Note: This document provides an interpretive synthesis of the research article *"Exposure scenario: Another important factor determining the toxic effects of PM2.5 and possible mechanisms involved"* (Zhou et al., *Environmental Pollution*, 2017). No new experimental data are presented.The focus of this interpretation is on mechanistic comparison between acute high-dose and repeated low-dose PM2.5 exposure scenarios.

1. Purpose and Hypothesis

- Air pollution by ambient particulate matter has become one of the most serious environmental and public health challenges in many developing and developed countries. In particular, increasing airborne fine particulate matter with an aerodynamic diameter less than 2.5 um (PM2.5) has a profound impact on public health.
- In places like Chennai or Kolkata, the mean concentration of PM2.5 is around 90 μg/m³. People living in these long-polluted areas are more likely to be in a long-term, slightly polluted PM2.5 exposure scenario (repeated exposure).
- In addition, acute haze episodes or smog events with high concentrations of PM2.5 (more than 500 μg/m³ , sometimes over 1000 μg/m³) now occur frequently in some areas.
- Therefore, the population may be subjected to two distinct PM2.5 exposure scenarios, repeated low-level and short-term high-level exposure, with different health effects.
- Exposure to PM2.5, including both acute exposure at extremely high levels and repeated exposure at lower levels, is strongly associated with many pulmonary diseases.
- Several studies have demonstrated that long-term low-level exposure to particles or nanoparticles elicits dissimilar biological responses compared to shortterm high-level exposure
- All these findings sμggest that in addition to particle size and chemical composition, exposure scenario may be an important factor determining the toxic effects of PM2.5.
- The hypothesis addressed in the study is that even brief exposure to higher concentrations of PM2.5 induces extensive oxidative damage causing injury and metabolic collapse in a short time, while repeated exposure to low PM2.5 levels causes chronic and low-grade enhancement of reactive oxygen species(ROS-chemically reactive molecules that induce oxidative damage to cellular

macromolecules) which triggers cycles of damages and repairs mediated by adaptive responses.

- The study compares the responses of the human bronchial epithelial cell line BEAS-2B to two distinct PM2.5 exposure scenarios to examine whether exposure is an important parameter determining the health effects of PM2.5.
- To address this, the study examines how distinct PM2.5 exposure scenarios differentially shape oxidative stress responses, cellular damage, and long-term adaptive mechanisms at the cellular level.

2. Exposure Models & Dose Logic

- In the acute exposure model, cells were treated with PM2.5 suspension at 0, 6, 12, 24, 48, or 96 μg/cm² for 24 h, while in the repeated exposure model cells were treated daily with PM2.5 suspension at 0, 1.5, 3, or 6 μg/cm² for consecutive 10 days, with passage every 3 or 4 days.
- These values were designed using multiple-path particle dosimetry (MPPD) software. The study needs deposition of particulate per unit surface area, but typically, the values measured are particulate present in unit volume of air. The MPPD software convert PM2.5 per unit volume area to PM2.5 deposition per unit area.

PM2.5 concentration(μg/m³)	Contact Concentration(μg/cm²) Resting	Contact Concentration(μg/cm²) Heavy Exercise
30	1.47 * 10^-3	4.367 * 10^-3
100	4.879 * 10^-3	1.462 * 10^-2
200	9.759 * 10^-3	2.924 * 10^-2
500	2.44 * 10^-2	7.29 * 10^-2

1000	4.90 * 10^-2	1.46 * 10^-1

- Based on these data as well as real-world exposure scenarios and considering risk factors, they performed acute exposure experiments on cultured epithelial cells using suspension concentrations about 1000-2000-fold greater than contact concentrations (6-96 μg/cm² compared to 4.88 10^-3 ~ 4.90 10^-2 μg/cm²) for 24 h, and repeated exposure experiments at 0-6 μg/cm² for 10 days.

3. Ultrastructural Changes

- Acute Exposure
  - Mitochondria swelling, cristae disruption, vacuolization, and ER dilation (ER becomes swollen and expanded) in all acute exposure groups except for the lowest dose group (6 μg/cm²)
  - Additionally, two types of autophagosomes, initial autophagic vacuoles (AVi)(early, double membrane vescicles capturing cytoplasmic cargo, lacking lysosomal enzymes and acidity) and late/degradative autophagic vacuoles (AVd)(formed after AVi fusion with lysosomes containing hydrolases), were observed in the three highest dose groups (24, 48, and 96 μg/cm²)
  - TEM (Transmission Electron Microscope) images of the 12 μg/cm² group provided clear evidence of phagophore formation, which would finally expand to form autophagosomes.

- Repeated Exposure
  - Severe ultrastructural changes in cell organelles are not observed after repeated exposure to PM2.5 for 10 days.
  - Occasional disruption of mitochondrial cristae in the 1.5 and 6 μg/cm² groups.
  - AVds were observed in all repeated exposure groups.
  - Some lamellar bodies (LB) appeared in the 3 and 6 μg/cm² groups.
  - Lysosomes were found in the two highest repeated dose groups, especially in the 6 μg/cm² group.

    - Endocytosis (taking up substances from outside) of PM2.5s was evident in both acute and repeated exposure groups, especially in higher-dose groups.

4. Oxidative Stress & DNA Damage

- Regardless of exposure conditions, the levels of intracellular ROS were increased by PM2.5 treatment.
- Acute Exposure
    - ROS levels were about 0.5 times, 1 time and 2.5 times higher than control in the 6 μg/cm² group, the 12-48 μg/cm² groups, and the 96 μg/cm² group, respectively.
- Repeated Exposure
    - Repeated exposure to PM2.5 did not induce such drastic increases but still caused 2%-58% increase in 1.5-6 μg/ cm2 groups.

- ROS accumulation can disrupt many cellular processes by nonspecifically attacking macromolecules, such as proteins, lipids, and DNA. Further, ROS-induced DNA damage is thoμght to be a seminal initiating event in the pathologenesis of many diseases, so the authors investigated the impact of PM2.5 exposure on DNA damage.
- ROS can oxidize DNA bases and cause base mismatches, strand breaks, mutations. The DNA damage can be seen with comet assay. The longer the tail of the comet, the more the DNA damage.
- They observed significant increases in %T (proportion of DNA in the tail) in all acute exposure groups except the 6 μg/cm² group after 24 h, but only slight increases in repeated exposure groups.

- Collins pioneered the Comet Assay technique. Collins developed a standardized method to categorize the extent of that damage by observing the shape of the "comet" under a microscope.

Class	Appearance	Damage Level	Description	Visual Score(for 100 comets)
Class 0	Tight Sphere	None / Negligible	No tail visible; all DNA remains in the nucleoid head.	0
Class 1	Small Blur	Low	A very short tail, barely longer than the head diameter.	100
Class 2	Clear Tail	Medium	The tail is distinct and roμghly equal in intensity to the head.	200
Class 3	Long Tail	High	The tail is longer and more intense than the head.	300
Class 4	Fan/Ghost	Severe	Almost all DNA is in the tail; the head is small or invisible.	400

- Acute Exposure
    - Significant increases in %T (proportion of DNA in the tail) in all acute exposure groups except the 6 μg/cm² group after 24 h.
    - The visual score for the 96 μg/cm² group reached 262, more than five times that for the 6 μg/cm² group after 10 days of exposure
    - Massive, immediate DNA damage
- Repeated Exposure
    - only slight increases in repeated exposure groups
    - Mild but persistent DNA damage

5. DNA Damage Response & Cell Fate Decisions

- Acute Exposure
    - dose-dependent increases in the protein levels of PARP-1(a DNA damage sensor involved in coordinating repair responses) and P21(a p53-dependent cell-cycle inhibitor that mediates checkpoint arrest), and the ratios of phospho-P53(activated P53) to P53(tumor suppressor that enforces damage-responsive cell-fate decisions) and H2A.X to γH2A.X(γH2A.X, a marker of DNA double-strand breaks
    - No effect in the level of cleaved Caspase-3(the executioner protease of apoptosis).
    - At the cellular level, no obvious changes in cell cycle distribution were observed.
    - Necrosis rate increased significantly in a dose-dependent manner in the exposure groups.
    - In acute high-dose PM2.5 exposure, ROS levels spike very fast, DNA damage is extensive and immediate, mitochondria and ER are damaged at the same time, ATP drops sharply, so even thoμgh P53 is activated, P21 is induced, DNA damage markers (γ-H2AX, PARP-1) increase, the damage comes faster than checkpoints can act.
    - So, there's no effective cell-cycle arrest, no time or energy for orderly apoptosis and the cell collapses into necrosis.

- Repeated Exposure
    - induced cell cycle arrest at S phase
    - increased the proportion of apoptotic cells
    - enhanced the level of cleaved Caspase-3 and cleaved PARP-1 as well as the ratio of γH2A.X to H2A.X in cells
    - the expression levels of P21 were actually decreased in a dose-dependent manner
    - In repeated low-dose PM2.5 exposure, ROS increases slowly and moderately; damage accumulates over days, not hours; ATP levels drop moderately; not catastrophicall; now checkpoints have time and energy to function.
    - So, the cell can decide to pause, repair and (if damage persists) die properly.

6. Autophagy & Energy Failure

- Acute Exposure
    - dramatically increased the proportion of necrotic cells, especially in the 96 μg/cm² group.

    - decreased the ratio of pmTOR(activated mTOR) to mTOR(central to regulating cell growth, metabolism, and survival)
    - elevated the expression of LC3-B(crucial protein in cellular autophagy), indicating autophagy initiation rather than completion
    - P62/ SQSTM1 binds to LC3-B and leads to a decrease in P62/SQSTM1 protein level during autophagy
    - increased accumulation of P62/ SQSTM1 protein, indicating inhibition of the autophagic flux
    - Autophagic flux blockade prevents clearance of damaged mitochondria and proteins
    - Impaired mitochondrial turnover contributes to severe ATP depletion
    - Energy failure under acute exposure shifts cell death toward autophagy-dependent necrosis rather than apoptosis
    - Acute PM2.5 exposure caused a dose-dependent collapse of cellular ATP levels, particularly at ≥24 μg/cm²

- Repeated Exposure
    - Repeated exposure did not obviously influence the expression of autophagic flux-related proteins (LC3-B, P62/SQSTM1).
    - Absence of P62 accumulation sμggests functional autophagic flux
    - Degradative autophagic vacuoles and lysosomes observed by TEM indicate ongoing organelle turnover
    - Preserved autophagic flux supports cell survival under chronic low-dose stress

- The ability to complete autophagic flux under repeated exposure preserves cellular energy balance, whereas flux blockade during acute exposure leads to ATP collapse and necrotic cell death.

7. Mitochondria & ER Stress / UPR

- Mitochondrial function and the unfolded protein response (UPR-cellular defense system in the endoplasmic reticulum that activates when misfolded proteins build up, signaling ER stress)are of particular importance for maintenance of cellular homeostasis under oxidative stress

- Morphological alterations in mitochondria and ER sμggested the possibility of mitochondrial dysfunction and increased UPR.

- Acute Exposure
    - increased the protein levels of NRF-1(transcription factor regulating genes for mitochondrial biogenesis), mtTFA(protein for mitochondrial DNA (mtDNA) transcription, replication, and maintenance), and PGC-1a (master regulatory protein crucial for cellular energy metabolism), indicating an attempted compensatory mitochondrial biogenesis response to oxidative damage
    - increase in transcriptional level of GRP 78(protein that helps fold proteins, manage UPRs) in a dose-dependent manner but did not have substantial effects on the transcription of ERS sensor genes except slight perturbations in several dose groups (slight increases in ATF-6(crucial protein sensor) and PERK(crucial stress sensor) transcription in the 12 μg/cm² group and decreases in IRE1a and PERK(sensor for ER stress) transcription in the 96 μg/cm² group)
    - This dissociation sμggests that ER stress exceeded the adaptive capacity of the unfolded protein response, resulting in non-functional UPR signaling
    - Despite increased mitochondrial biogenesis signaling, severe mitochondrial structural damage and ATP depletion indicate failure of functional recovery

- Mitochondrial dysfunction and ER stress responses are tightly coupled under oxidative stress, jointly determining whether cells attempt recovery or enter adaptive remodeling.

- Repeated Exposure
    - did not affect PGC-1a or mtTFA protein level and actually decreased NRF-1 protein expression. In addition, the transcriptional levels of GRP 78 and the three ERS sensor genes were all up-regulated after 10 days of PM2.5 treatment.
    - Suppression of mitochondrial biogenesis reflects long-term metabolic adaptation rather than acute compensation
    - Sustained activation of ER stress sensors indicates a functional and coordinated UPR

- Acute PM2.5 exposure triggers emergency mitochondrial and ER stress responses that fail to restore homeostasis, whereas repeated exposure

establishes a coordinated stress-adaptive state that supports long-term survival under chronic oxidative pressure.

8. Epigenetic Regulation (Long-term risk)

- Acute Exposure
    - Acute PM2.5 exposure increased DNMT1(enzyme that copies and maintains DNA methylation patterns during cell division) and SIRT1(enzyme that regulates gene expression) levels and decreased DNMT3B (crucial enzyme responsible for *de novo* DNA methylation) level in a dose dependent manner
    - This epigenetic profile is consistent with DNA repair–associated chromatin regulation rather than stable gene silencing
    - did not have any significant effect on DNMT3A protein level.
    - These changes reflect an immediate and reversible epigenetic response to oxidative DNA damage

- Repeated Exposure
    - Repeated exposure to PM2.5 gradually inhibited DNMT1 protein expression but enhanced DNMT3B protein level, while there were no obvious changes in SIRT1 protein expression
    - This shift favors de novo DNA methylation over maintenance methylation
    - Such reprogramming is associated with long-term transcriptional silencing rather than DNA repair
    - Stable epigenetic reprogramming under chronic PM2.5 exposure may contribute to persistent alteration of gene expression and increased carcinogenic risk
- Acute PM2.5 exposure activates epigenetic mechanisms linked to DNA repair and stress resolution, whereas repeated exposure promotes long-lasting epigenetic reprogramming with potential pathological consequences.

9. ATP & Inflammatory Signaling

- Acute Exposure
    - Acute exposure to PM2.5 induced about 11% and 32% reduction of cellular ATP levels, respectively, in the 6 and 12 μg/cm² groups
    - Dramatically decreased ATP levels in the 24, and 48, 96 μg/cm² groups.
    - Severe ATP depletion under acute high-dose exposure limits energy-dependent apoptotic pathways, favoring necrotic cell death

- Repeated Exposure
    - After repeated exposure to PM2.5 for 10 days, ATP levels in BEAS-2B cells were about 13%-24% less than control
    - Moderate but sustained ATP reduction reflects chronic metabolic stress rather than acute energetic collapse

- PM2.5 treatment induced dose-dependent increases in IL-6(a crucial cytokine) protein levels but did not influence the expression of IL-1b(pro-inflammatory cytokine) in both acute and repeated exposure models.
- IL-6 and IL-1β are inflammatory cytokines with distinct roles in acute and chronic inflammatory signaling
- Selective induction of IL-6 without IL-1β sμggests activation of stress-associated inflammatory signaling rather than inflammasome-driven acute inflammation
- The IL-6 level in the acute 96 μg/cm² group was 0.66 times higher than that in the highest repeated dose group.
- The markedly higher IL-6 level under acute exposure reflects necrosis-associated inflammatory release, whereas repeated exposure induces a lower, sustained inflammatory state
- Together, ATP depletion and inflammatory signaling patterns indicate that acute PM2.5 exposure provokes energy collapse–driven necrosis and strong inflammation, while repeated exposure sustains low-grade metabolic stress and chronic inflammatory signaling.

10. Integrated Mechanistic Model

This study demonstrates that the toxic effects of PM2.5 are strongly determined by the exposure scenario, rather than dose alone. Under acute high-dose exposure, PM2.5 induces a rapid burst of intracellular ROS, leading to simultaneous damage to

mitochondria, the endoplasmic reticulum, and DNA. Althoμgh cells initiate compensatory responses such as DNA repair signaling, mitochondrial biogenesis, and autophagy, the magnitude and speed of damage overwhelm these protective mechanisms. Autophagic flux becomes blocked, cellular ATP levels collapse, and cells predominantly undergo autophagy-dependent necrotic death, accompanied by strong inflammatory signaling.

In contrast, repeated low-dose exposure results in sustained low-grade ROS accumulation that produces milder but persistent cellular stress. In this scenario, DNA damage checkpoints, autophagy, and the unfolded protein response remain functionally active, allowing cells to manage damage throμgh regulated cell-cycle arrest, apoptosis of severely damaged cells, and long-term adaptation. However, this adaptive state is accompanied by suppressed mitochondrial biogenesis, chronic metabolic strain, and stable epigenetic reprogramming. Consequently, while repeated exposure does not cause immediate cytotoxic collapse, it promotes long-term alterations in cellular homeostasis that may increase the risk of chronic disease and carcinogenesis.

11. Conclusion

In summary, this study highlights exposure scenario as a critical determinant of PM2.5-induced cellular toxicity. Acute high-dose exposure overwhelms cellular defense mechanisms, leading to rapid organelle failure, energy collapse, necrotic cell death, and pronounced inflammatory signaling. In contrast, repeated low-dose exposure elicits a coordinated adaptive response that preserves short-term cell viability throμgh functional checkpoint control, autophagy, and stress-response pathways.

Importantly, this apparent adaptation is accompanied by persistent metabolic stress and stable epigenetic reprogramming, indicating that survival under chronic PM2.5 exposure comes at a biological cost. These long-term alterations may contribute to progressive tissue dysfunction and increased disease risk despite the absence of acute cytotoxicity. Together, these findings demonstrate that PM2.5 toxicity cannot be fully understood by dose alone, and that the temporal pattern of exposure plays a central role in shaping cellular fate and long-term pathological outcomes.