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Effects of Aerosol Residues Benzene and Vinyl Chloride on A549 Lung Epithelial Cells

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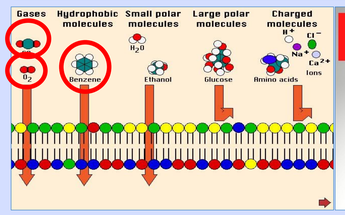
Abstract

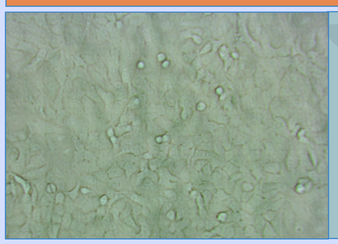
Aerosols are very prevalent in today's society (febreeze, spray sunscreens, hairsprays and spray deodorants). However, recent research has identified benzene and vinyl chloride residues in aerosol products (Vrodjack et al., 2021. Guatam et al., 2022. Sugaya et al., 2023). Benzene and vinyl chloride have been studied fairly well independently. However, no research has been done on the possible synergistic activity of these chemicals. Also, no study has been done on if inhaling them in the dosage levels present in aerosol products induces apoptosis. We performed a novel study to test if benzene and vinyl chloride levels present in a single dose of an aerosol can induce apoptosis in A549 cells. We observed that A549 cells subjected to benzene and/or vinyl chloride expressed signals of stress, such as, reactive oxygen species production, caspase cascade activation, and upregulation of the INOS gene. We also observed that benzene and vinyl chloride together had synergistic toxicity effects on the A549 cells.

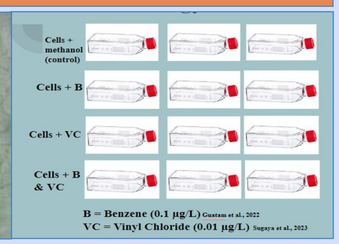


Introduction

- Benzene and vinyl chloride residues are being detected in aerosol products (Vrodjack et al., 2021. Guatam et al., 2022. Sugaya et al., 2023)
- Benzene and vinyl chloride can diffuse into our lung epithelial cells and induce apoptosis and spread elsewhere through the bloodstream.
- Studies have observed the general toxicity of benzene and vinyl chloride
- Possible synergistic toxicity between benzene and vinyl chloride has yet to be investigated
- We performed a novel experiment to see if a singular "dose" of an aerosol contains enough benzene/vinyl chloride to induce apoptosis in A549 cells

Methods





1. Cultured A549 (from ATCC) cells in DMEM medium, incubated at 37 C with 5% CO_2
2. Treated cells with 0.1 µg/L benzene and/ or 0.01 µg/L vinyl chloride for 24 hours
3. The treated A549 cultures were analyzed for different stress signal expression
   - 3A. An ABCAM ROS kit was used to test levels of reactive oxygen species. The fluorescent dye was oxidized by intracellular ROS. FLow cytometry was utilized to detect the % change in fluorescence.
   - 3B. The SYBR GREEN® qRT-PCR protocol and the Quanta Biosystems qScript One-Step Fast Low-Rox qRT-PCR kit was used on RNA that was extracted from treated cells and ran through qRT-PCR instrument to detect targets of INOS, COX2, KRAS, and ERK. 50 ng of total RNA were used in each reaction. GAPDH was amplified as an internal control.
   - 3C. A caspase 3 assay was performed to identify the apoptotic caspase cascade. NucView 488 dye fluorescently probed any present caspase 3. Flow cytometry was utilized to detect the florence.

Results

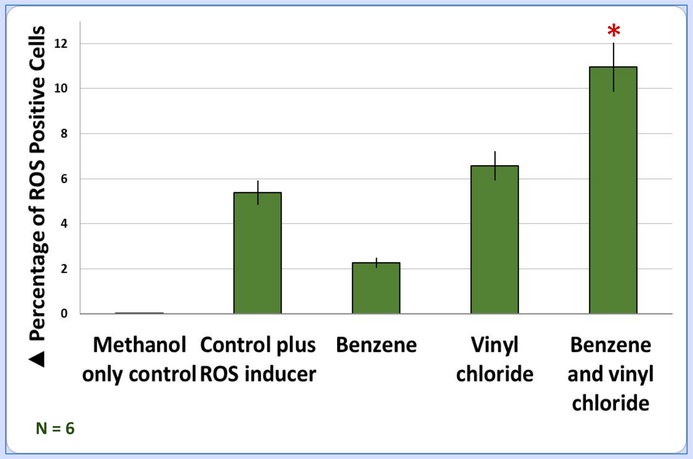


Figure 1 (above). Graph showing the ΔΔ values for Benzene, Vinyl Chloride and B&VC treatment averages of % of ROS positive cells (visualized by flow cytometry) when normalized to and then subtracted from negative control values. The benzene and vinyl chloride treatment was significantly different from the control. Furthermore, the benzene treatment and the vinyl chloride treatment were significantly different from the B&VC treatment.

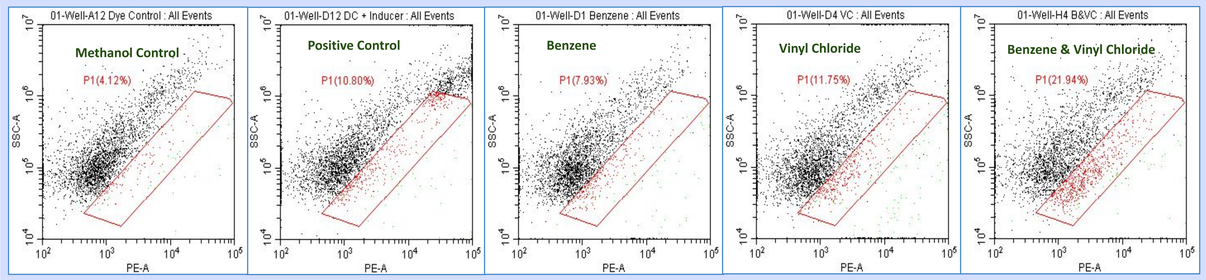


Figure 2 (above). Flow cytometry was done to view the % change in fluorescence of the ROS label. Cells that are tagged with the oxidized ROS label appear as red events. Cells that did not produce ROS are shown as black events. The negative control trial showed 4.12% of red events, meaning that a low number of cells were producing ROS. The ROS inducer PC trial showed 10.8% of red events. The B&VC trial showed 21.94% of the events were fluorescent, meaning that many more cells (relative to the control) were producing ROS after being treated with B&VC.

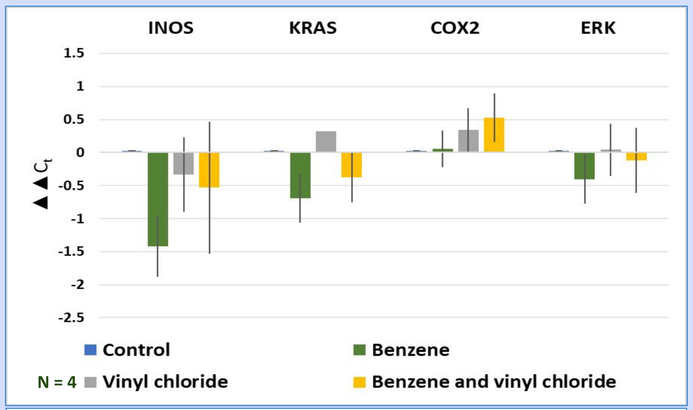


Figure 3 (above). Graph showing the ΔΔCt values for COX2, ERK, KRAS, and INOS gene expression based on qRT-PCR results when normalized to and then subtracted from GAPDH values. Significant differences were not observed. However, a trend of upregulation is seen with INOS expression and a downregulation of COX2 is seen

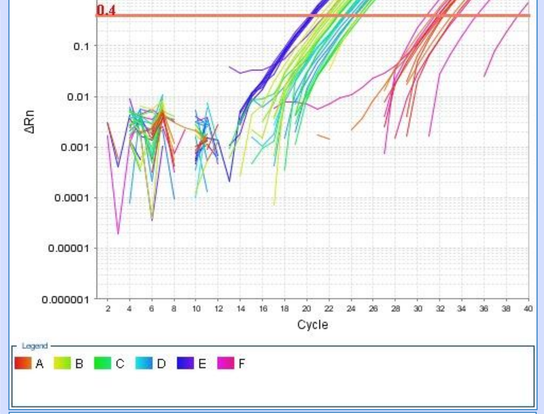


Figure 4 (above). Plots showing qRT-PCR results for cellular stress markers COX2, ERK, KRAS and INOS in A549 cells exposed to all four treatments. Data for $\Delta\Delta C_T$ calculations are from the threshold (red line) cycle numbers.

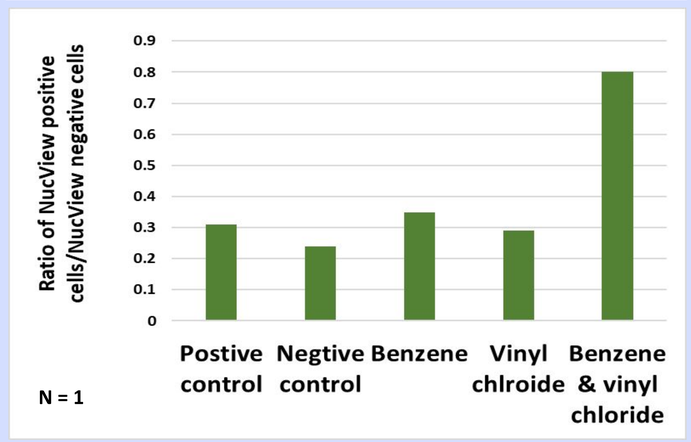
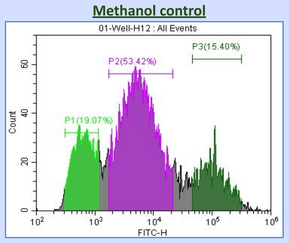


Figure 5 (above). Graph showing the ΔΔ values for a Benzene, Vinyl Chloride and B&VC treatment ratios of NucView 488 positive cells/NucView 488 negative cells when normalized to and then subtracted from negative control values.

Methanol control



Benzene and Vinyl Chloride

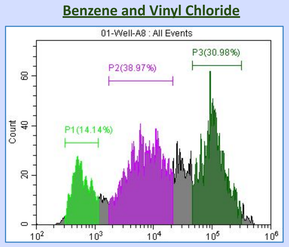


Figure 6 (left). The cells were incubated with NucView 488 label. Caspase 3 that is present within apoptotic cells cleaves the NucView 488 label, changing the fluorescence. Flow cytometry used to detect differences. Well H12, negative control treatment, shows 15.4% of the events were with the cleaved NucView 488 label. Well A8, the benzene and vinyl chloride treatment, shows 30.9% of the events had the cleaved NucView 488 label.

Conclusions

- When treating A549 cells with benzene and vinyl chloride together, synergistic levels of ROS are produced.
- When treating A549 cells with benzene and vinyl chloride together, a trend of inducing synergistic levels of caspase 3 activation is seen.
- When treating A549 cells with benzene and vinyl chloride together, a trend of inducing synergistic levels of the INOS gene is seen.
- Benzene and vinyl chloride both being present in aerosols increases the toxicity of the product.
- One "dose" of an aerosol product contains enough benzene and vinyl chloride to induce apoptotic effects in A549 cells.
- Some aerosols have been voluntarily recalled, but regulations concerning benzene and vinyl chloride presence in aerosol products needs to be more strict and more products need to be recalled.

Acknowledgments

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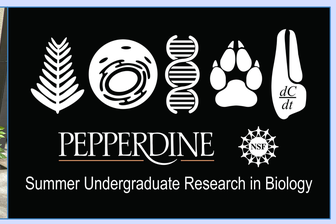

Literature

Balmanno K, Cook SJ. 2009. Tumor cell survival signaling by the ERK1/2 pathway. *Cell death and differentiation.* Vol (16): 368-377.

Bironaite D, Siegel D, Moran J, Weksler B, Ross D. 2004. Stimulation of endothelial production and apoptosis by phenolic metabolites of benzene in human bone marrow endothelial cells. *Chemico-biological interactions.* Vol (149): 37-49.

Carthew P, Clapp C, Gutsell S. 2009. Exposure based waiving: The application of the toxicological threshold of concern (TTC) to inhalation exposure of aerosol ingredients in consumer products.

Feng X, Liang T, Gao J, Kong D, Ge R, Li Q. 2017. Heme oxygenase-1, a key enzyme for the cytoprotective actions of halophenols by upregulating Nrf2 expression via activating Erk1/2 and PI3K/AKT in Ea.hy926 cells. *Oxidative medicine and cellular longevity.* Vol (17): 1-13.

Girardi P, Barbiero F, Baccini M, Comba P, Pirastu R, Mastrangelo G, Ballrin M, Biggeri A, Fedeli U. 2022. *Internationol journal of environmental research and public health.* Vol (19).

Guatam A, Cressey P. 2022 Health risk assement: benzene in cosmetics. *ESR.*

Huff J, Hasemen J, DeMarini D, Eustis S, Maronpot R, Peters A , Persing R, Chrisp C, Jacobs A. 1989. Multiple-site carcinogenity of benzene in Flscher 344 rats and B6C3F mice. *Environmental helath perspetives.* Vol (82): 125-163.

Mascelloni M, Delgado-Sabroti J, Hodeges N, Harrison R. 2015. Study of gaseous benzene effects upon A549 lung epithelial cells using a novel exposure system. *Toxicology letters.* Vol (237): 38-45.

Mogel I, Baumann S, Bohme A, Kohajda T, Bergen M, Simon J, Lehman I. 2011. The aromatic volatile organic compounds toluene, benzene, and styrene induce COX-2 and prostaglandins in human lung epithelial cells via oxidative stress and p38 MAPK activation. *Toxicology.* Vol(289): 28-37.

Silva, F., Starostina, I., Vitalievna Ivanova, V., Rizvanov, A., Oliveira, P. J., & Pereira, S. P. (2016). Determination of cell mass and viability using a tandem resazuring/sulforhodamine B assay. *CurrentProtocols in Toxicology, 68,* 2.24.1-2.24.15,1002.

Sugaya N, Inoue K, Tahara M, Kawakami T. 2022 Analysis and risk assessment of vinyl chloride emitted from aerosol products. *Journal of environmental science and health.* Vol (58:4): 284-294.