattori H, Nakashima T, Kikuta A, Matsuyoshi H, Takaki M, Effects of formaldehyde on cardiovascular system in in situ rat hearts, *Basic & clinical pharmacology & toxicology*, 105 (2009) 271–280. [PubMed: 19558560]
- [31]. Tani T, Kogi K, Horiguchi Y, Inhibitory effects of formaldehyde inhalation on the cardiovascular and respiratory systems in unanesthetized rabbits, *Japanese journal of pharmacology*, 40 (1986) 551–559. [PubMed: 3735804]
- [32]. Smith LE, Inhalation of the Photochemical Smog Compound Peroxyacetyl Nitrate, *American journal of public health and the nation's health*, 55 (1965) 1460–1468.
- [33]. Niemann B, Rohrbach S, Miller MR, Newby DE, Fuster V, Kovacic JC, Oxidative Stress and Cardiovascular Risk: Obesity, Diabetes, Smoking, and Pollution: Part 3 of a 3-Part Series, *J Am Coll Cardiol*, 70 (2017) 230–251. [PubMed: 28683970]
- [34]. Hazari MS, Haykal-Coates N, Winsett DW, Krantz QT, King C, Costa DL, Farraj AK, TRPA1 and sympathetic activation contribute to increased risk of triggered cardiac arrhythmias in hypertensive rats exposed to diesel exhaust, *Environmental health perspectives*, 119 (2011) 951–957. [PubMed: 21377951]
- [35]. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Lunts A, Kreyling W, Cox C, Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats, *J Toxicol Environ Health A*, 65 (2002) 1531–1543. [PubMed: 12396867]
- [36]. LeBlanc AJ, Moseley AM, Chen BT, Frazer D, Castranova V, Nurkiewicz TR, Nanoparticle inhalation impairs coronary microvascular reactivity via a local reactive oxygen species-dependent mechanism, *Cardiovasc Toxicol*, 10 (2010) 27–36. [PubMed: 20033351]
- [37]. Zhan Y, Luo Y, Deng X, Grieneisen ML, Zhang M, Di B, Spatiotemporal prediction of daily ambient ozone levels across China using random forest for human exposure assessment, *Environ Pollut*, 233 (2018) 464–473. [PubMed: 29101889]

A.

B.

C.

Figure 1.

Pre-ischemic heart rate (A), left ventricular developed pressure (LVDP) (B), and coronary flow rate (C) in hearts isolated from mice exposed to SA-G. Hearts were isolated immediately or 20 h after exposure to filtered air (FA) or SA-G for 4 h as described in METHODS. Data are means $\pm$ SE. $n = 5-8$ in the FA and SA-G groups. * $p < 0.05$; compared with FA control group 20 h after exposure.

A.

B.

Figure 2.

Pre-ischemic cardiac contractility assessed by maximum dP/dt (A) and minimum dP/dt (B) in hearts isolated from mice exposed to SA-G. Hearts were isolated immediately or 20 h after exposure to filtered air (FA) or SA-G for 4 h as described in METHODS. Data are means $\pm$ SE. $n = 5-8$ in the FA and SA-G groups. * $p = 0.05$; compared with FA control group 20 h after exposure.

A.

B.

C.

Figure 3.

Pre-ischemic heart rate (A), left ventricular developed pressure (LVDP) (B), and coronary flow rate (C) in hearts isolated from mice exposed to SA-Is. Hearts were isolated immediately or 20 h after exposure to filtered air (FA) or SA-Is for 4 h as described in METHODS. Data are means ± SE. $n = 5-8$ in the FA and SA-Is groups. * $p < 0.05$; compared with FA control group immediately after exposure.

A.

B.

Figure 4.

Pre-ischemic cardiac contractility assessed by maximum dP/dt (A) and minimum dP/dt (B) in hearts isolated from mice exposed to SA-Is. Hearts were isolated immediately or 20 h after exposure to filtered air (FA) or SA-Is for 4 h as described in METHODS. Data are means $\pm$ SE. $n = 5-8$ in the FA and SA-Is groups. * $p < 0.05$; compared with FA control group immediately after exposure.

Table 1.

Average concentrations of 20 most abundant hydrocarbons in the chamber prior photochemical reactions in simulated atmospheres generated from gasoline (SA-G) or isoprene (SA-Is) precursors.

SA-G			SA-Is		
Compound	Concentration (ppb)	%	Compound	Concentration (ppb)	%
Isoprene	5225	40.0	Isoprene	22852	100
Toluene	1135	8.6			
2-Methylpentane	663	5.0			
Ethanol	650	4.9			
m, p-Xylene ^a	641	4.9			
n-Hexane	437	3.3			
3-Methylpentane	367	2.8			
Isopentane	341	2.6			
1,2,4-TMB	202	1.5			
Ethylbenzene	175	1.3			
Methylcyclopentane	167	1.3			
n-Pentane	166	1.3			
o-Xylene	153	1.2			
Cyclohexane	135	1.0			
m-Ethyltoluene	123	0.9			
2,3-Dimethylbutane	112	0.9			
2-Methyl-2-butene	97	0.7			
n-Butane	96	0.7			
trans-2-Pentene	89	0.7			
1,3,5-TMB	85	0.6			
Others	2091	15.8			

^a m-Xylene and p-Xylene co-elute and are reported as a single concentration.

Table 2.

Average particle mass and gas concentrations measured in the exposure chamber.

	SA-G		SA-Is	
	FA	SA-G	FA	SA-Is
Calculated AQHI ^a		99.7		75.0
PM mass concentration (filter) ($\mu\text{g}/\text{m}^3$)	0.0	304.0	11.3	160.0
O ₃ (ppb)	2.0	360.7	4.3	246.3
NO ₂ (ppb)	0.0	584.1	0.0	508.6
Estimated gas-phase products ($\mu\text{gC}/\text{m}^3$) ^b	0.0	2,959	0.0	5,069
Humidity (%)	45.3	38.9	42.4	43.5
Temperature (F)	74.4	74.5	72.4	72.1

^a AQHI, air quality health index[4].^b Gas-phase reaction products estimated from reacted HC-SOA carbon. Details in Krug et al [22]

Table 3.

Average concentrations of aldehyde and PAN-analogue compounds measured in the exposure chamber.

Analyte (ppb)	SA-G	SA-Is
Formaldehyde	491.9	1192.7
Acetaldehyde	79.0	43.9
Acetone	39.7	45.8
Propionaldehyde	33.3	51.1
Methacrolein	66.4	270.8
2-Butanone	12.3	10.0
Glyoxal	34.0	37.1
Methyl Glyoxal	125.0	129.6
PAN [*]	158.77	309.03
PPN [*]	15.13	54.72
MPAN [*]	n/a	65.45

^{*} peroxyacetic nitric anhydride (PAN), peroxypropionic nitric anhydride (PPN), and peroxyacetic nitric anhydride (MPAN). Note: Acrolein, crotonaldehyde, butyraldehyde, benzaldehyde, valeraldehyde, m-tolualdehyde, and hexaldehyde were among the non-detectable aldehydes.

Table 4.

Hemodynamics of isolated mouse hearts after exposure.

Parameters	SA-G				SA-Is			
	FA-0 h	SA-G -0 h	FA-20 h	SA-G -20 h	FA-0 h	SA-Is -0 h	FA-20 h	SA-Is -20 h
HR (bpm)	349±22	320±12	358±32	428±106	355±19	336±21	318±20	325±39
LVDP (cmH ₂ O)	84.3±4.6	68.3±6.6	79.3±7.4	54.0±12.7 *	83.9±7.4	52.2±5.7 #	59.8±9.2 #	60.7±8.8
dP/dt _{max} (cmH ₂ O/sec)	3073±243	2640±225	2650±258	1864±328 *	3044±219	2025±169 #	2474±410	2265±259
dP/dt _{min} (cmH ₂ O/sec)	-2644±283	-2388±225	-2238±243	-2125±431	-2748±274	-1758±205	-2255±387	-2083±328
Coronary flow rate (mL/min)	1.9±0.1	1.3±0.3	2.6±0.3	1.5±0.2 *	2.8±0.3	3.1±0.8	3.0±0.4	2.4±0.3
%LVDP	29.7±3.9	24.9±5.3	32.4±8.6	28.0±4.3	26.0±6.1	31.9±5.6	23.3±6.1	29.7±6.9

Values are means ± SE. HR=heart rate, LVDP=left ventricular developed pressure; dP/dt_{max}=maximum 1st derivative of the change in left ventricular pressure/time; dP/dt_{min}=minimum 1st derivative of the change in left ventricular pressure/time; %LVDP, recovery of LVDP after 20 min of ischemia and 60 min of reperfusion. n=5–8 in each group.

* $p < 0.05$ compared with FA control group 20 h post exposure.

$p < 0.05$ compared with FA control group immediately post exposure.