Analysis of 19 urinary biomarkers of oxidative stress, nitrative stress, metabolic disorders, and inflammation using liquid chromatography–tandem mass spectrometry

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Abstract
Environmental chemical exposures have been associated with cancer, diabetes, hormonal and immunological disorders, and cardiovascular diseases. Some direct effects of chemical exposure that are precursors to adverse health outcomes, including oxidative stress, nitrative stress, hormonal imbalance, neutrophilia, and eosinophilia, can be assessed through the analysis of biomarkers in urine. In this study, we describe a novel methodology for the determination of 19 biomarkers of health effects: malondialdehyde (MDA), 8-isoprostaglandin-F2α (8-PGF2α), 11-β-prostaglandin-F2α (11-PGF2α), 15-prostaglandin-F2α (15-PGF2α), 8-iso-15-prostaglandin-F2α (8,15-PGF2α), 8-hydroxy-2′-deoxyguanosine (8-OHdG), 8-hydroxyguanosine (8-HdG), 8-hydroxyguanine (8-HG), dityrosine (diY), allantoin (Alla), and two metabolic products of 4-hydroxynonenal (HNE), namely 4-hydroxy-2-nonenal glutathione (HNE-GSH) and 4-hydroxy-2-nonenal mercapturic acid (HNE-MA) (in total, 12 oxidative stress biomarkers, OSBs); 8-nitroguanosine (8-NdG), 8-nitroguanine (8-NG), and 3-nitrotyrosine (3NT) (3 nitrative stress biomarkers, NSBs); chlorotyrosine (CY) and bromotyrosine (BY) (2 inflammatory biomarkers); and the advanced glycation end-products (AGEs) Nε-carboxymethyllysine (CML) and Nε-carboxyethyllysine (CEL) (2 metabolic disorder biomarkers). Since these biomarkers are triggered by a variety of environmental insults and produced by different biomolecular pathways, their selective and sensitive determination in urine would help broadly elucidate the pathogenesis of diseases mediated by environmental factors.

Keywords ROS · RNS · Oxidative stress · Biomarkers · AGEs · Urine

Introduction
Environmental factors are known to contribute to a variety of adverse health outcomes in human and wildlife populations [1–4]. Nevertheless, molecular mechanisms underlying health effects due to exposure to environmental stressors remain poorly understood. Exposure to environmental chemicals can inflict oxidative stress, nitrative stress, inflammation, and metabolic disorders, which are early events that occur well before the clinical diagnosis of diseases such as atherosclerosis, cancer, or neurodegenerative disorders [5–8]. Monitoring and assessment of early warning biomarkers of diseases are crucial to understanding pathogenesis following environmental insults. In particular, health-effect biomarkers can be useful in illuminating relationships among environmental exposures, human physiology, and disease.

Biomarkers of health effects are commonly assessed by analyzing biospecimens such as blood or urine. Urine is widely used in monitoring exposure and effect biomarkers of a variety of environmental chemicals of concern. Studies have reported urinary biomarkers of oxidative stress, nitrative stress, eosinophilia, neutrophilia, and metabolic disorders as means to elucidate disease pathogenesis [9, 10].

Oxidative and nitrative stresses are associated with several diseases [8]. It is well known that concentrations of
these stress markers increase in body tissues and fluids following exposure to environmental toxicants [11–16]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by the body at basal levels from metabolism, and these reactive species are normally neutralized by antioxidants. However, excessive production of ROS (e.g., superoxide anion \([O_2^-]\), hydrogen peroxide \([H_2O_2]\)) and RNS (e.g., nitric oxide \([NO]\), peroxynitrite \([ONOO^-]\)) above the antioxidant capacity of a biological system can arise from factors such as environmental stressors, medication, diet, smoking, and acute exercise. Excess ROS leads to oxidative stress, resulting in oxidative damage to biomolecules such as DNA and protein. The free radical \(O_2^-\) can react with NO to form \(ONOO^-\), a potent, diffusible radical that can react with amino acids, contributing to protein modifications such as nitration and nitrosylation. High levels of ROS and/or RNS can also cause lipid peroxidation, DNA damage, and oxidation/nitration of proteins that can activate inflammatory response, fibrosis, or cell death [8–10]. Therefore, a thorough understanding of the mechanisms that lead to perturbations in oxidative/nitratative status and their relationship to pro-inflammatory, hypertrophic, fibrotic, and apoptotic pathways would aid the development of strategies to counteract disease development and progression.

The quantitative determination of free radicals/ROS that contribute to oxidative/nitratative stress (ONS) is challenging due to their highly reactive nature and resulting transient lifespan in body tissues and fluids. However, the free radicals react with various biomolecules, and the products of these reactions can be detected in urine, blood, or feces. For instance, dityrosine (diY) and nitrotyrosine (NY) [17] are the products of protein damage caused by ROS and RNS, respectively. Oxidative damage to DNA by ROS can generate 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-hydroxyguanine (8-HG), whereas that by RNS can yield 8-nitroguanine (8-NG). Reactions of ROS and RNS with RNA produce 8-hydroxyguanosine (8-HdG) and 8-nitroguanosine (8-NdG), respectively [12, 14, 18]. The biomarkers of oxidative damage to polyunsaturated fatty acids (PUFA) by ROS are malondialdehyde (MDA) and 4-hydroxynonenal (HNE) [19, 20]; the oxidation of arachidonic acid (a fatty acid found in the liver, brain, and glands) can produce prostaglandin isomers, 8-isoprostaglandin \(F_{2\alpha}\) (8-PGF\(_{2\alpha}\)), 11β-prostaglandin \(F_{2\alpha}\) (11-PGF\(_{2\alpha}\)), 15-prostaglandin \(F_{2\alpha}\) (15-PGF\(_{2\alpha}\)) and 8−iso-15-prostaglandin \(F_{2\alpha}\) (8,15-PGF\(_{2\alpha}\)) [21]; and uric acid oxidation yields allantoin (Alla) [22]. All of these reaction products are commonly excreted in urine.

Inflammation is a part of body’s defense in response to injury or infection, but chronic inflammation, which can result from exposure to environmental chemicals, plays a key role in many disorders, such as autoimmune diseases and asthma [23–25]. Eosinophils and neutrophils are two types of white blood cells that are activated to fight diseases, infections, and inflammation. Eosinophilia and neutrophilia are conditions in which the number of eosinophils or neutrophils, respectively, increases above the normal levels in affected tissue [23, 26]. Under these conditions, neutrophil myeloperoxidase and eosinophil peroxidase overproduce halogenated species such as HOCI or HOBr that react with tyrosine, increasing chlorotyrosine (CY) [27, 28] and bromotyrosine (BY) [28] levels, which are recognized as inflammatory biomarkers (IBs) [17, 28].

Exposure to various environmental pollutants is linked to metabolic disorders [29]. Evidence indicates that metabolic disorders may be mediated through oxidative stress. Advanced glycation end-products (AGEs; proteins or lipids that become glycated from exposure to sugars) such as \(N^\epsilon\)-MDA-\(d_2\) (CML) and \(N^\epsilon\)-carboxyethyllysine (CEL) are biomarkers of metabolic disorders or diseases [20].

Earlier methods to analyze health-effect biomarkers entailed testing for a single class of chemicals at a time. However, the simultaneous determination of urinary concentrations of multiple biomarkers of health effects, such as oxidative stress (OSBs), nitrative stress (NSBs), eosinophilia (EB), and neutrophilia (NB) along with AGES, would help advance the knowledge of disease pathogenesis. Because different biomarkers are generated in organisms from various molecular pathways, after exposure to multiple radicals (or radical-producing stressors) that damage proteins, lipids, uric acid, DNA, RNA, or sugars, concurrent and broader testing can provide comprehensive information about the events mediating disease progression (Fig. 1; Table 1).

In addition, evidence supports interplay between biomarkers [20], and therefore, simultaneous analyses of multiple biomarkers are crucial for comprehensive understanding of disease pathogenesis. For example, although the generation of AGES (glycation of proteins or lipids) is known to be mediated by elevated ROS [30], the interactions involved are poorly understood due to the fact that analytical methods to measure multiple effect biomarkers simultaneously were not available. Moreover, selection of reliable biomarkers of a disease is possible only when multiple biomarkers are analyzed simultaneously.

In this study, we developed a sensitive and selective analytical method for simultaneous determination of 16 effect biomarkers—namely, 11 biomarkers that report levels of oxidative stress, diY, 8-OHdG, 8-HG, 8-HdG, 8-PGF\(_{2\alpha}\), 11-PGF\(_{2\alpha}\), 15-PGF\(_{2\alpha}\), 15-PGF\(_{2\alpha}\), 8,15-PGF\(_{2\alpha}\), MDA, 4-hydroxynonenal glutathione (HNE-GHS), and 4-hydroxynonenal mercapturic acid (HNE-MA); three for nitrative stress, NY, 8-NG, and 8-NdG; one each for eosinophil (BY) and neutrophil (CY) activation. The determination of Alla and two biomarkers of metabolic disorders (CML and CEL) was accomplished using dilute and shoot (D&S) method to enable analysis of 19 biomarkers in urine using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS).
This methodology is suitable for application in large-scale human biomonitoring studies.

**Materials and methods**

**Standards and chemicals**

HPLC-grade methanol, acetonitrile, water, and ethyl acetate were purchased from Fisher Scientific (Pittsburgh, PA, USA). Synthetic urine, 2,4-dinitrophenylhydrazine (DNPH; ≥ 99% purity), 8-hydroxy-2′-deoxyguanosine (8-OHdG; ≥ 98%), 3-chloro-4-hydroxy-2-timethyltyrosine (CY; 97%), and malondialdehyde tetrabutylammonium salt (MDA; 97%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The analytical standards 4-hydroxynonenal glutathione (trifluoroacetate salt) (HNE-GSH; ≥ 98%), 8-isoprostaglandin F2α (8-ISO-PGF2α; ≥ 98%), 8-iso-15-prostaglandin F2α (8,15-ISO-PGF2α; ≥ 98%), 8-hydroxy-2′-deoxyguanosine-15N5 (8-OHdG-15N5; 95%) were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Malondialdehyde-1,3-d2-bis(diethyl acetal) (MDA-d2; 98%) was from C/D/N Isotopes (Pointe-Claire, Quebec, Canada).

A working standard mixture containing 1000 ng mL−1 of each native compound was prepared in 50:50 (v/v) methanol:water. An internal standard mixture at a concentration of 1000 ng mL−1 was prepared from individual stock solutions of HNE-GS-d3, HNE-GHS-d3, MDA-d2, 8-PGF2α-d4, BY-13C9,15 N, NG-13C2,15 N, diY-13C12, NY-13C6, and 8-OHdG-15N5. Individual working standards containing Alla-13C2,15 N and CML-d4 were used for the determination of Alla and AGEs, respectively.

**Analysis**

The determination of urinary biomarkers of oxidative and nitrative stress, as well as inflammation, was accomplished through solid-phase extraction (SPE) followed by HPLC–MS/MS analysis as previously reported [31], with modifications. An earlier method was validated in our laboratory for seven OSBs, and the method here includes an additional 9 OSB/NSB biomarkers, for a total of 16 OSB/NSB/IB biomarkers. Separate methods were developed for the analysis of Alla and AGEs.

**Analysis of 16 urinary OSBs/NSBs/IBs**

An aliquot of 0.5 mL urine was transferred into a polypropylene (PP) tube, and 10 µL of a solution containing 1000 ng mL−1 of each internal standard, as well as 100 µL of 0.005 M DNPH (derivatization reagent) solution,
was added. Samples were incubated at room temperature (22 °C) for 30 min, and then, 1 mL of water was added in preparation for SPE. ABS ElutNexus cartridges (60 mg, 3 mL) (Agilent, Santa Clara, CA, USA) were cleaned and conditioned with 2 mL of methanol followed by 2 mL of HPLC-grade water. The derivatized sample then was passed through the cartridge and washed with 2 mL of 95:5 (v/v) water:methanol. Cartridges were vacuum dried and eluted with 1 mL methanol followed by 1 mL ethyl acetate, which were collected in the same tube. Eluates were dried under a gentle nitrogen stream at room temperature, reconstituted with 0.2 mL of 8:2 (v/v) water:methanol, and centrifuged, and finally, 20 µL of the sample was injected into the HPLC–MS/MS instrument.

The MS was operated in both positive and negative ionization modes, and compound-specific MS/MS parameters...
were optimized by infusion of individual standard solutions (Table 2). The most abundant MS/MS transition was selected as quantitative, and the second most abundant was selected as the qualitative ion, especially for those compounds that did not have corresponding labeled internal standard (and therefore required a confirmation ion). An Agilent 1260 HPLC instrument (Agilent, Santa Clara, CA, USA), connected with an Eclipse Plus C18 (particle size 3.5 µm; 4.6×10 mm) column (Agilent, Santa Clara, CA, USA) and a Betasil C18 guard column (particle size 5 µm; 4.6×10 mm), and interfaced with an ABSCIEX triple quadrupole 5500 MS (Sciex, Framingham, MA, USA), with a Turbo V ionization source was used in the analysis. Electrospray positive ionization conditions were set as follows: curtain gas (CUR) 50 psi, collision-activated dissociation gas (CAD) 10 psi, temperature 450 °C, nebulizer gas (GS1) 50 psi, and turbo ion spray voltage (IS) 4500 V. For the duration of the negative ionization, temperature was set at 500 °C and turbo ion spray voltage was set at −4500 V. Liquid chromatographic separation was accomplished at room

| Type                                      | Biomarker                  | Abbreviation | Source                          | Structure |
|-------------------------------------------|----------------------------|--------------|---------------------------------|-----------|
| Oxidative stress biomarkers (OSBs)        | Allantoin                  | Alla         | Oxidation of uric acid          | ![Structure](image1) |
|                                           | 8-Nitroguanosine           | 8-NdG        | Oxidation of RNA by RNS         | ![Structure](image2) |
| Nitrative stress biomarkers (NSBs)        | 8-Nitroguanine             | 8-NG         | Oxidation of DNA by RNS         | ![Structure](image3) |
|                                           | Nitrotyrosine              | NY           | Oxidation of proteins by RNS    | ![Structure](image4) |
| Eosinophilia biomarker (EB)               | Bromotyrosine              | BY           | Modification of tyrosine after activation by eosinophils | ![Structure](image5) |
| Neutrophilia biomarker (NB)               | Chlorotyrosine             | CY           | Modification of tyrosine after activation by neutrophils | ![Structure](image6) |
| Advanced glycation end-products (AGES)    | Nε-(1-Carboxymethyl)-L-lysine | CML         | Glyoxal reaction with lysine    | ![Structure](image7) |
|                                           | Nε-(1-Carboxyethyl)-L-lysine | CEL         | Methylglyoxal reaction with lysine | ![Structure](image8) |

ROS, reactive oxygen species; RNS, reactive nitrogen species; PUFA, polyunsaturated fatty acids
Table 2 Optimized HPLC–MS/MS parameters for the analysis of 19 biomarkers and corresponding internal standards determined in urine in this study

| Biomarker         | MW  | RT (min) | Mode | MRM transition (m/z) | DP (V) | EP (V) | CE (V) | CXP (V) |
|-------------------|-----|----------|------|----------------------|--------|--------|--------|---------|
| MDA-DNPH          | 234 | 11.6     | +    | 235 > 159, 235 > 189 | 30     | 8      | 30     | 3       |
| D₂-MDA-DNPH       | 236 | 11.6     | +    | 237 > 161           | 30     | 8      | 30     | 3       |
| HNE-GHS           | 463 | 8.9 + 9.1 + 9.4 | + | 464 > 308, 464 > 446 | 25     | 10     | 18     | 12      |
| D₂-HNE-GHS        | 466 | 8.9 + 9.1 + 9.4 | + | 467 > 308           | 25     | 10     | 18     | 12      |
| HNE-MA            | 319 | 12.4     | –    | 318 > 171           | –80    | –10    | –27    | –12     |
| D₂-HNE-MA         | 322 | 12.4     | –    | 321 > 174           | –80    | –10    | –27    | –12     |
| 8-PGF₂α           | 354 | 15.8     | –    | 353 > 193           | –40    | –8     | –30    | –12     |
| 11-PGF₂α          | 354 | 16.0     | –    | 353 > 193           | –40    | –8     | –30    | –12     |
| 8,15-PGF₂α        | 354 | 16.2     | –    | 353 > 193           | –40    | –8     | –30    | –12     |
| 15-PGF₂α          | 354 | 16.2     | –    | 353 > 193           | –40    | –8     | –30    | –12     |
| 8,15-PGF₂α        | 354 | 15.6     | –    | 353 > 193           | –40    | –8     | –30    | –12     |
| 15N₅-8-OHdG       | 288 | 6.2      | +    | 289 > 173           | 25     | 3      | 18     | 4       |
| 8-OHdG            | 283 | 6.2      | +    | 284 > 168, 284 > 140 | 25     | 3      | 18     | 4       |
| 8-HG              | 167 | 6.2      | +    | 168 > 140           | 25     | 10     | 21     | 12      |
| 8-HdG             | 299 | 5.9      | +    | 300 > 168, 300 > 140 | 25     | 10     | 21     | 12      |
| diY               | 360 | 5.5      | +    | 361 > 315, 361 > 283 | 35     | 10     | 22     | 12      |
| 13C₁₂-diY         | 372 | 5.5      | +    | 373 > 327           | 35     | 10     | 22     | 12      |
| 8-NG              | 196 | 6.3      | +    | 197 > 151           | 21     | 10     | 10     | 12      |
| 8-NdG             | 328 | 6.6      | +    | 329 > 151, 329 > 197 | 20     | 10     | 40     | 12      |
| NY                | 226 | 6.7      | +    | 227 > 117, 227 > 181 | 25     | 10     | 20     | 12      |
| 13C₁₂-NY          | 232 | 6.7      | +    | 233 > 122           | 25     | 10     | 18     | 12      |
| BY                | 259 | 6.5      | +    | 260 > 243           | 25     | 10     | 15     | 12      |
| 13C₁⁴N₁₂-BY       | 269 | 6.5      | +    | 270 > 252           | 30     | 10     | 16     | 12      |
| CY                | 215 | 6.2      | +    | 216 > 199           | 25     | 10     | 14     | 12      |
| Alla              | 158 | 4.2      | +    | 159 > 116, 159 > 99 | 30     | 3      | 10     | 3       |
| 13C₂₋₁⁴N₁₂-Ala    | 164 | 4.2      | +    | 165 > 120           | 30     | 3      | 10     | 3       |
| CEL               | 218 | 4.1      | +    | 219 > 84           | 30     | 7      | 25     | 12      |
| CML               | 204 | 4.0      | +    | 205 > 84, 205 > 130 | 40     | 7      | 20     | 12      |
| D₂-CML            | 208 | 4.0      | +    | 209 > 88           | 35     | 7      | 25     | 12      |

*Confirmation ion transition

DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, exit potential; MW, molecular weight; RT, retention time
temperature. The mobile phase consisted of 0.1% acetic acid in water (phase A) and 8:2 (v/v) methanol:acetonitrile containing 0.1% acetic acid (phase B). The flow was initially set at 100% A at 500 μL min⁻¹, held for 2 min, and then decreased to 50% A over the next 3 min, to 35% A over 6 min, to 30% A for 9 min, and to 0% over the final 1 min. The flow was then continued with 100% B (0% A) for 6 min. The gradient composition was then reverted to the original 100% A for the last 9 min to equilibrate the column, for a total run time of 36 min.

**Method validation**

The linearity, sensitivity, matrix effect, accuracy, and precision of the analytical method were assessed. The linear regression parameters were obtained from the analysis of urine spiked at concentrations near the expected limits of detection and quantification for the calculation of method limit of detection (mLOD) and method limit of quantification (mLOQ) for each biomarker. mLOD and mLOQ were calculated as 3 and 10 times of the $S_b/a$ respectively ($S_b$: intercept standard deviation, $a$: slope).

Method validation was accomplished through the analysis of synthetic urine and a pool of real urine, each fortified with standard analytes at three different concentrations (low, A; medium, B; high, C) and analyzed in triplicate for 3 days for the determination of precision and accuracy. The fortified concentrations of analytes for method validation were set based on the mLOD and expected concentrations in urine. For 8-PGF₂α, 11-PGF₂α, 15-PGF₂α, 8,15-PGF₂α, 8-OHdG, and 8-NdG, the low (A), medium (B), and high (C) fortification levels were 0.1, 1.0, and 10.0 ng mL⁻¹, respectively. For 8-HG, 8-HdG, diY, 8-NG, NY, BY, CY, MDA and HNE-GHS, the fortification levels were A, 0.5 ng mL⁻¹; B, 5.0 ng mL⁻¹; and C, 10.0 ng mL⁻¹; and those for HNE-MA were A, 5.0 ng mL⁻¹; B, 10.0 ng mL⁻¹; and C, 50.0 ng mL⁻¹. The analysis of variance (ANOVA) of the data obtained from three replicate samples analyzed over 3 different days was used to calculate intra-day and inter-day precision (RSD, %) at the three levels. Accuracy was calculated in terms to recovery (%) at the low-medium–high fortification levels. The method validation for linearity (coefficient of determination, $R^2$), sensitivity (mLOD and mLOQ), precision (intra-day and inter-day RSD, %), and accuracy (% recovery) at the three fortification levels (A, B, and C) is shown in Table 3.

**Determination of allantoin and creatinine**

Due to the high polarity of Alla and Cr, and their high concentrations relative to those of other target analytes in urine, these two biomarkers were determined by following a method described earlier [22].

### Table 3

| Biomarker | Intra-day repeatability (%) | Inter-day repeatability (%) | Recovery (%) | mLOD (ng mL⁻¹) | mLOQ (ng mL⁻¹) | $R^2$ |
|-----------|-----------------------------|-----------------------------|--------------|----------------|----------------|-------|
|           | A   | B   | C   | A   | B   | C   | A   | B   | C   | A   | B   | C   |       |
| MDA-DNPH  | 11  | 6   | 1   | 15  | 11  | 3   | 91  | 102 | 115 | 0.08 | 0.27 | 0.9992 |
| HNE-GHS   | 9   | 6   | 5   | 7   | 6   | 5   | 101 | 115 | 113 | 0.03 | 0.11 | 0.9998 |
| HNE-MA    | 6   | 7   | 4   | 7   | 4   | 4   | 105 | 105 | 102 | 0.36 | 1.20 | 0.9998 |
| 8-PGF₂α   | 7   | 8   | 2   | 9   | 9   | 5   | 94  | 96  | 94  | 0.013 | 0.04 | 0.9998 |
| 11-PGF₂α  | 13  | 3   | 6   | 5   | 5   | 5   | 119 | 116 | 117 | 0.014 | 0.05 | 0.9998 |
| 15-PGF₂α  | 7   | 6   | 9   | 6   | 10  | 14  | 109 | 111 | 110 | 0.013 | 0.04 | 0.9998 |
| 8,15-PGF₂α| 6   | 8   | 9   | 4   | 9   | 3   | 89  | 91  | 100 | 0.013 | 0.05 | 0.9998 |
| 8-OHdG    | 6   | 4   | 6   | 2   | 3   | 3   | 103 | 110 | 107 | 0.023 | 0.08 | 0.9998 |
| 8-HG      | 8   | 9   | 7   | 6   | 8   | 5   | 104 | 106 | 95  | 0.03  | 0.11 | 0.9994 |
| 8-HdG     | 3   | 9   | 1   | 4   | 10  | 10  | 100 | 100 | 90  | 0.04  | 0.12 | 0.9994 |
| diY       | 8   | 10  | 9   | 6   | 8   | 11  | 114 | 114 | 102 | 0.06  | 0.20 | 0.9992 |
| 8-NG      | 11  | 7   | 6   | 9   | 14  | 5   | 88  | 95  | 88  | 0.05  | 0.18 | 0.9998 |
| 8-NdG     | 9   | 12  | 8   | 3   | 5   | 14  | 92  | 109 | 107 | 0.03  | 0.09 | 0.9994 |
| NY        | 6   | 4   | 4   | 12  | 11  | 4   | 98  | 91  | 82  | 0.05  | 0.15 | 0.9998 |
| BY        | 10  | 7   | 7   | 12  | 9   | 11  | 89  | 109 | 99  | 0.06  | 0.19 | 0.9998 |
| CY        | 8   | 10  | 6   | 7   | 10  | 8   | 94  | 106 | 110 | 0.09  | 0.30 | 0.9992 |
| CML       | 3   | 7   | 3   | 7   | 7   | 9   | 97  | 94  | 91  | 0.13  | 0.43 | 0.9998 |
| CEL       | 3   | 8   | 4   | 14  | 14  | 12  | 93  | 91  | 90  | 0.18  | 0.59 | 0.9996 |
Determination of AGEs in urine

CML and CEL were analyzed using a D&S method. Urine samples were diluted at 1:100 with HPLC-grade water; 10 μL of an internal standard, CML-d4 (1000 ng mL⁻¹), was added, and the samples were injected on the HPLC–MS/MS instrument. The MS parameters were optimized as described above. A Luna C18 (3 µm, 4.6 × 100 mm; Phenomenex, Torrance, CA, USA) column connected to a Betasil C18 guard column (5 µm; 4.6 × 10 mm) was used for chromatographic separation. The mobile-phase gradient flow consisted of 100% A (0.1% acetic acid in water) held for 4 min, increased to 100% B (0.1% acetic acid in methanol) over 1 min and held for 2 min (for column cleaning), and then returned to the initial 100% A over 1 min and held for 4 min (for column stabilization). The mobile-phase flow was set at 350 µL min⁻¹. Total analysis time was 12 min, and the MS data were acquired from 2 to 6 min after the start of flow.

Instrument parameters and select chromatograms of target analytes are shown in Table 2 and Fig. 2, respectively. Method validation for CML and CEL was performed by fortifying synthetic and real urine samples with standard analytes at three different concentrations and classifying their concentrations as low, 1 ng mL⁻¹; medium, 10 ng mL⁻¹; or high, 50 ng mL⁻¹. The results for precision, sensitivity, and accuracy of the method are presented in Table 3.

Urine samples

For method development and validation, a pool of real urine from 10 volunteers (50% males and 50% females) was used. Urine samples collected from healthy volunteers in Albany, NY, USA, in 2018 (n = 20, 10 males and 10 females; ages 11–56 years) were analyzed according to the method developed, to demonstrate its feasibility. Urine samples were kept at −80 °C until analysis. Approval of the Institutional Review Board of New York State Department of Health was obtained for the analysis of targeted biomarkers in urine.

Results and discussion

Methods to analyze effect biomarkers in human specimens have thus far been focused on assessing a single class of chemicals, with similar physicochemical properties, at a time. Recently, however, we reported the simultaneous analysis of seven urinary biomarkers of the oxidation of lipids, proteins, DNA, and uric acid and highlighted the feasibility and utility of monitoring multiple biomarkers of health effects so as to elucidate adverse outcome pathways[25]. In the current study, we augmented that method to include biomarkers of nitrative stress, eosinophilia, neutrophilia, and metabolic disorders to help describe interactions between these effect biomarkers in disease pathogenesis.

From analytical point of view, and in regard to structural similarities of the target compounds, the 19 biomarkers can be grouped as (i) modified tyrosines: diY, CY, BY, and NY; (ii) DNA/RNA lesion products: 8-OHdG, 8-HG, 8-HdG, 8-NG, and 8-NdG; (iii) aldehyde derivatives: MDA, HNE-GHS, and HNE-MA; (iv) prostaglandin isomers: 8-PGF₂α, 11-PGF₂α, 15-PGF₂α, and 8,15-PGF₂α; and (v) highly polar AGE biomarkers: CML, CEL, and Alla.

Method performance

The sensitivity, accuracy, and precision of the method were determined through the analysis of synthetic and pooled urine samples fortified with target chemicals at three different concentrations (see the "Materials and methods" section for details). An optimal precision and accuracy were demonstrated for all biomarkers (Table 3). The intra-day RSD of the method was <13% and the inter-day RSD was <15% for all of the target analytes. Recoveries of target analytes spiked into urine matrices ranged from 91% for 8,15-PGF₂α, NY, and CEL to 116% for 11-PGF₂α. The sensitivity of the method, expressed as mLOD and mLOQ, is acceptable based on the expected concentrations of the target analytes in urine. The mLODs ranged from 0.1 to 1 ng mL⁻¹ for low concentrations, 1 to 10 ng mL⁻¹ for medium concentrations, and 10 to 100 ng mL⁻¹ for high concentrations.
from 0.013 ng mL\(^{-1}\) for PGF\(_{2\alpha}\) isomers to 0.36 ng mL\(^{-1}\) for HNE-MA (Table 3).

No significant matrix effects were observed when slopes of calibration graphs were constructed using synthetic urine and real urine samples (\(\alpha = 0.05\)) matched against the standards prepared in solvent.

**Application of the method**

We applied the method to analyze urine samples collected from 20 healthy volunteers (10 male, 10 female) from the Albany area of New York State. Of the 19 biomarkers assayed, we detected 8 in 100% of the samples: MDA, HNE-MA, 8-OHdG, 8-HdG, Alla, diY, CML, and CEL. 8-HG (95%), 8-PGF\(_{2\alpha}\) (90%), and 11-PGF\(_{2\alpha}\) and
8,15-PGF2α (80%) were frequently detected. Other OSB biomarkers were found in <75% of samples. The modified tyrosine NY was found in 90% of samples, whereas 8-NG and 8-NdG were found in 55% and 45% of samples, respectively. Inflammation biomarkers (CY and BY) were found in 90–95% of samples. The metabolic disorder biomarkers CML and CEL were found in all urine samples (Fig. 3).

The concentrations of Alla were significantly higher [22] (6300 μmol mol Cr⁻¹) than those of other OSBs. MDA and HNE-MA showed median concentrations of 11.9 and 5.5 μmol mol Cr⁻¹, respectively. Whereas HNE-GHS was found only at trace levels (median below LOQ), its metabolite HNE-MA occurred at concentrations of up to 128 ng mL⁻¹. The two biomarkers of nucleic acid oxidation, 8-NG and 8-NdG, were found at similar median concentrations (0.35 μmol mol Cr⁻¹), while NY was found at a median concentration of 0.14 μmol mol Cr⁻¹. BY and CY levels were similar (0.27 and 0.20 μmol mol Cr⁻¹), whereas the concentrations of AGEs ranged from 2.33 μmol mol Cr⁻¹ for CEL to 4.35 μmol mol Cr⁻¹ for CML (Table 4).

The concentrations and profiles of individual health-effect biomarkers may be used to elucidate the health status of individuals (Fig. 3). Notably, the distribution pattern of the 19 biomarkers we tested was quite varied among 20 healthy individuals. Further studies should focus on establishing baseline levels and profiles for these biomarkers in a healthy population and assessing what factors affect their concentrations in urine.

**Significance of effect biomarkers in disease conditions**

**Biomarkers of protein damage: diY, NY, CY, and BY**

Oxidative stress, nitrative stress, neutrophilia, and eosinophilia can all damage proteins and generate modified tyrosines, namely diY, NY, CY, and BY, respectively. These four compounds have been measured individually using immunoassays and gas chromatography (GC)/LC–MS methods [17, 28, 32]. For example, all four have been previously assessed in diabetic patients [17] and asthmatic individuals [28], and NY, CY, and BY have been measured in urine from pregnant woman to elucidate their association with exposure to estrogenic chemicals [13]. Urinary NY concentrations are associated with BPA exposure [16] (Table 5).

**DNA and RNA lesion products**

Oxidized species of the nucleoside guanosine include 8-OHdG, 8-HG, and 8-NG, which are biomarkers of DNA damage, as well as 8-HdG and 8-NdG, biomarkers of RNA damage. 8-OHdG is the OSB commonly measured in environmental health investigations. Studies have linked exposure to phthalates [14, 33–35], BPA [15, 16], organophosphate esters (OPEs) [36], or pesticides [37, 38] with elevated urinary 8-OHdG. Simultaneous determination of 8-OHdG and 8-NG has been used to assess link between oxidative and/or nitrative damage and exposures to per- and polyfluoroalkyl substances [12], phthalates [14], or BPA...
Oxidized serum guanine (e.g., 8-OHdG, 8-HG, 8-HdG) has been associated with COVID-19 mortality [39], and elevated serum levels of 8-HG, 8-HdG, or 8-NdG have been linked to mortality associated with traumatic brain injury [18].

Aldehyde derivatives: MDA, HNE-GHS, and HNE-MA

The oxidation of lipids results in the formation of an array of biomolecules that are excreted in urine. Oxidation of polyunsaturated acids (PUFA) generates aldehydes, such as MDA and 4-hydroxynonenal (4-HNE). Because aldehydes are...
highly reactive, their direct determination in urine is challenging. The most commonly used method to detect MDA is the thiobarbituric acid (TBA) assay, but this is known to lack selectivity. Accurate and selective determination of urinary MDA has been successfully accomplished through its derivatization with DNPH followed by HPLC–MS/MS analysis of the stable product, MDA-DNPH. Urinary MDA concentrations have been associated with phthalate exposures and with thyroid dysfunction [14] and diabetes [40]. Similarly, MDA levels have been positively correlated with exposure to brominated diphenyl ethers [41] or organophosphate pesticides and neonicotinoid insecticides [37, 38].

4-HNE forms an adduct with the antioxidant glutathione, producing 4-HNE-GHS [19, 42], which in turn is metabolized and excreted as 4-HNE-MA conjugate in urine. Studies have associated 4-HNE-MA with thyroid dysfunction and phthalate exposure [33] and with adverse fetal outcomes and bisphenol exposure [15, 43]. No studies have reported the simultaneous analysis of both HNE derivatives, along with MDA, and this study is the first to report the concurrent measurement of these biomarkers.

**Prostaglandin isomers: 8-PGF2α, 11-PGF2α, 15-PGF2α, and 8,15-PGF2α**

The oxidation of arachidonic acid by ROS yields F2-isoprostanes, which comprise 64 different isomers. The reported method focused on the determination of four bioactive forms of F2-isoprostanes: 8-PGF2α, 11-PGF2α, 15-PGF2α, and 8,15-PGF2α. 8-PGF2α is the prostaglandin most frequently analyzed in studies relating thyroid dysfunction [33], birth outcomes [44], and inflammation [35]. The four isomers were simultaneously determined in a case-control study linking diabetes and phthalate exposure, showing that 8-PGF2α was positively correlated with urinary phthalate metabolites and high odd ratios for diabetes and two prostaglandin isomers, 8-PGF2α and 15-PGF2α [34].

**Highly polar AGE biomarkers: CML, CEL, and Alla**

AGEs such as CML, CEL, and Alla are highly polar molecules (with XLogP3 values −2.2, −5.2, and −5.3, respectively) and are typically present at measurable concentrations in urine. Therefore, the identification and quantification of AGEs and Alla could be accomplished by a simple D&S method with optimized LC–MS/MS parameters. Notably, Alla is produced by the reaction of uric acid, an important antioxidant, with ROS. It has been reported that Alla concentrations do not correlate with those of uric acid, but are positively correlated with the concentrations of other OSBs [22]. Nevertheless, very few studies have reported urinary concentrations of Alla in relation to adverse health effects.

AGEs are linked to metabolic disorders and oxidative stress [20, 45, 46] and are generated under high glucose concentrations (hyperglycemia) via a nonenzymatic glycation pathway (Maillard reaction). During glycation, glucose binds with proteins, making cells stiffer, less pliable, and subject to premature aging. Glycation can be accelerated by heat or oxidative stress. AGEs such as CML have been used as biomarkers of chronic diseases, such as diabetes, atherosclerosis, and renal failure. Although CML can be present in diet, those levels are negligible compared with the magnitude of endogenous formation [47]. CML is formed when glyoxal (formed from oxidation of lipids and sugars) reacts with lysine. Similarly, methylglyoxal produces CEL, which can interact with AGE receptors (RAGEs) and disrupt cellular signaling [46]. Significant relationships between AGEs and diabetes, aging, cardiovascular and neurological diseases, and cancer have been documented in the literature [45, 47]. To the best of our knowledge, however, little is known about any association between exposure to organic contaminants and AGE formation.

**Conclusions**

Here, we describe a method for the simultaneous analysis of 16 biomarkers of oxidative stress, nitrative stress, and inflammation; in addition, two metabolic disorder biomarkers and alla were measured in urine for a total of 19 effect biomarkers analyzed. The capability to analyze multiple biomarkers of health effects simultaneously that we demonstrated here could help elucidate the biological pathways underlying various types of pathogenesis. Also noteworthy is that when we used this method to analyze urine from 20 healthy individuals, we detected all 19 of these biomarkers of concern in some or all of those individuals.

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**Declarations**

**Conflict of interest** The authors declare no competing interests.

**References**

1. Zeliger HI (2016) Causes, mechanisms and prevention of environmental diseases. Dual Diagnosis Open Access 01:1–18. https://doi.org/10.21767/2472-5048.100001
2. Flies EJ, Mavoa S, Zosky GR, Mantzioris E, Williams C, Eri R, Brook BW, Buettel JC. Urban-associated diseases: candidate
diseases, environmental risk factors, and a path forward. Environ Int. 2019;133: 105187. https://doi.org/10.1016/j.envint.2019.105187.

3. Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. Environmental pollutants and child health—a review of recent concerns. Int J Hyg Environ Health. 2016;219:331–42. https://doi.org/10.1016/j.ijheh.2016.05.001.

4. Liu Y, Wang J, Huang Z, Liang J, Xia Q, Xia Q, Liu X. Environmental pollutants exposure: a potential contributor for aging and age-related diseases. Environ Toxicol Pharmacol. 2021;83:103575. https://doi.org/10.1016/j.etap.2020.103575.

5. Kelly FJ, Fussell JC (2019) Metabolomics as a tool to unravel the oxidative stress-induced toxicity of ambient air pollutants. In: Oxidative Stress: Eustress and Distress. Elsevier, pp 463–488. https://doi.org/10.1016/B978-0-12-818606-0.00024-9.

6. Hu X, He L, Zhang J, Qiu X, Zhang Y, Mo J, Day DB, Xiang J, Gong J. Inflammatory and oxidative stress responses of healthy adults to changes in personal air pollutant exposure. Environ Pollut. 2020;263:114503. https://doi.org/10.1016/j.envpol.2020.114503.

7. Jana K, C. P, Environmental toxicants induced male reproductive disorders: identification and mechanism of action. Toxic Drug Test. 2012. https://doi.org/10.5772/92969.

8. Roberts RA, Laskin DL, Smith CV, Robertson FM, Allen EMG, Doorn JA, Slikker W. Nitrative and oxidative stress in toxicology and disease. Toxicol Sci. 2009;112:4–16. https://doi.org/10.1093/toxsci/kfp179.

9. Gasparovic AC, Zarkovic N, Bottari SP. Biomarkers of nitro-oxidation and oxidative stress. Curr Opin Toxicol. 2018;7:73–80. https://doi.org/10.1016/j.coxtox.2017.10.002.

10. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, Knight AR, Taylor EL, Oettrich J, Ruskovska T, Gasparovic AC, Cuadrado A, Weber D, Poulsen HE, Grune T, Schmidt HHW, Ghezzi P. Clinical relevance of biomarkers of oxidative stress. Antioxidants Redox Signal. 2015;23:1144–70. https://doi.org/10.1089/ars.2015.6317.

11. Aggarwal V, Tuli HS, Varol A, Thakral F, Yerer MB, Sak K, Varol M, Jain A, Khan MA, Sethi G (2019) Role of reactive oxygen species in cancer progression: molecular mechanisms and recent advancements. Biomolecules 9: https://doi.org/10.3390/biom91110735.

12. Lin CY, Lee HL, Hwang YT, Su TC. The association between total serum isomers of per- and polyfluoroalkyl substances, lipid profiles, and the DNA oxidative/nitrative stress biomarkers in middle-aged Taiwanese adults. Environ Res. 2020;182: 109064. https://doi.org/10.1016/j.envres.2019.109064.

13. Putthayatappa M, Banker M, Zeng L, Goodrich JM, Domino SE, Dolinoy DC, Meeher JD, Pennathur S, Song PXX, Padmanabhan V. Maternal exposure to environmental disruptors and sexually dimorphic changes in maternal and neonatal oxidative stress. J Clin Endocrin Mol Metab. 2020;105:492–505. https://doi.org/10.1210/clinem/dg2063.

14. Huang PC, Waits A, Chen HC, Chang WT, Jaakkola JJK, Bin HH. Mediating role of oxidative/nitrosative stress biomarkers in the associations between phthalate exposure and thyroid function in Taiwanese adults. Environ Int. 2020;140: 105751. https://doi.org/10.1016/j.envint.2020.105751.

15. Huang YF, Wang PW, Huang LW, Lin MH, Yang W, Chen HC, Yu KP, Chen ML. Interactive effects of nonylphenol and bisphenol A exposure with oxidative stress on fetal reproductive indices. Environ Res. 2018;167:567–74. https://doi.org/10.1016/j.envres.2018.08.007.

16. Veiga-Lopez A, Pennathur S, Kannan K, Patitsalou HB, Dolinoy DC, Zeng L, Padmanabhan V. Impact of gestational bisphenol A on oxidative stress and free fatty acids: human association and interspecies animal testing studies. Endocrinology. 2015;156:911–22. https://doi.org/10.1210/en.2014-1863.

17. Kato Y, Dozaki N, Nakamura T, Kitamoto N, Yoshida A, Naito M, Kitamura M, Osawa T. Quantification of modified tyrosines in healthy and diabetic human urine using liquid chromatography/tandem mass spectrometry. J Clin Biochem Nutr. 2009;44:67–78. https://doi.org/10.3146/jcnn.08-08-15.

18. Lorente L, Martin MM, González-Rivero AF, Pérez-Cejas A, Abreu-González P, Ramos L, Argueso M, Cáceres JJ, Solé-Violán J, Alvarez-Castillo A, Jiménez A, García-Marín V. Association between DNA and RNA oxidative damage and mortality of patients with traumatic brain injury. Neurocrit Care. 2020;32:790–5. https://doi.org/10.1007/s12028-019-00800-w.

19. Alary J, Guéraud F, Cravedi JP. Fate of 4-hydroxynonenal in vivo: disposition and metabolic pathways. Mol Aspects Med. 2003;24:177–87. https://doi.org/10.1016/S0098-2997(03)00012-8.

20. Il D, Scarbrough P, Spasojevic I. Urinary biomarkers of oxidative stress. Clin Chim Acta. 2012;413:1446–53. https://doi.org/10.1016/j.cca.2012.06.012.

21. Aszyk J, Kot J, Tkachenko Y, Woźniak M, Bogucka-Kocka A, Kot-Wasilik A. Novel liquid chromatography method based on linear weighted regression for the fast determination of isoprostane isomers in plasma samples using sensitive tandem mass spectrometry detection. J Chromatogr B Anal Technol Biomed Life Sci. 2017;1051:17–23. https://doi.org/10.1016/j.jchromb.2017.02.021.

22. Martínez-Moral M-P, Kannan K (2019) Alantoin as a marker of oxidative stress: inter- and intraindividual variability in urinary concentrations in healthy individuals. Environ Sci Technol Lett 6: https://doi.org/10.1021/acs.estlett.9b00142.

23. Isa KNM, Hashim Z, Jalaludin J, Than LTL, Hashim JH. The effects of indoor pollutants exposure on allergy and lung inflammation: an activation state of neutrophils and eosinophils in spumum. Int J Environ Res Public Health. 2020;17:1–18. https://doi.org/10.3390/ijerph17155413.

24. Vetranov AM, Laskin DL, Archer F, Syed K, Gray JP, Laskin JD, Nwebebe N, Weinberger B. Inflammatory effects of phthalates in neonatal neutrophils. Pediatri Res. 2010;68:134–9. https://doi.org/10.1203/PDR.0b013e3181e5c177.

25. Loffredo LF, Berdnikovs MECS (2020) Endocrine disruptor bisphenol a (BPA) triggers systemic para-inflammation and is sufficient to induce airway allergic sensitization in mice. Nutrients 12: https://doi.org/10.3390/nu12020343.

26. Kovalszki A, Weller PF, Israel B. Eosinophilia. Prim Care. 2016;43:607–17. https://doi.org/10.1016/j.pop.2016.07.010. Eosinophilia.

27. Buss IH, Sentihmohan R, Darlow BA, Mogridge N, Kettle AJ, Winterbourn CC. 3-Chlorotyrosine as a marker of protein damage by myeloperoxidase in tracheal aspirates from preterm infants: association with adverse respiratory outcome. Pediatr Res. 2003;53:455–62. https://doi.org/10.1203/01.PDR.0000050655.25689.CE.

28. Mita H, Higashi N, Taniguchi M, Higashi A, Kwagishii Y, Akiyama K. Urinary 3-bromotyrosine and 3-chlorotyrosine concentration in urine and plasma proteins in aspirin-induced asthma after intravenous aspirin challenge. Clin Exp Allergy. 2004;34:931–8. https://doi.org/10.1111/j.1365-2222.2004.01968.x.

29. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev. 2009;30:293–342. https://doi.org/10.1210/er.2009-0002.
Environ Sci Technol 2018;52:6647–55. https://doi.org/10.1021/acs.est.8b00883

Gaut JP, Byun J, Tran HD, Heinecke JW. Artifact-free quantification of free 3-chlorotyrosine, 3-bromotyrosine, and 3-nitrotyrosine in human plasma by electron capture – negative chemical ionization gas chromatography mass spectrometry and liquid chromatography – electrospray ionization tandem mass spectrometry. Anal Biochem. 2002;259:252–9. https://doi.org/10.1006/abio.2001.5469

Zhang M, Deng YL, Liu C, Chen PP, Luo Q, Miao Y, Cui FP, Wang LQ, Jiang M, Zeng Q. Urinary phthalate metabolite concentrations, oxidative stress and thyroid function biomarkers among patients with thyroid nodules. Environ Pollut. 2021;272: 116416. https://doi.org/10.1016/j.envpol.2021.116416.

Li AJ, Martínez-Moral M-P, Al-Malki AL, Al-Ghamdi MA, Al-Bazi MM, Kumosani TA, Kannan K (2019) Mediation analysis for the relationship between urinary phthalate metabolites and type 2 diabetes via oxidative stress in a population in Jeddah, Saudi Arabia. Environ Int 122: https://doi.org/10.1016/j.envint.2019.01.082

Van'TErve TJ, Rosen EM, Barrett ES, Nguyen RHN, Sathyanarayana S, Milne GL, Calafat AM, Swan SH, Ferguson KK. Phthalates and phthalate alternatives have diverse associations with oxidative stress and thyroid function among adults. Environ Int. 2019;130: 104904. https://doi.org/10.1016/j.envint.2018.11.007

Li AJ, Martínez-Moral MP, Kannan K. Temporal variability in urinary pesticide concentrations in repeated-spot and first-morning-void samples and its association with oxidative stress in healthy individuals. Environ Int. 2019;130: 104904. https://doi.org/10.1016/j.envint.2019.104904.

Li AJ, Martínez-Moral MP, Kannan K. Variability in urinary neonicotinoid concentrations in single-spot and first-morning void and its association with oxidative stress markers. Environ Int. 2020;135: 105415. https://doi.org/10.1016/j.envint.2019.105415.

Lorenti L, Martín MM, González-Rivero AF, Pérez-Cejas A, Cáceres JJ, Perez A, Ramos-Gómez L, Solé-Violán J, Marcos y Ramos JA, Ojeda N, Jiménez A (2021) DNA and RNA oxidative damage and mortality of patients with COVID-19. Am J Med Sci 1–6. https://doi.org/10.1016/j.amjms.2021.02.012

Li AJ, Martínez-Moral MP, Al-Malki AL, Al-Ghamdi MA, Al-Bazi MM, Kumosani TA, Kannan K. Mediation analysis for the relationship between urinary phthalate metabolites and type 2 diabetes via oxidative stress in a population in Jeddah, Saudi Arabia. Environ Int. 2019;126:153–61. https://doi.org/10.1016/j.envint.2019.01.082.

Zhang H, Yang X, Li X, Cheng Y, Zhang H, Chang L, Sun M, Zhang Z, Wang Z, Niu Q, Wang T. Oxidative and nitrosative stress in the neurotoxicity of polybrominated diphenyl ether-153: possible mechanism and potential targeted intervention. Chemosphere. 2020;238: 124602. https://doi.org/10.1016/j.chemosphere.2019.124602.

Völk W, Alvarez-Sánchez R, Weick I, Mally A, Dekant W, Pähler A. Glutathione conjugates of 4-hydroxy-2(E)-nonenal as biomarkers of hepatic oxidative stress-induced lipid peroxidation in rats. Free Radic Biol Med. 2005;38:1526–36. https://doi.org/10.1016/j.freeradbiomed.2005.02.015.

Wang YX, Liu C, Shen Y, Wang Q, Pan A, Yang P, Chen YJ, Deng YL, Lu Q, Cheng LM, Miao XP, Xu SQ, Lu WQ, Zeng Q. Urinary levels of bisphenol A, F and S and markers of oxidative stress among healthy adult men: variability and association analysis. Environ Int. 2019;123:301–9. https://doi.org/10.1016/j.envint.2018.11.071.

Ferguson KK, Cantonwine DE, Rivera-González LO, Loch-Caruso R, Mukherjee B, Anzalota Del Toro LV, Jiménez-Vélez B, Calafat AM, Ye X, Alshawabkeh AN, Cordero JP, Meeker JD. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. Environ Sci Technol. 2014;48:7018–25. https://doi.org/10.1021/es502076j.

Ighodaro OM. Biomedicine & Pharmacotherapy Molecular pathways associated with oxidative stress in diabetes mellitus. Biomed Pharmacother. 2018;108:656–62. https://doi.org/10.1016/j.biopha.2018.09.058.

Vistoli G, Maddis D De, Cipak A, Zarkovic N, Carini M, Aldini G, Maddis D De, Cipak A, Zarkovic N, Carini M, Aldini G. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): an overview of their mechanisms of formation. Free Rad Res. 2013;47:3–27. https://doi.org/10.3109/10701576.2013.815348

End-products G, Zeng C, Li Y, Ma J, Niu L, Tay FR. Clinical / translational aspects of advanced. Trends Endocrinol Metab. 2019;30:959–73. https://doi.org/10.1016/j.tem.2019.08.005.

AitBamai Y, Bastiaensen M, Araki A, Goudarzi H, Konno S, Ito S, Miyashita C, Yao Y, Covacci A, Kishi R. Multiple exposures to organophosphate flame retardants alter urinary oxidative stress biomarkers among children: the Hokkaido Study. Environ Int. 2019;131: 105003. https://doi.org/10.1016/j.envint.2019.105003.

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