Urban Air Pollution Nanoparticles from Los Angeles: Recently Decreased Neurotoxicity

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Abstract

Background: Air pollution is widely associated with accelerated cognitive decline at later ages and risk of Alzheimer’s disease (AD). Correspondingly, rodent models demonstrate the neurotoxicity of ambient air pollution and its components. Our studies with nano-sized particulate matter (nPM) from urban Los Angeles collected since 2009 have shown pro-amyloidogenic and pro-inflammatory responses. However, recent batches of nPM have diminished induction of the glutamate receptor GluA1 subunit, Iba1, TNF\textsubscript{a}, A\textsubscript{B}_{42} peptide, and white matter damage. The same methods, materials, and mouse genotypes were used throughout.

Objective: Expand the nPM batch comparisons and evaluate archived brain samples to identify the earliest change in nPM potency.

Methods: Batches of nPM were analyzed by \textit{in vitro} cell assays for NF-\textkappa\textsubscript{B} and Nrf2 induction for comparison with \textit{in vivo} responses of mouse brain regions from mice exposed to these batches, analyzed by PCR and western blot.

Results: Five older nPM batches (2009–2017) and four recent nPM batches (2018, 2019) for NF-\textkappa\textsubscript{B} and Nrf2 induction showed declines in nPM potency after 2017 that paralleled declines of \textit{in vivo} activity from independent exposures in different years.

Conclusion: Transcription-based \textit{in vitro} assays of nPM corresponded to the loss of \textit{in vivo} potency for inflammatory and oxidative responses. These recent decreases of nPM neurotoxicity give a rationale for evaluating possible benefits to the risk of dementia and stroke in Los Angeles populations.

Keywords: Air pollution, Alzheimer’s disease, microglia, mouse brain, ultrafine particulate matter

INTRODUCTION

Exposure to particulate matter (PM) in urban air pollution is associated with accelerated cognitive decline and increased risk of Alzheimer’s disease (AD) in many populations [1–4]. Urban airborne
PM is a mixture from diverse sources that can vary widely in chemical composition and size distribution. Rodent models for the neurotoxicity of urban air document neurite atrophy, glial activation, and oxidative damage in association with increased production of amyloid peptides, inflammatory responses, and Nrf2-mediated antioxidant response [2, 5–7]. Experimental studies have used PM from diverse sources: concentrated total ambient urban PM [8, 9], ambient air from a traffic tunnel [10], size-selected PM collected on filters [11–13], and dust-storm PM [14]. Air pollution components include exposure to diesel exhaust particles [5, 15, 16], zinc nanoPM [17], iron-coated silica nanoPM [18], carbon-nanotubes [19], and ozone [7, 20].

Protocols used by our laboratory since 2010 [21] expose rodents to nPM, a subfraction of ultrafine PM0.2 [1, 11, 21–35]. Urban ultrafine PM (PM0.2) are continuously collected on filters from the same urban freeway corridor in Los Angeles. Filters are sonicated in water to yield an eluate we designated as nPM in distinction from total ultrafine PM [21]. The nPM subfraction of PM0.2 has equivalent oxidative activity to total PM0.2 assayed in vitro [36], despite its lower content of transition metals and the absence of polycyclic aromatic hydrocarbons [11]. Exposure of mice to nPM for 3–12 weeks can activate microglia in multiple brain regions [25, 28, 35]; induce pro-inflammatory cytokines in brain (TNFα and IL-1β) [21, 25, 28]; decrease neuronal glutamate receptor subunit GluA1 in hippocampus and cortex [1, 21, 28]; and cause atrophy of hippocampal CA1 neurites [1, 28]. The nPM and other air pollution components are also pro-amyloidogenic in AD-transgenic mice [1, 7, 10] and in wild-type rodents: the Aβ42 peptide was increased by diesel exhaust in F344 rats [37] and by nPM in C57BL/6 mice [36]. The combination of nPM with chronic cerebral hypoperfusion from bilateral carotid artery stenosis (BCAS) caused increased white matter damage and activated microglia [27].

We recently characterized seven nPM batches collected from 2016 to 2018 for inflammatory cytokine responses and lipid peroxidation in cultured monocytes [36]. Several recent batches of nPM showed lower activity for NF-κB induction and lipid peroxidation that corresponded to decreased in vivo microglial activation and amyloidogenic response [36]. Here, we extend these findings to a wider time range of nPM batches, 2009–2020. The nPM batches are compared for in vivo inflammatory responses, glutamate signaling, amyloid production, and white matter damage in mouse models. A new in vitro assay was developed for simultaneously assessing the activation of Nrf2 and NF-κB in monocytes, which are increased in lung by chronic exposure to air pollution [38].

METHODS AND MATERIALS

Reagents

RPMI 1640 cell culture media and TRIzol reagent were from Thermal Fisher (Rockford, IL). QUANTIBlue was from InvivoGen (San Diego, CA); reverse transcription reagent and qPCR master mixture were from BioPioneer Inc (San Diego, CA); other chemicals, were from Sigma-Aldrich (St. Louis, MO). Antibodies of Iba1, MAG, and dMBP were from Abcam (Cambridge, MA). Aβ42 was assayed by ELISA Meso Scale Discovery (Rockville, MD).

Collection and preparation of nPM

All nPM batches were collected from Los Angeles city within 150 m downwind of the I-110 Freeway [39]. These aerosols represent a mix of fresh ambient PM mostly from vehicular traffic. PM0.2 was collected on Zeflour PTFE filters (Pall Life Sciences, Ann Arbor, MI) with a High-Volume Ultrafine Particle Sampler [39] at 400 L/min flow. Filter-deposited nPM was eluted by sonication into deionized sterile water and stored in −20°C. Table 1 shows the collection dates of nPM batches.

Animals and nPM exposure

Animal procedures were approved by the University of Southern California (USC) Institutional Animal Care and Use Committee (IACUC). Young adult C57BL/6J male and female mice were purchased from Jackson Laboratories. Mice were exposed to nPM on the same protocol used since 2010 [11]. For each exposure, mice were transferred from home cages into sealed exposure chambers with ample ventilation. The nPM group was exposed to re-aerosolized nPM at 300 μg/m³, while controls received filtered air in parallel chambers. Exposure schedules were 5 h/d, 3 d/wk; the duration varied by experiment, 3 to 15 wk (Table 1). Mass concentration of the re-aerosolized nPM exposure stream was gravimetrically assessed on filters in parallel to the exposure stream before and after exposures. The nebulizer maintained a similar size distribution of re-aerosolized PM0.2. In one study, mice were...
Table 1

<table>
<thead>
<tr>
<th>Batch</th>
<th>Collection date</th>
<th>Genotype and Sex</th>
<th>Exposure duration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Nov-Jan, 2008-2009</td>
<td>M</td>
<td>10 wk</td>
<td>[21], Fig. 2B</td>
</tr>
<tr>
<td>2010a</td>
<td>Mar, 2010</td>
<td>M</td>
<td>10 wk</td>
<td>[22, 40]</td>
</tr>
<tr>
<td>2010b</td>
<td>Mar-Apr, 2010</td>
<td>F</td>
<td>Prenatal 10 wk</td>
<td>[23, 24, 28, 29, 31]</td>
</tr>
<tr>
<td>2011</td>
<td>Mar-Jun, 2011</td>
<td>M (LDL−/−)</td>
<td>10 wk</td>
<td>[41]</td>
</tr>
<tr>
<td>2012</td>
<td>Aug-Sep, 2012</td>
<td>M, M (ApoE-TR), F (EFAD)</td>
<td>1, 4, 9 d, 15 wk, 15 wk</td>
<td>[1, 25, 33]</td>
</tr>
<tr>
<td>2015a</td>
<td>Oct-Dec, 2015</td>
<td>M</td>
<td>3 wk</td>
<td>[27, 35]</td>
</tr>
<tr>
<td>2015b</td>
<td>Nov-Feb, 2015-2016</td>
<td>M (rat)</td>
<td>28 wk, prenatal to adult 5 mo</td>
<td>[30]</td>
</tr>
<tr>
<td>2016a</td>
<td>Feb-Apr, 2016</td>
<td>M</td>
<td>10 wk</td>
<td>Table 2, Fig. 2B</td>
</tr>
<tr>
<td>2016b</td>
<td>Oct-Dec, 2016</td>
<td>M (J20 hAPPswe)</td>
<td>10 wk</td>
<td>[42]</td>
</tr>
<tr>
<td>1</td>
<td>Apr-June, 2016</td>
<td>In vitro only</td>
<td></td>
<td>[36], Fig. 1A, B</td>
</tr>
<tr>
<td>2</td>
<td>June-Aug, 2016</td>
<td>M</td>
<td>prenatal, or 8 wk</td>
<td>[34–36, 43], Fig. 2A, C, G, H</td>
</tr>
<tr>
<td>3</td>
<td>May-Sep, 2016</td>
<td>In vitro only</td>
<td></td>
<td>Fig. 1A, B</td>
</tr>
<tr>
<td>4</td>
<td>Nov-Jan, 2016-2017</td>
<td>Caenorhabditis elegans</td>
<td></td>
<td>[44]</td>
</tr>
<tr>
<td>5</td>
<td>May-Jun, 2017</td>
<td>M</td>
<td>3 wk</td>
<td>[11, 33]</td>
</tr>
<tr>
<td>6</td>
<td>Jan-Mar, 2018</td>
<td>M, F</td>
<td>8 wk</td>
<td>[33, 36]</td>
</tr>
<tr>
<td>7</td>
<td>May-Jul, 2018</td>
<td>F</td>
<td>8 wk</td>
<td>Fig. 2G</td>
</tr>
<tr>
<td>8</td>
<td>Feb-Mar, 2019</td>
<td>F</td>
<td>10 wk</td>
<td>Fig. 2, D, E, F</td>
</tr>
<tr>
<td>9</td>
<td>Sep-Nov, 2019</td>
<td>M, F</td>
<td>3 wk</td>
<td>Table 2, Fig. 2G, 2H</td>
</tr>
<tr>
<td>10</td>
<td>Jan-Mar, 2020</td>
<td>In vitro only</td>
<td></td>
<td>Fig. 1A, B</td>
</tr>
</tbody>
</table>

C57BL/6 mice and designated transgenic strains, and Sprague-Dawley rats were exposed to nPM at 300 μg/m3, 5 h/d, 3 d/wk for the stated number of weeks.

Fig. 1. In vitro biological activities of nPM batches. A) NF-κB induction; LPS, lipopolysaccharide (5 ng/ml). B) Nrf2 induction; SF, sulforaphane (5 μM). *p < 0.05 versus control, four wells per assay plate. C) Co-plot of NFκB and Nrf2: R² = 0.94, p = 0.0001; bolded numbers are batch ID since 2018 (Batch 6–10). The correlation of NFκB and Nrf2 was analyzed by two-tailed Pearson correlation analysis. D) Historical change of air pollutants. Levels of PM2.5, ozone and NO2 in central LA (5 miles from nPM collection site) from 2009 to 2020 showed linear trends: PM2.5: R² = 0.26, p = 0.09; O3: R² = 0.58, p = 0.004; NO2: R² = 0.83, p = 0.001.

given bilateral carotid artery stenosis (BCAS) at 4 weeks before the final nPM exposure [45]. After euthanization by cardiac perfusion under anesthesia, brains were dissected, frozen on dry ice, and stored at −80°C.

mRNA

Cerebral cortex (alternate hemispheres) was extracted for RNA with TRizol reagent, followed by qPCR with specific primers [11].
Fig. 2. *In vivo* brain responses to nPM by batch for 120 to 150 h (see Table 1 for exposure details and primary citations). A) Microglial Iba1 immunostaining in hippocampus; Batch 2 data are shown from our initial report [36]. B) TNFα mRNA in cerebral cortex; Batch 2009 [21]. C) Aβ42 peptide in cerebral cortex; Batch 2 data [36]. D) Microglial Iba1 immunostaining in corpus callosum; Batch 2015a [35]. E) MAG (myelin-associated glycoprotein) immunostaining in corpus callosum; Batch 5 data [Q. Liu and W.J. Mack, unpublished]. F) dMBP (debris of myelin basic protein) immunostaining in corpus callosum [Q. Liu and W.J. Mack, unpublished]. G) GluA1 in olfactory bulb; Batch 2 and Batch 9: mRNA by qPCR, Batch 7: protein by western blot. H) IL-1β mRNA in olfactory bulb. Mean ± SD, N = 7–10, *p < 0.05, **p < 0.01.

I) Novel Object in Context (NOIC) score for memory assessment. Batch comparisons were from exposures for 8 or 10 weeks conducted in different years. The standardized effect sizes differed across batch collection years (*p = 0.001); effect sizes in 2018 and 2019 were smaller than prior years (*p = 0.003).
Immunohistochemistry

Iba1 [45], myelin-associated glycoprotein (MAG), and debris of myelin basic protein (dMBP) [26] were immunostained by standard procedures in cited references.

**NF-κB and Nrf2 activity**

To simultaneously assay both activities, we engineered THP-1 Blue NF-κB reporter monocytes by transfection with a Nrf2 reporter. This THP-1 line expresses the reporter NF-κB-SEAP (secreted embryonic alkaline phosphatase) regulated by 5-tandem IkB cis-elements [36]. The NF-κB reporter measures activity but cannot differentiate which NF-κB family member causes activation. THP-1 NF-κB/Nrf2 reporters were developed by transducing THP-1 Blue NF-κB monocytes with ARE Luciferase Reporter Lentivirus (BPS Bioscience, San Diego, CA). The ARE Luciferase reporter is regulated by 3-tandem antioxidant-response elements. Cells were grown at density of $4 \times 10^5$–$1 \times 10^6$ cells/ml under 5% CO$_2$/37°C in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and antibiotics (1% penicillin/streptomycin, 100 μg/ml normocin, 10 μg/ml blasticidin). Cells were treated with 5 μg/ml nPM for 8 h; NF-κB activity was assayed by QUANTI-Blue and Nrf2/luciferase activity by luminometry; both were calculated relative to vehicle (H$_2$O). This new cell line is available on request.

**Behavioral testing**

Novel Object in Context (NOIC) test was used to assess hippocampal dependent object and context recognition [46].

**Statistical analysis**

Data were analyzed by GraphPad Prism v.7 and Stata v.16.1 (College Station, TX). Batch group comparisons were analyzed by either Student t-test (for 2 groups) or one-way ANOVA (>2 groups). Pairwise comparisons of later batches to the first batch were corrected for multiple hypothesis testing using the False Discovery Rate of Benjamini, Krieger and Yekutieli [47]. For trend analysis, a time variable (in months since first sample batch) was calculated according to the nPM collection date, with Batch 1 represented as time 0. The date for each batch used the midpoint of the date range (Table 1); the month time variable ranged from 0–47 months. Linear regressions evaluated NF-κB and Nrf2 activity as dependent variables, and months as the independent variable. For effect size analysis of each data collection period in Fig. 2A-I, standardized treatment effect sizes (nPM versus control) were calculated as the mean difference between groups, divided by the pooled standard deviation. The standardized effect sizes were compared over collection year, using a mixed effects linear model specifying a fixed effect for collection year, and a random effect for study (Fig. 2A-I). Historical data 2009–2020 of PM2.5, ozone and NO$_2$ was obtained from Chemical Speciation Network (CSN) provided by the US Environmental Protection Agency (US EPA) [48] for a site in Los Angeles city (1630 North Main St., Los Angeles, CA 90012), 5 miles from the nPM collection site (3400 South Hope St., Los Angeles, CA 90089). p < 0.05 was considered significant for all statistical tests.

**RESULTS**

**In vitro activity**

Inflammation and oxidative stress are two putative mechanisms of how nPM causes neurotoxicity. In vitro activity of nPM was assessed by its capacity to induce two key transcription factors: NF-κB, the master transcriptional regulator of inflammatory response, and Nrf2, which controls the antioxidant response to oxidative stress [49]. The reporter genes were transfected into a monocyte cell line, as a model for respiratory tract monocyte/macrophages that directly receive inhaled PM [36]. Exposure of NF-κB/Nrf2 reporter cells to nPM increased the activities of both NF-κB and Nrf2, with dose-dependence over a 40-fold range (0.25 –10 μg/ml, data not shown).

The nPM batches differed in potency for inducing NF-κB (Fig. 1A) and Nrf2 (Fig. 1B). Both transcriptional responses were strongly correlated ($r=0.97$, Fig. 1C). The trend analysis showed a significant decrease in the induction of NF-κB and Nrf2 by collection dates ($p<0.01$ for both NF-κB and Nrf2). For both NF-κB and Nrf2, Batches 2 and 3 were significantly higher than Batch 1 ($p<0.05$), and Batches 6–10 (2018-2019) were significantly lower than Batch 1. These data suggest that the potency of nPM to induce NF-κB and Nrf2 varies by batch and has declined since 2017.

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declined weakly (p = 0.09), while the decline of NO₂ was significant (3.6%/y, p = 0.001). Opposing these declines was a significant increase of ozone (1.8%/y, p = 0.004).

In vivo responses

Five older nPM batches (2009–2017) and four recent nPM batches (2018, 2019) (Table 1) were compared from independent 8- or 10-week exposure experiments in different years (Fig. 2A–I). Each panel shows the statistically significant response of earlier nPM batches for comparison with null response to recent nPM. Microglial Iba1 (Fig. 2A) did not respond to recent batches. The mRNAs of RelA/p65 (NF-κB1, the p105/p50 member of NF-κB family), and TLR4 responded minimally to nPM, and was decreased by most batches (Table 2). IL-1β mRNA responses in vivo declined weakly (p = 0.09), while the decline of NO₂ was significant (3.6%/y, p = 0.001). Opposing these declines was a significant increase of ozone (1.8%/y, p = 0.004).

To extend the examples of Fig. 2, we examined mRNA responses of archived cerebral cortex from mice exposed to different nPM Batches (Table 2). NFκB1, the p105/p50 member of NF-κB family, gave the most consistent response to nPM, and was decreased by most batches (Table 2). IL-1β was increased by Batch 2016a but not by others. MyD88, an adaptor protein in the TLR4-NFκB pathway, was decreased by Batches 5 and 9 in males exposed for 3 weeks, whereas MyD88 did not respond to other batches. GluA1 mRNA was decreased in cortex by Batches 2016a and 5, confirming findings in hippocampus [1, 21, 28], whereas GluA1 mRNA did not respond to recent batches. The mRNAs of RelA/p65 (NF-κB family), and TLR4 responded minimally to all nPM batches. Taken together, Table 2 findings parallel batch responses of Fig. 1 and Fig. 2, suggesting that nPM batches collected since 2018 have decreased neurotoxicity. This comparison is limited by two factors: exposures varied from 3 to 10 weeks (Table 1) and some batches were not available because of depletion in prior studies.

**DISCUSSION**

Air pollution PM varies widely in bioactivity/toxicity by sites of collection and season, as shown in prior studies. Some sites have much higher PM levels than others, some PM samples are more toxic than others, and some sites have more or less neurotoxicity by sites of collection and season, as shown in prior studies. Some sites have much higher PM levels than others, some PM samples are more toxic than others, and some sites have more or less neurotoxicity by sites of collection and season, as shown in prior studies.
by the DTT assay and other biochemical and in vitro cell models [36, 50–55]. The present data extend these findings by comparing the in vitro Nrf2 and NF-κB transcriptional responses with in vivo neurotoxic effects caused by batches of PM0.2 collected over a decade at the same site in urban Los Angeles. These findings show extensive variations in the biological potency of the nPM subfraction of ambient PM0.2, assessed by the same methods, materials, and mouse genotype from the same sources. The new cell-based assay for induction of Nrf2 and NF-κB may be useful to evaluate the potency of PM collected from ambient air in animal models of air pollution.

The in vitro potency of nPM to induce NF-κB and Nrf2 signaling with a monocyte reporter declined in five batches collected after 2016 (Fig. 1). NF-κB and Nrf2 were chosen for in vitro assays because these transcription factors are key mediators of inflammatory, detoxification, and anti-oxidative responses. The transcriptional gene reporter for NF-κB does not identify the relative contribution of its five subunits (RelA/p65, NFκB1/p50, NFκB2/p52, RelB and RelC) that act by forming hetero- or homo-dimers [54–56], which could include a decrease of the NFκB1 mRNA as observed in vivo.

The in vivo responses of cerebral cortex were expanded to four recent batches collected after 2017 (Batches 6,7,8,9) (Fig. 2, Table 2). Batches 6–9 did not increase TNFα, microglial Iba1, Aβ42, and dMPB (myelin degeneration), or decrease GluA1 (glutamate receptor subunit), unlike earlier batches. The decreasing neurotoxicity of recent batches since 2016 was consistently observed in four brain regions: cerebral cortex, corpus callosum, hippocampus, and olfactory bulb. These observations further document the declining in vitro potency of recent batches and confirm our initial findings that nPM batches vary in the potential to cause inflammatory response and neurotoxic effects [36].

In contrast, some other cerebral cortex mRNA responses were maintained in recent batches, e.g., NFκB1 mRNA was still decreased by Batches 8 and 9 (Table 2). Batch 5 (2017) and Batch 9 (2019) showed respectively higher and lower potency in vitro, nonetheless both decreased Myd88 mRNA in cortex. Moreover, a separate transcriptomic analysis of male C57BL/6 mice showed Batch 6 induced extensive responses of Nrf2 and NF-κB mediated pathways [33]. A hierarchy of atherogenic response to air pollutants was described by Araujo and Nel [56], in which antioxidant defenses responded to lower activity of PM, followed by inflammation and cytotoxicity at higher PM activity. The nPM batches that did not induce brain inflammatory cytokines (Fig. 2, Table 2), but still altered Nrf2 and NF-κB [33] may be consistent with this framework. We are planning to compare Batch 6 with Batch 2 for transcriptomic responses of cerebral cortex.

Unexpectedly, the early 2012 Batch did not alter the Aβ42 in cerebral cortex of ApoE-TR cerebral cortex, but still decreased mRNA for amyloid production (APP, Psen1) and Aβ42 peptide clearance (C3, Vav3) [33]. Note that these data for transgenic mice cannot be compared with the wildtype B6 which was examined in a different study with another nPM batch (Fig. 2C). Current data do not resolve the cause of inconsistent effects of nPM on the brain and whether they are attributable to exposure duration, sex, or difference in PM0.2 composition. Nor do epidemiological associations of air pollution components with dementia risks [1–4] show how ubiquitous inflammatory processes may cause brain aging to go from bad to worse.

The cognitive impact of air pollution must consider multiple interacting factors and neurotoxic pathways [57] including and beyond the glial and neuronal responses being studied in this growing field. The decreased impact of nPM on glial and neuronal responses gives a rationale for examining Los Angeles elderly populations for possible benefits to cognition. The declining activity of nPM per μg by our measures since 2016 does not parallel the modest declines of PM2.5 and NO2 and is opposite to the stronger trend for increased ozone (Fig. 1D). However, the mass of the nPM fraction is less than 25% of the total PM2.5 at the nPM collection site [58]. We must also consider the concurrent increase of ozone 2009–2020 (Fig. 1D) [20, 59], which was specifically associated with impaired executive function of Los Angeles elderly [60].

The nPM subfraction of urban air pollution is a highly complex mixture, in which altered batch differences of trace components could alter in vivo potency. Batches 1–7 varied widely in composition without obvious historical trends for copper, iron, or other transition metals, or toxic organic species [36]. Hierarchical clustering by nPM batch showed weak associations of endotoxin with NF-κB activation in vitro but did not identify correlations of any chemical element with in vitro activities [36]. A more comprehensive analysis of nPM components, including the chemical status of transition metals on particle surface, the microbial components and other factors, may explain the cause of the unequal toxicity of nPM.
We must also consider the chemical state of transition elements. For example, Los Angeles PM2.5 contain multiple species of iron in combination with carbonaceous material ranging from crystalline iron to several types of ferrihydrates [61]. These different iron species arise from engine combustion and from abrasion of brake linings. Additional redox active meals arise from industrial and natural sources [62, 63]. We suggest a broader discussion of urban air pollution components in experimental models that will consider particle surface chemistry as well as composition. A systematic approach could identify broadly shared anthropogenic and natural components that might serve as a standard in comparing experimental findings from the expanding research on air pollution neurotoxicity.

In conclusion, we show that nPM batches from Los Angeles have recently decreased potency for neurotoxic responses relevant to neurodegeneration and AD risk in the same mouse model. The declines in ambient PM2.5 and NO2 of Los Angeles air pollution 2009–2020 (Fig. 1D) in combination with decreased neurotoxicity give a rational for examining possible parallel changes in human brain health. A decline in nPM potency could impact the hospital admission rates or discharge outcomes for patients presenting with stroke or cerebral hypoperfusion in the Los Angeles basin.

**Limitations and strengths**

These post hoc comparisons of nPM by batch (Table 2) were limited by availability of archived nPM and cerebral cortex tissues. The *in vivo* comparisons in Fig. 2 were from independent experiments at different times. Cognitive deficits from earlier nPM batches were not evaluated for the recent batches. Caveats recognized, the data shows progressively declining potency of nPM during the past decade of sampling in urban Los Angeles.

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**SUPPLEMENTARY MATERIAL**

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**REFERENCES**


Acute exposure to diesel exhaust impairs adult neurogenesis in mice: Primocine in males and protective effect of pioglitazone. Arch Toxicol 92, 1815-1829.


markers of neurodegenerative disease. J Neuroinflammation 8, 105.


