

Comparison of direct exposure of human lung cells to modern engine exhaust particles

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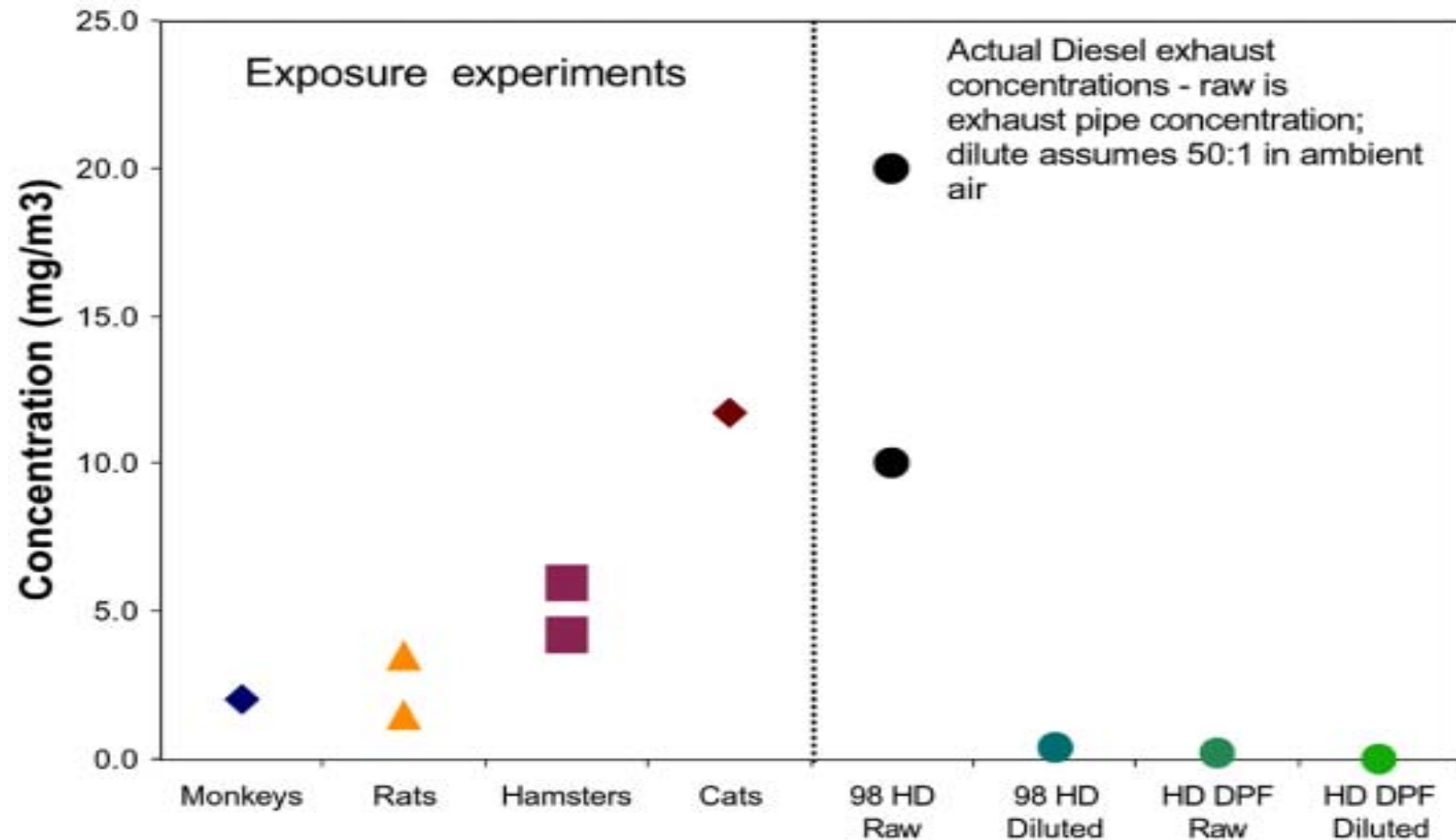
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We saw need to move beyond studies using unrealistic exposure levels and “old-tech soot”*...lung cell assay seemed well-suited

***relatively high SOF and larger size distribution**



Objectives

- **To continue experiments with cellular exposure/response to particles from vehicles *using new engine/exhaust emissions control***
- **To observe difference in the response of cells, normal or primed, to particles**

Materials and Methods

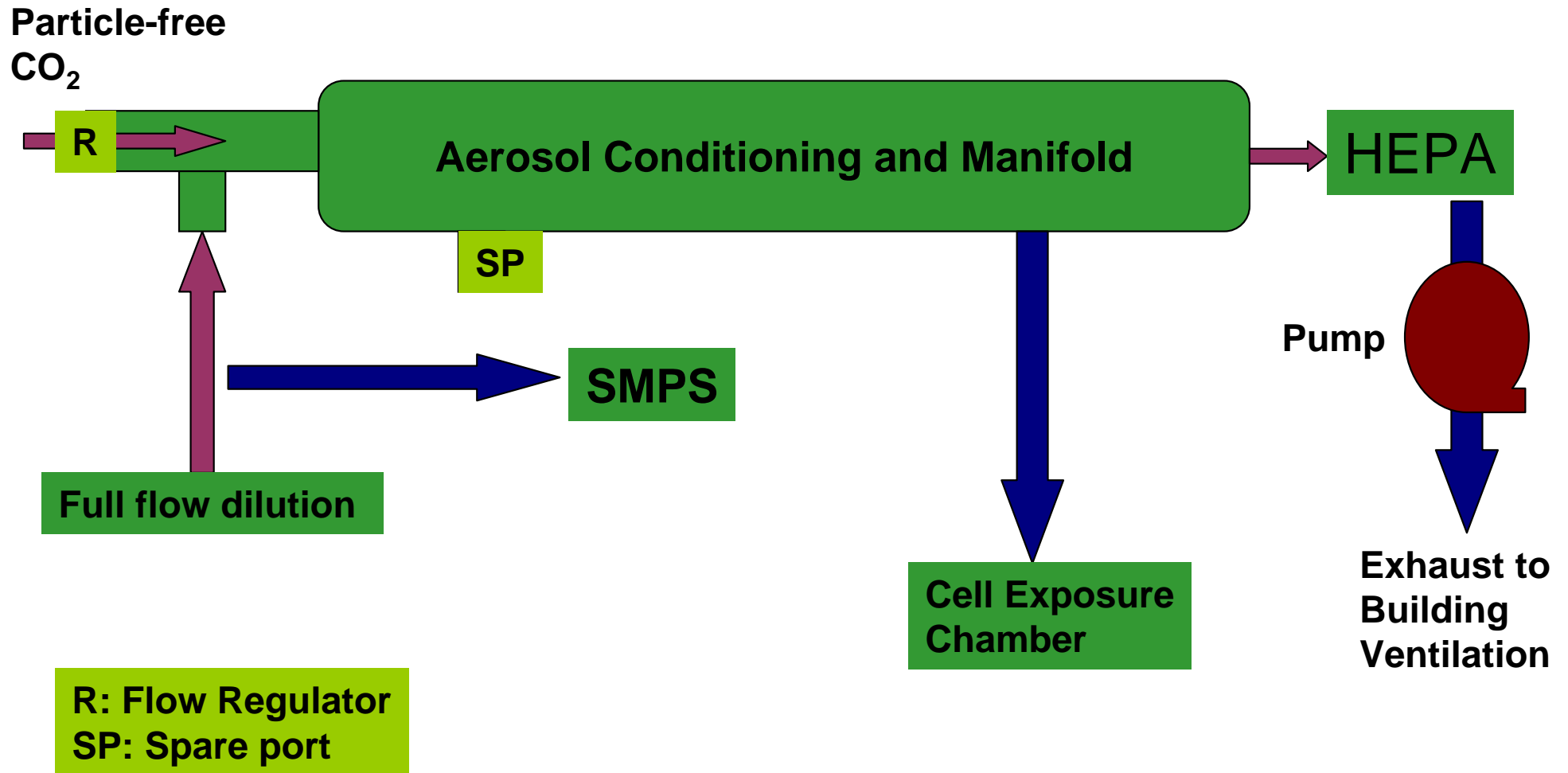
- **Cells**

- Human lung carcinoma cell line, A549, epithelial from ATCC
- Density - 80,000 cells per each transwell membrane
- Incubation period before exposure: 48 hours
- Primed cells: add 25 ng/cc Human Tumor Necrosis Factor (TNF)- α agent for 24 hours before replacing medium for exposure experiments
- Placed on top of a COSTAR transwell filter membrane with medium underneath in a well throughout exposure experiment
- Number of cells counted right before an exposure experiment

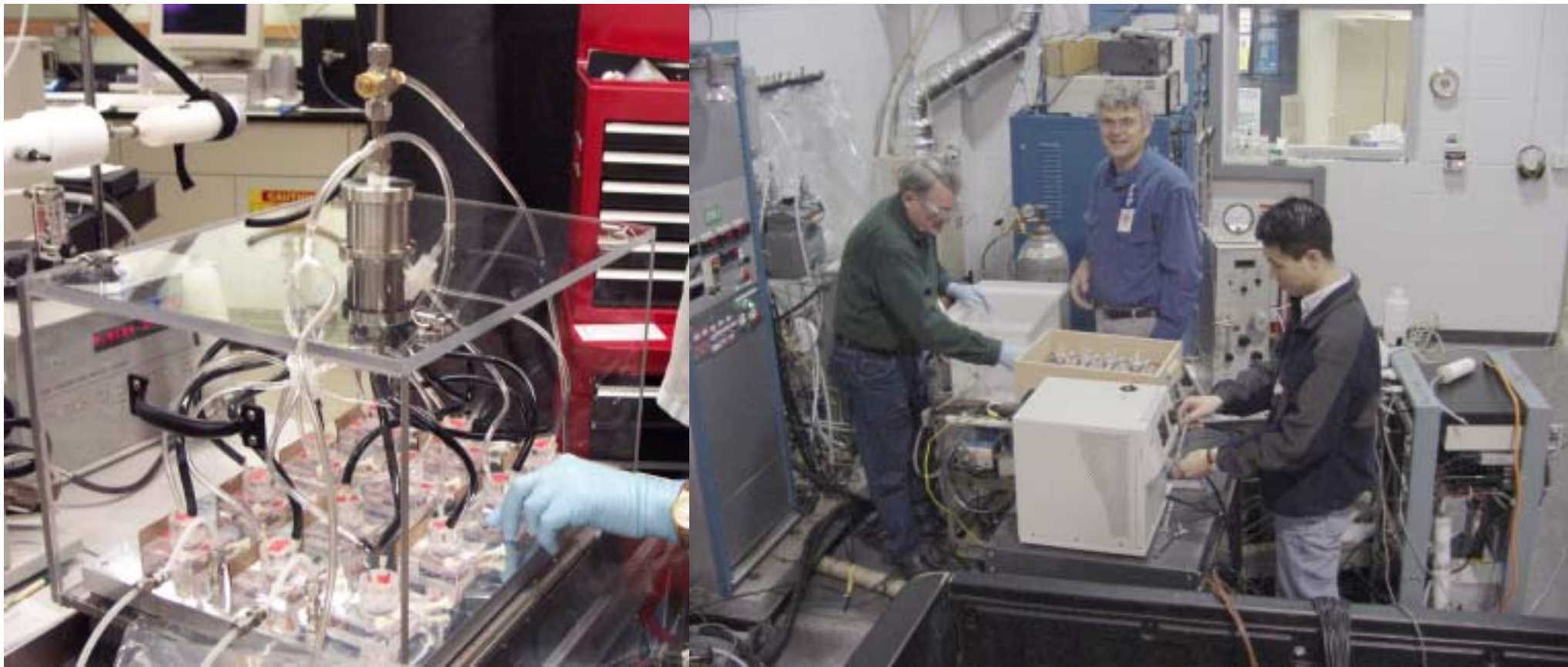
Materials and Methods (cont.)

- **Vehicle Generated Particles**
 - 1999 Chevrolet Sierra gasoline, three-way catalyst
 - 1999 Mercedes-Benz A170 CDI equipped with a CDPF, ECD-1 fuel
 - Cold LA4 + 6 Hot LA4/US06
- Measured by scanning mobility particle sizer
- **ELISA - IL 8**
- **Lactate dehydrogenase (LDH)**
- **Electrical resistance measured**

Block Diagram of Cell Exposure

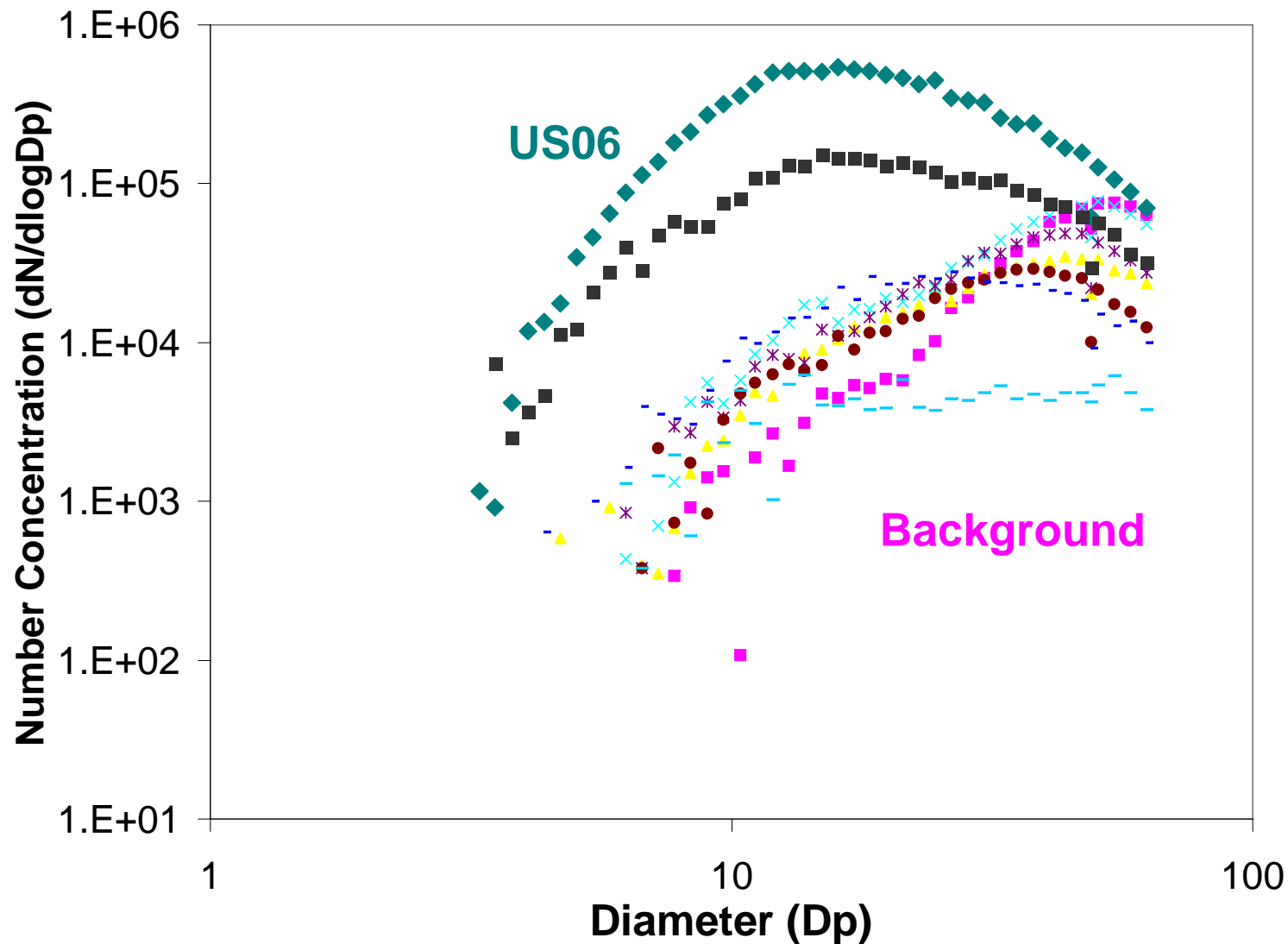


Apparatus can do 12 exposures

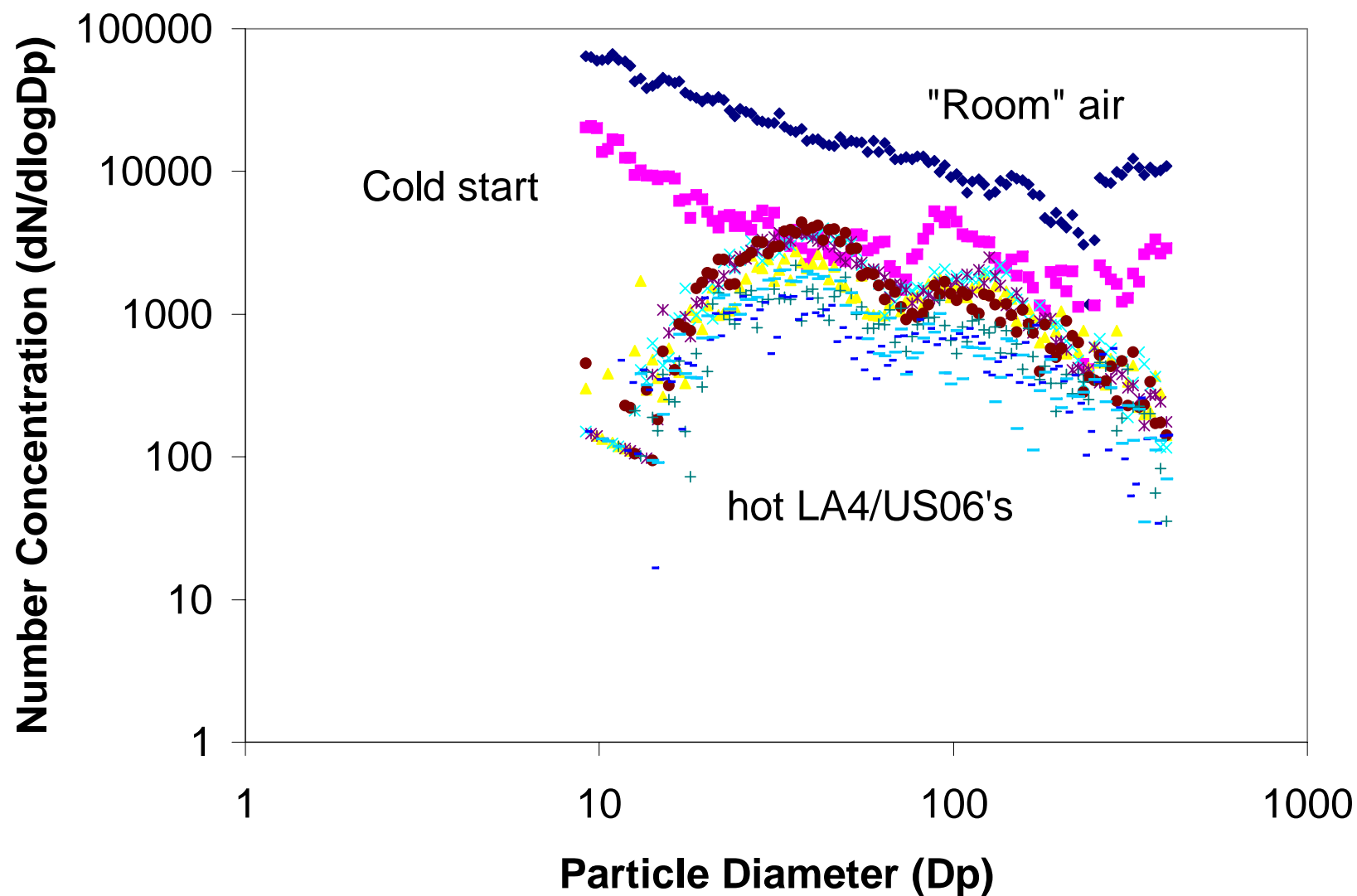


Results

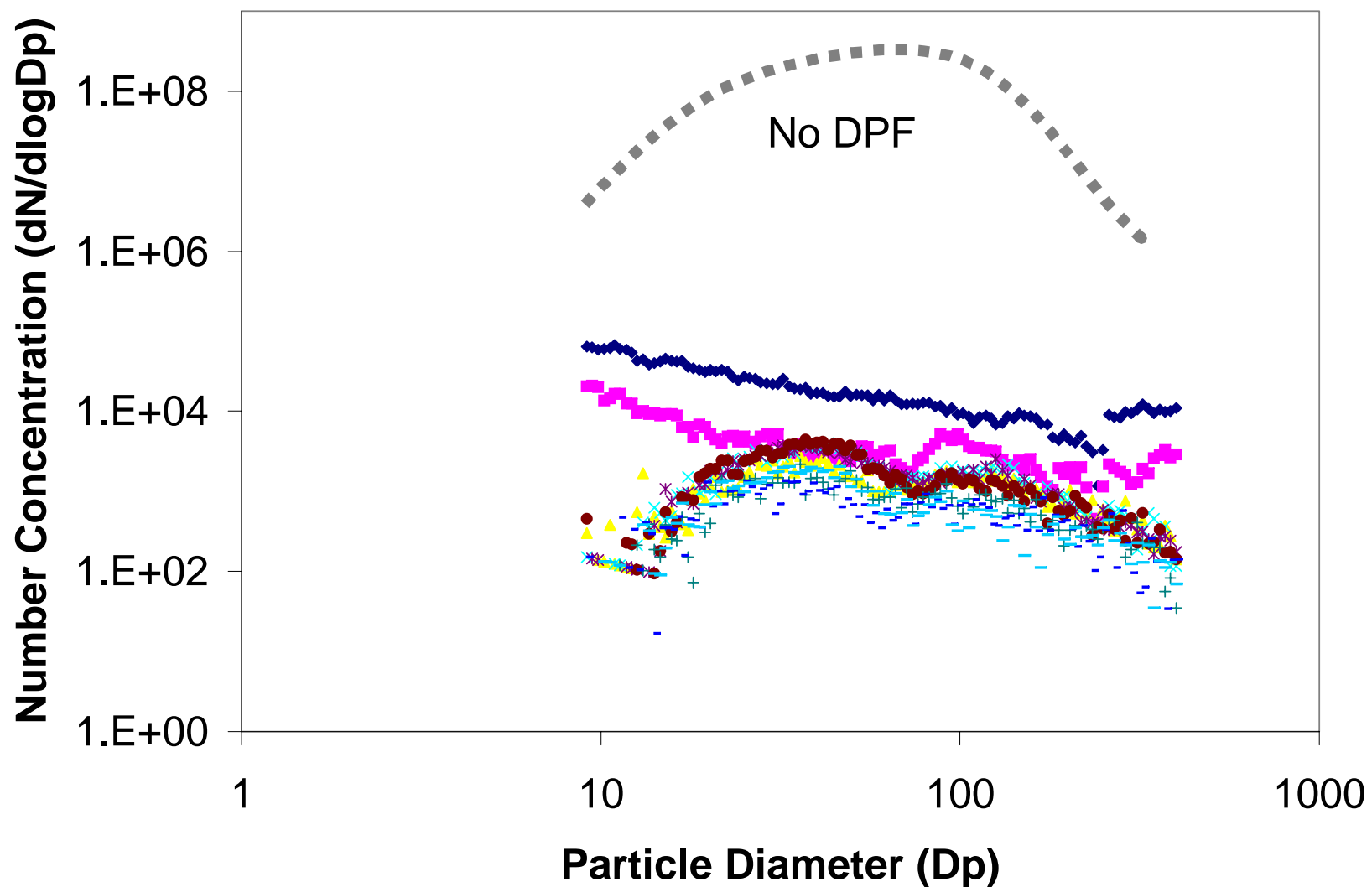
Cold start leads to aerosol formation during US06



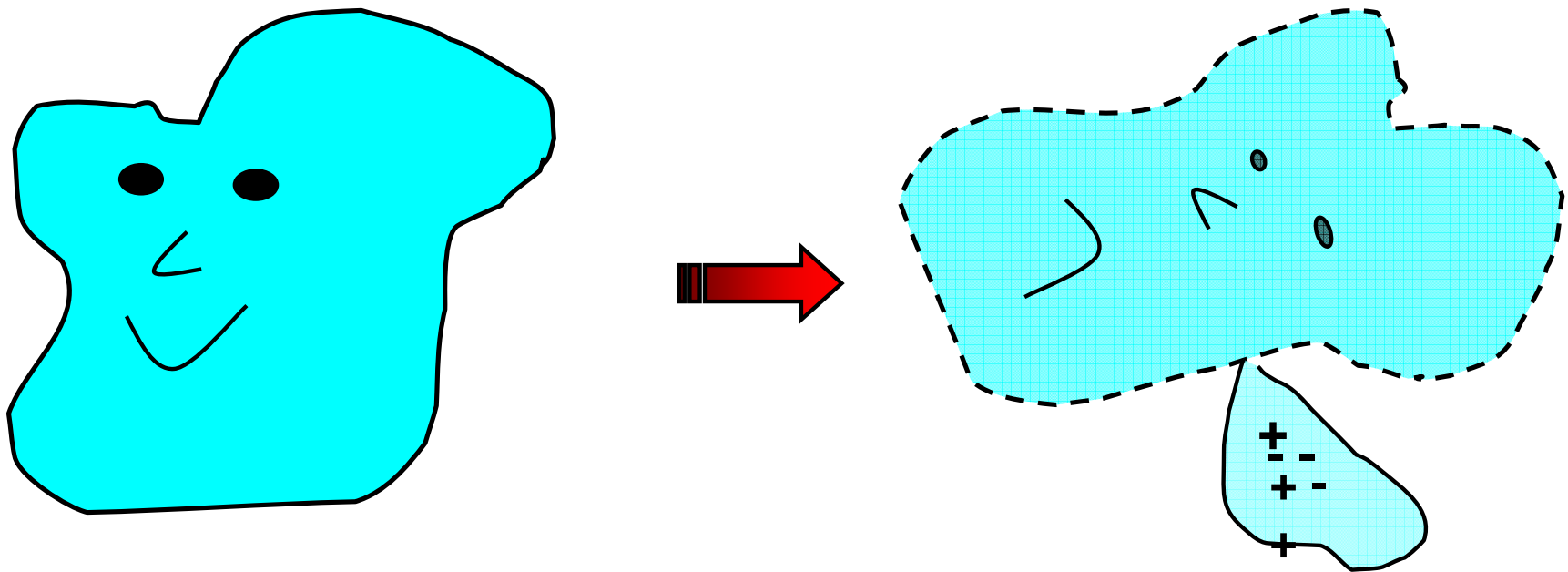
DPF “cleans” room air



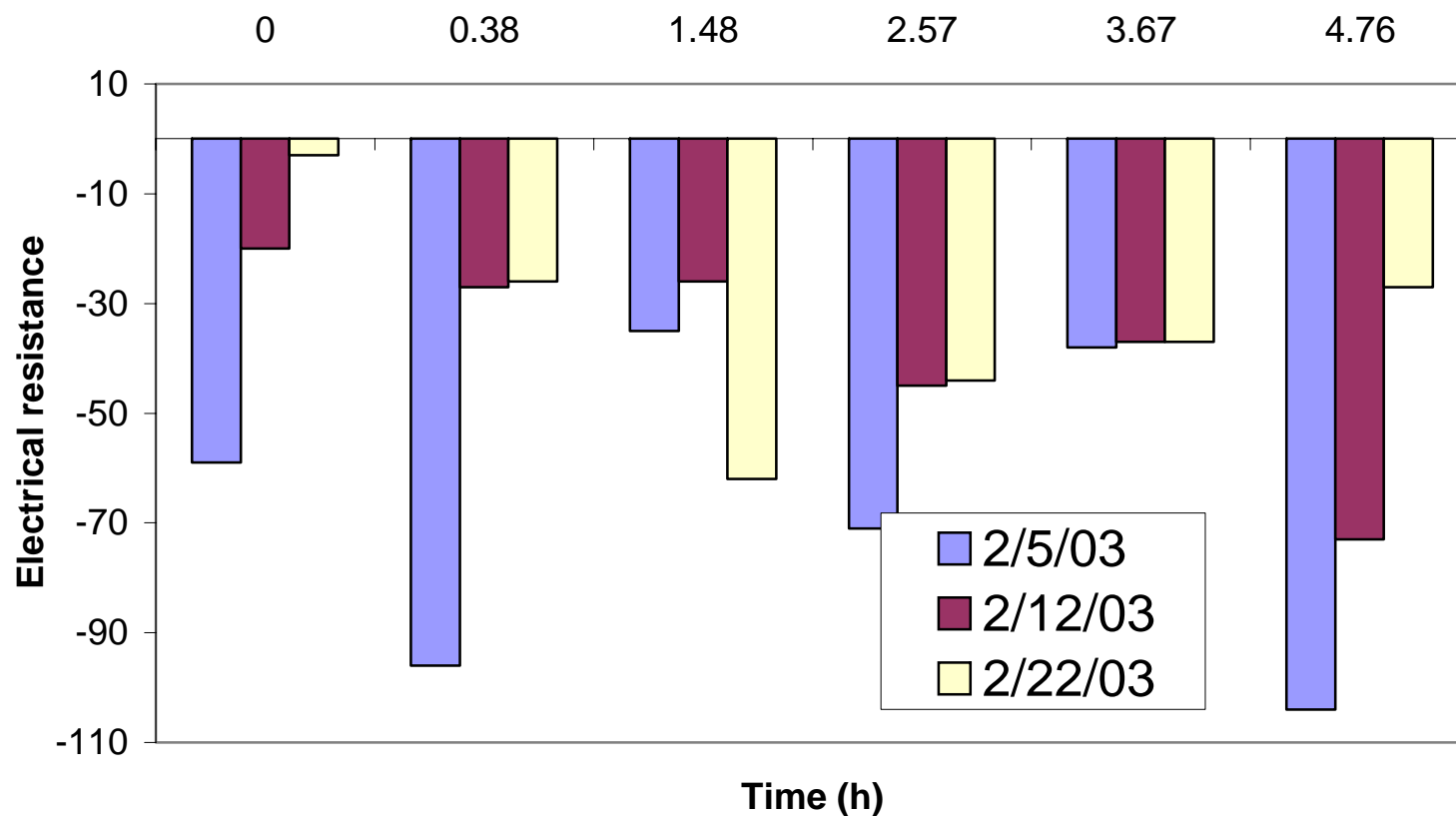
DPF removes most particles



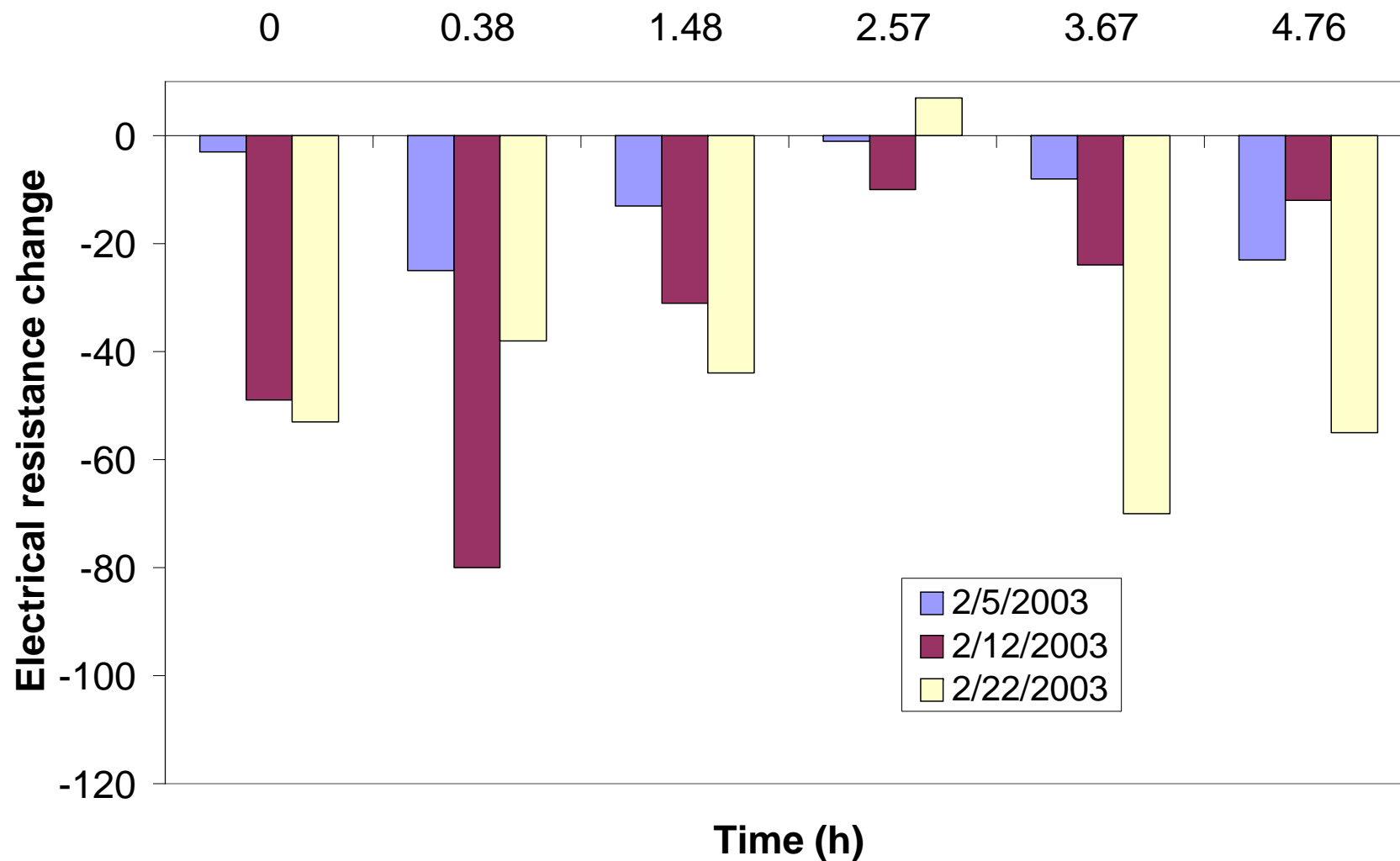
Electrical resistance decreases as cells die or lyse, also lose contact



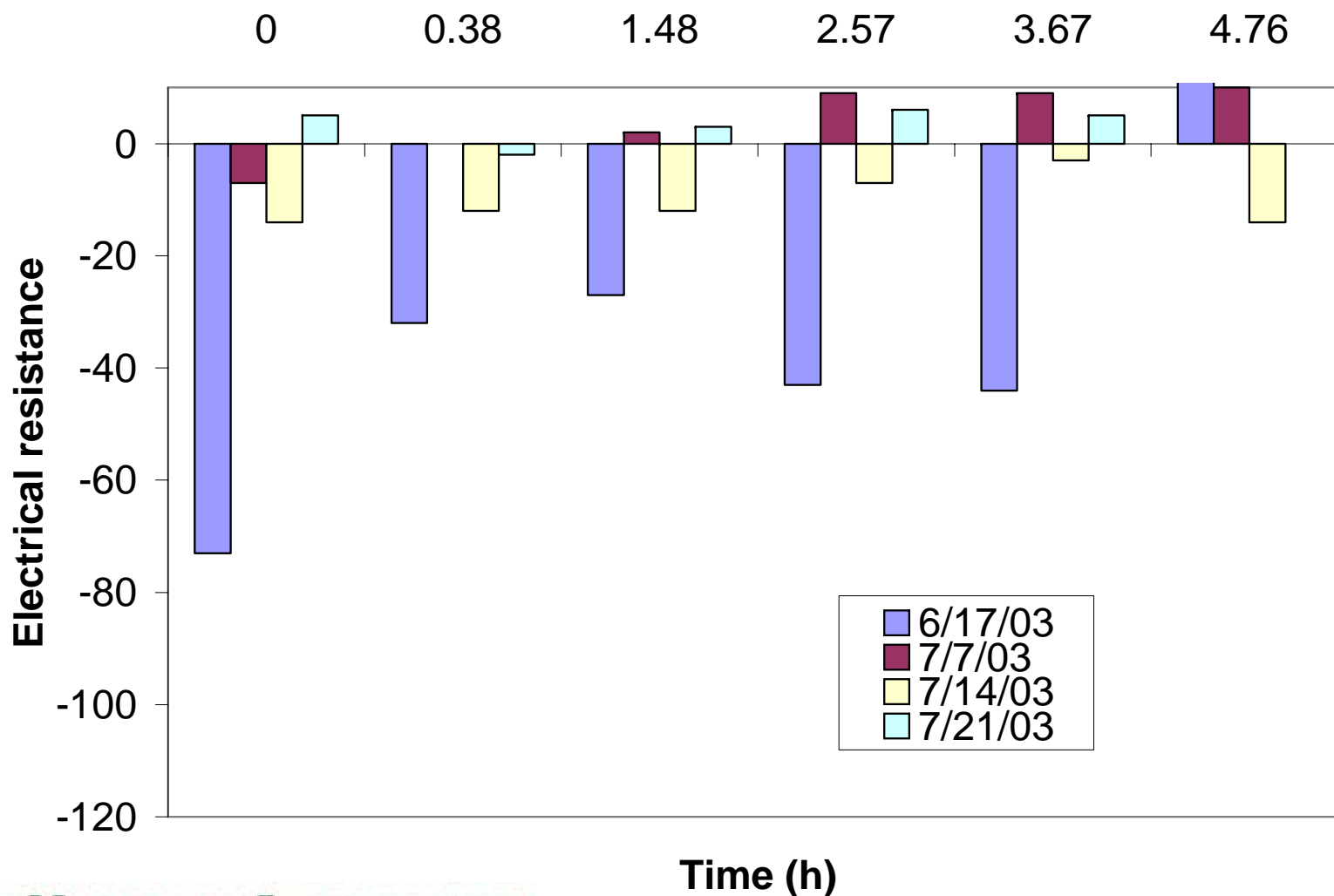
Change (>20%) in Electrical resistance indicates possible cell loss after exposure – gasoline, unprimed



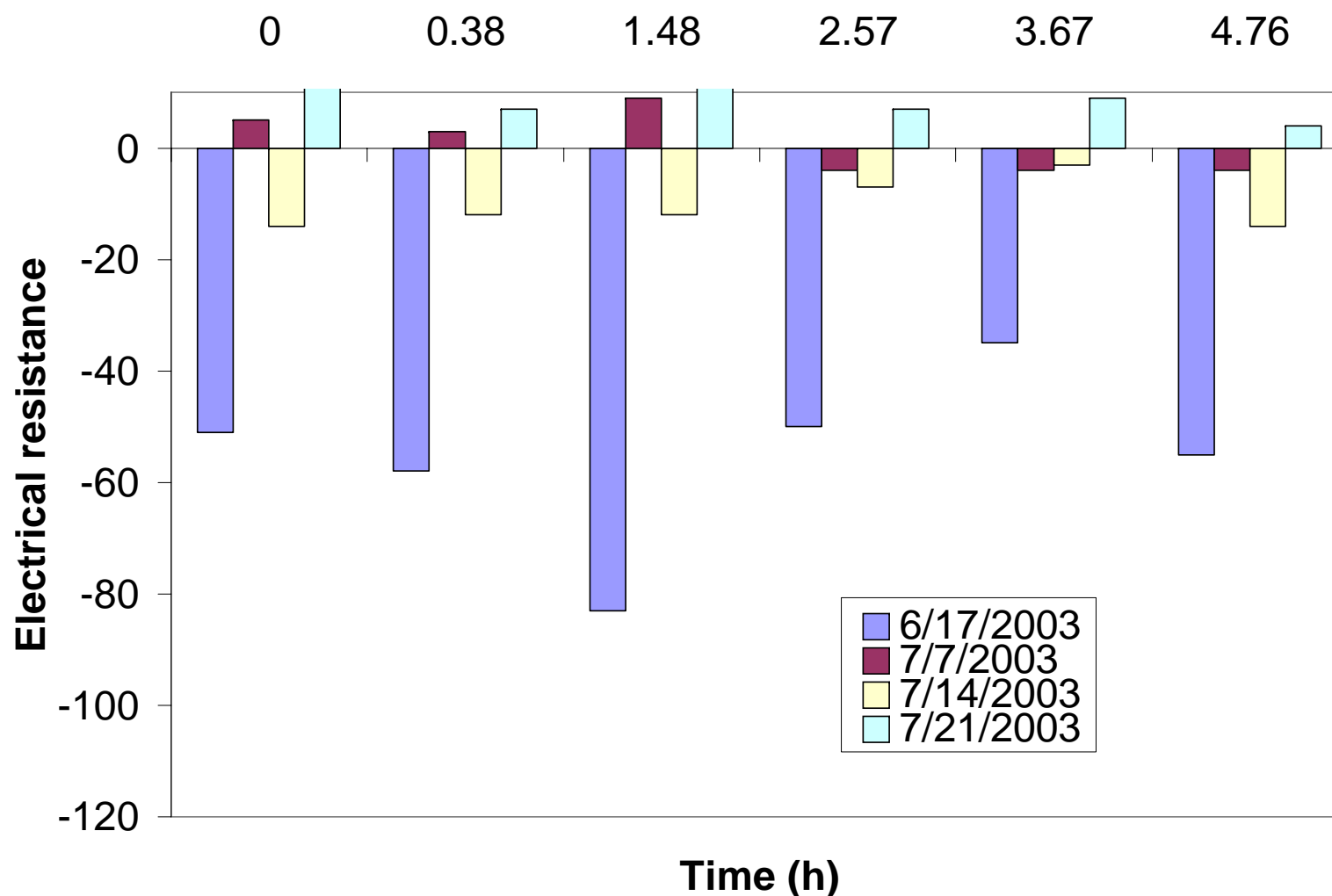
Change (>20%) in Electrical resistance indicates possible cell loss after exposure – gasoline SI , primed



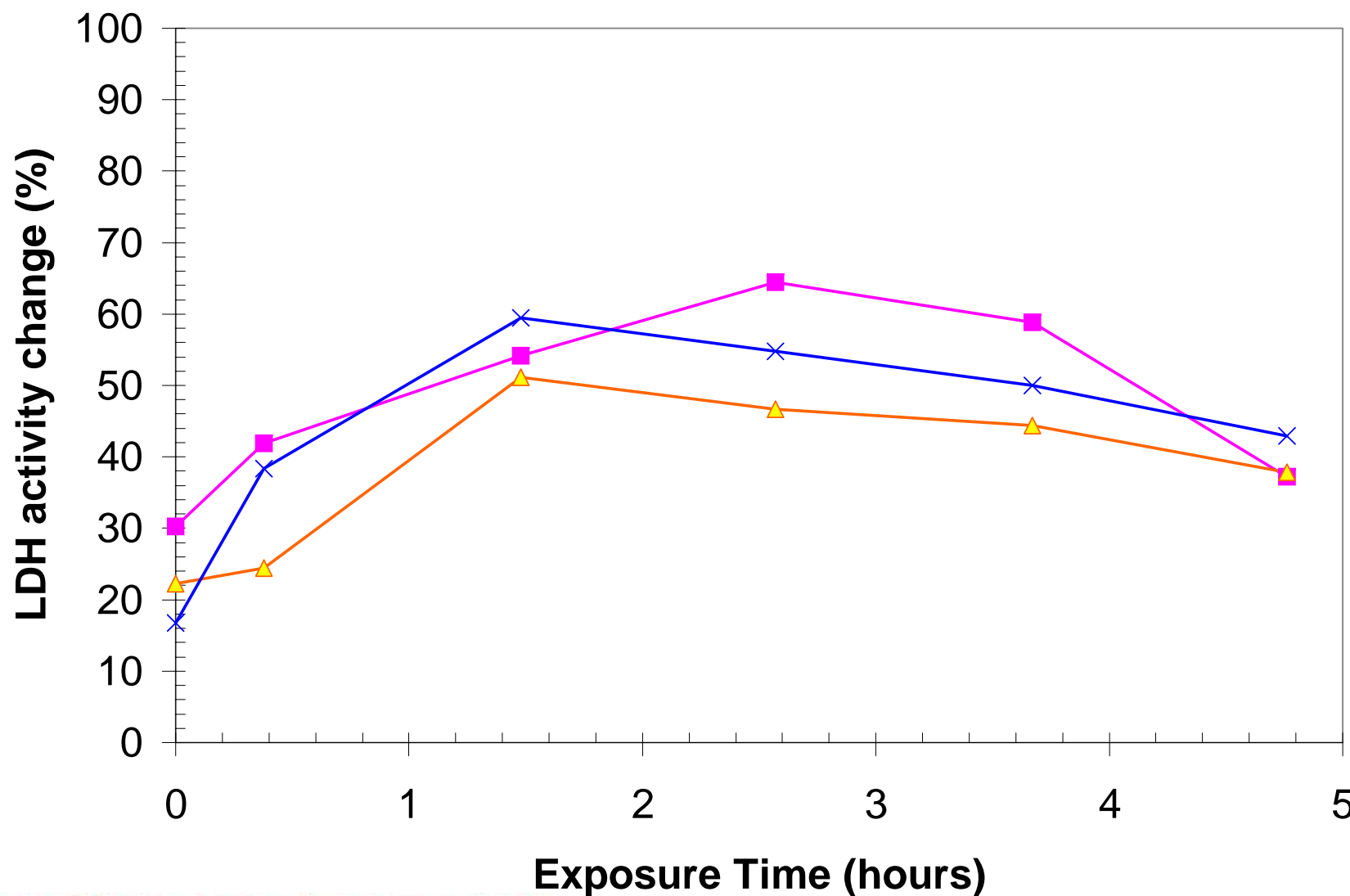
Change in electrical resistance indicates some cell growth after exposure, some loss of cells – diesel unprimed



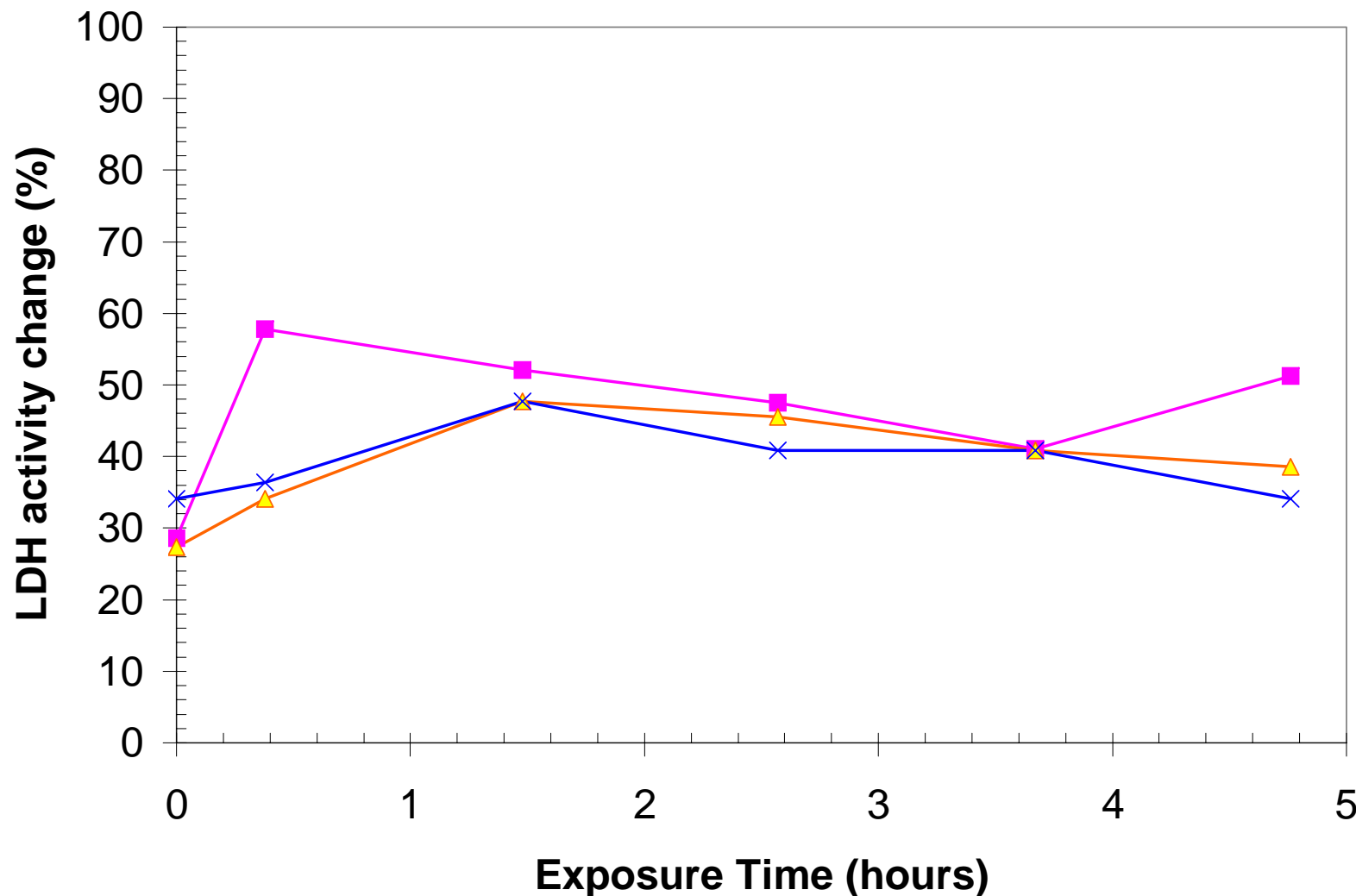
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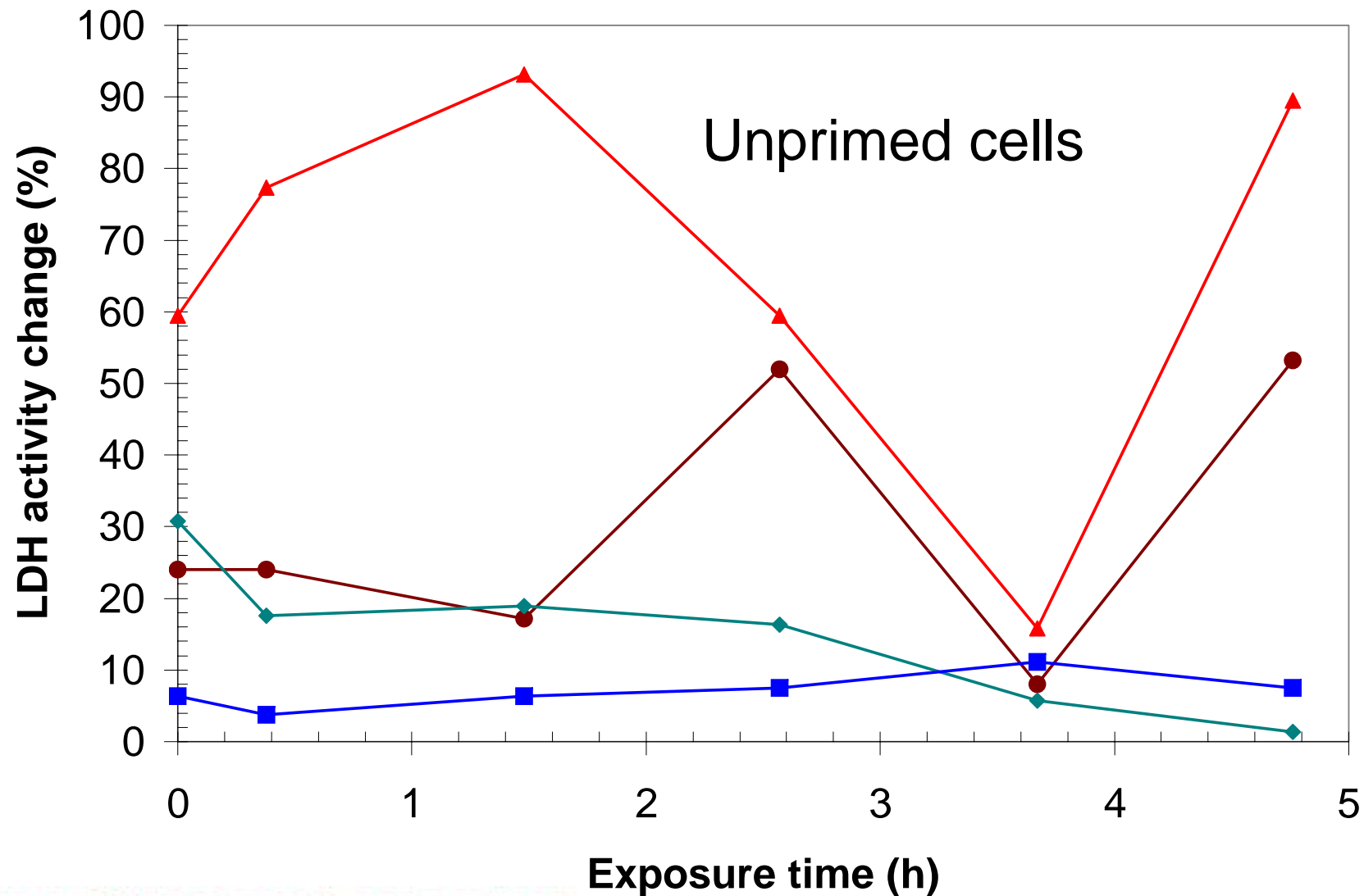
Lactate dehydrogenase production increases slightly during test – gasoline unprimed



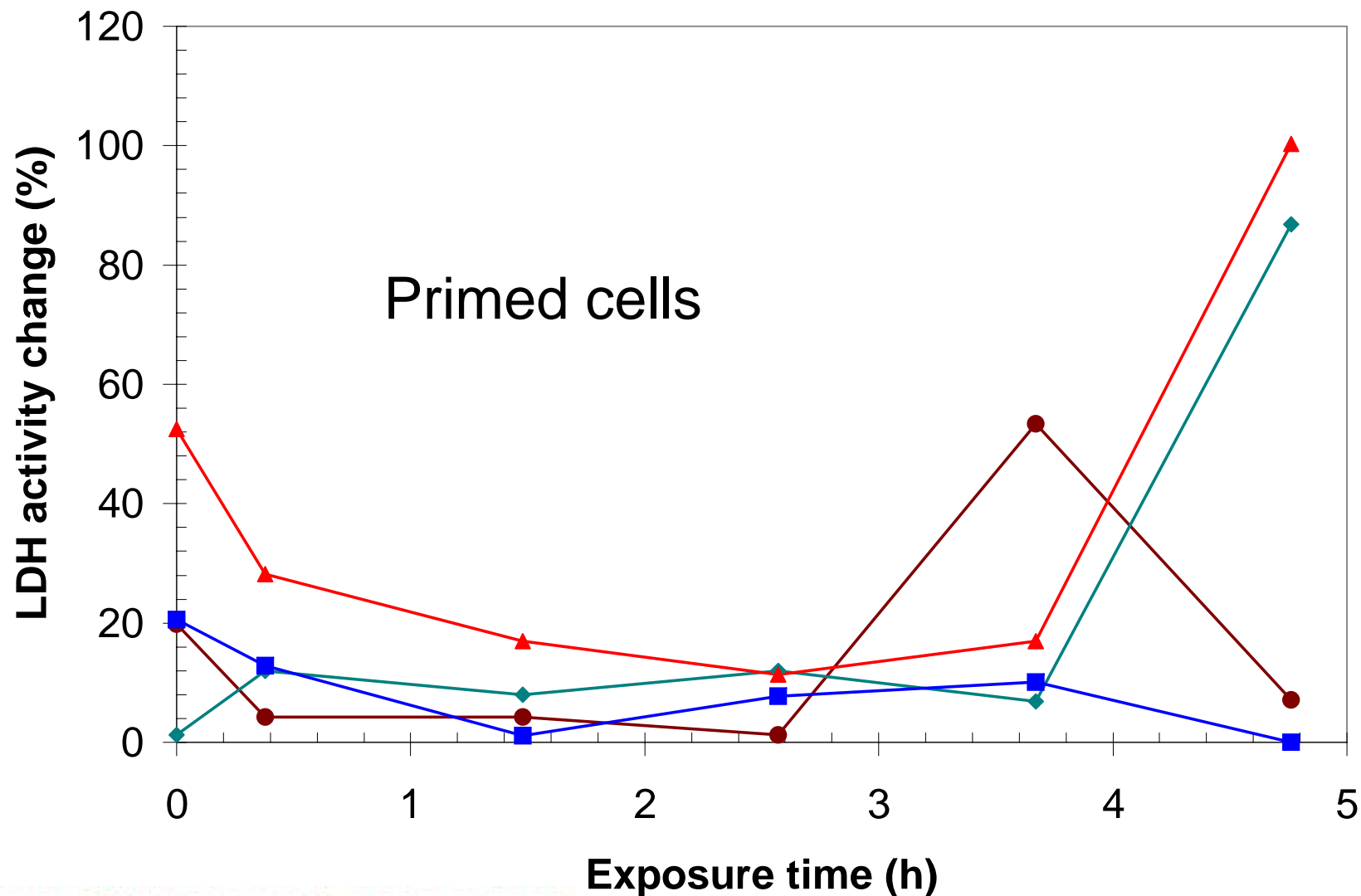
Lactate dehydrogenase production shows little increase for primed cells– gasoline SI



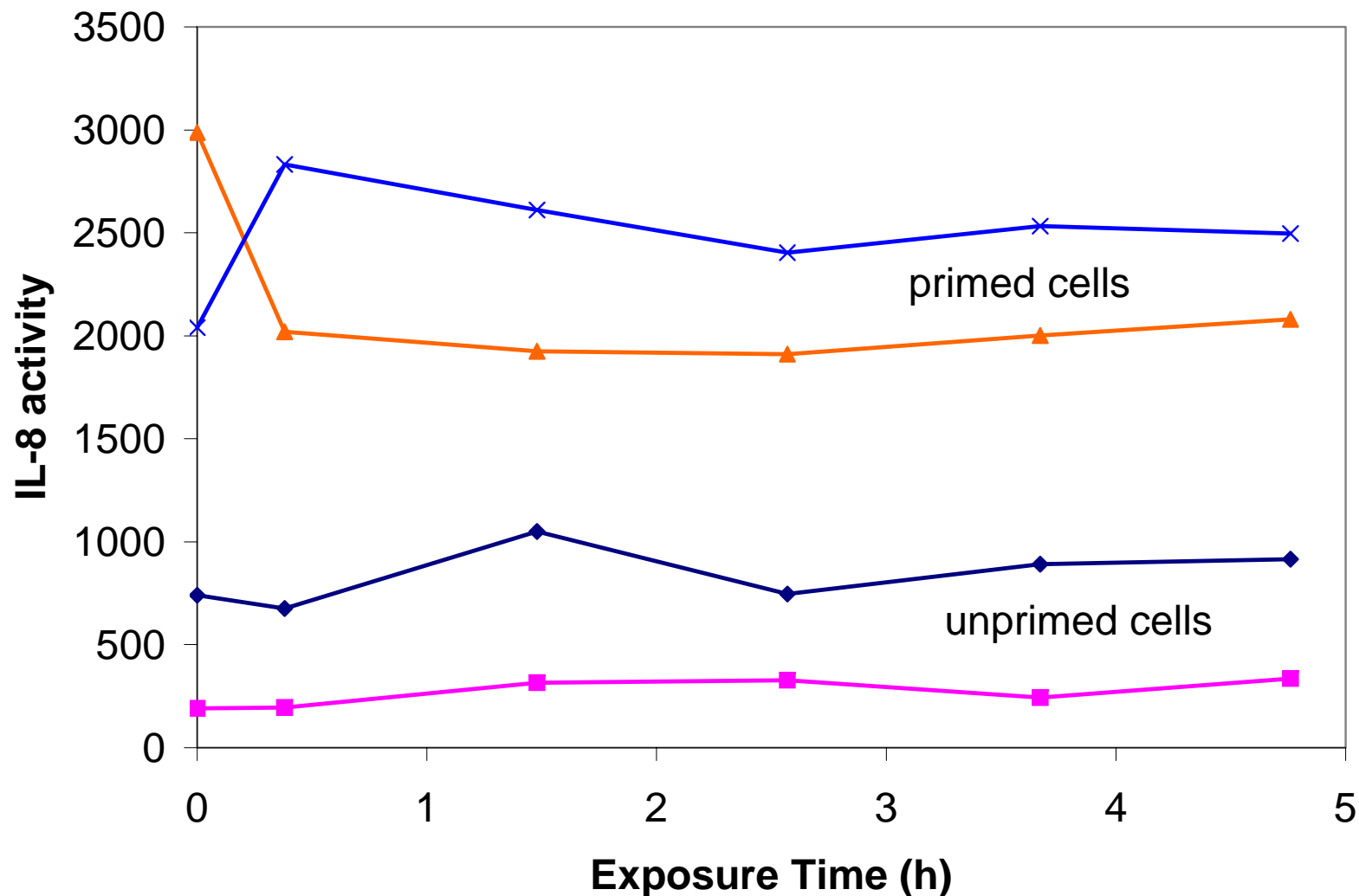
Lactate dehydrogenase production not conclusive during diesel exposure



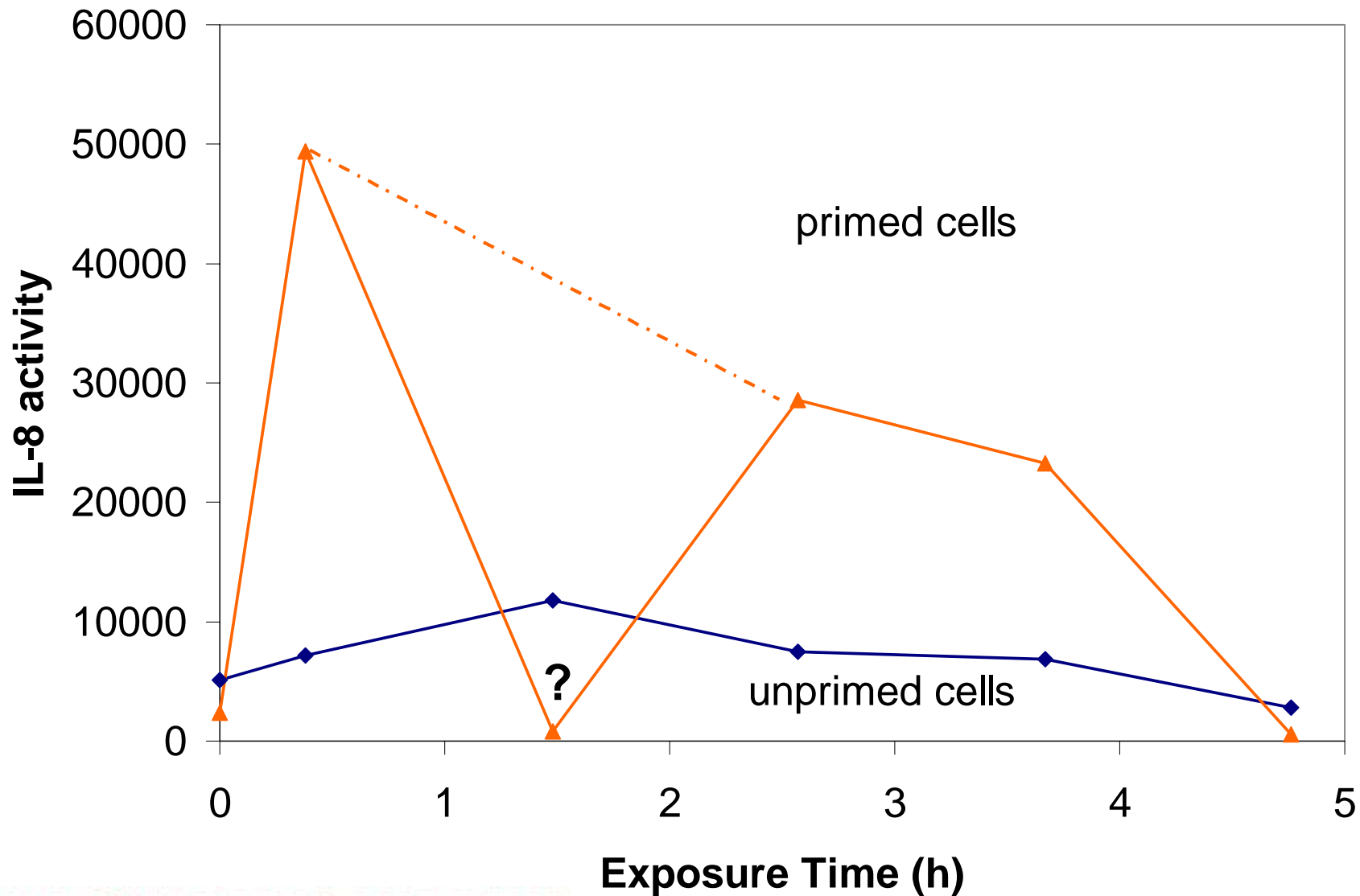
Lactate dehydrogenase production not conclusive during diesel exposure



IL-8 production remains flat during test – gasoline



IL-8 production not conclusive during diesel exposure



Summary

- **PM levels, particle counts very low for these studies**
- **Cellular toxicity measures gave noisy results**
- **Although not conclusive, little effect seen on cells from either exhaust**
- **Increase in resistance consistent with cell growth seen by LRRI (Seagrave et al., 2000) with diesel exhaust exposure**