Cytotoxic Effects of N,N-Diethyl-Meta-Toluamide (DEET) on Sinonasal Epithelia

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Abstract
Although the etiology of chronic rhinosinusitis remains unknown, environmental factors including airborne pollutants and toxicants are postulated to contribute to its pathogenesis. However, the precise pathomechanisms with which environmental toxicants may contribute to chronic rhinosinusitis are not fully understood. The purpose of this pilot study is to examine the cytotoxic effects of N,N-diethyl-meta-toluamide (DEET), a commonly used pesticide, on sinonasal epithelial cells (SNECs). Sinus mucosa was obtained from 3 subjects without a history of chronic rhinosinusitis. Cultured SNECs were exposed to various concentrations of DEET (0-5 mM) for 6 days. Cell viability, proliferation, and morphologic changes were assessed using the MTT colorimetric dye assay and the Incucyte Live Cell Monitoring System. Statistically significant dose-dependent reduction in cell viability and proliferation was observed between exposure and control groups (P < .05) at all concentrations tested. Dose-dependent cellular morphological changes were also seen. These findings indicate that DEET exposure induces dose-dependent cytotoxicity in sinonasal epithelia.

Keywords
chronic rhinosinusitis, epithelia, toxicants, pesticide, chemicals

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Although the etiology of chronic rhinosinusitis (CRS) remains unknown, environmental factors, including airborne toxicants, are postulated to contribute to its pathogenesis.1,2 Occupational and combat zone chemical exposures are associated with increased prevalence of CRS.3-13 Farmers with pesticide exposures have a significantly higher incidence of sinusitis compared to matched controls (n = 196).4 A cross-sectional epidemiologic study of 261 healthy volunteers revealed a dose-response relationship between level of pesticide exposure and reported sinusitis independent of age, sex, and smoking status.6 N,N-diyethyl-meta-toluamide (DEET) is a commonly used insect repellent sprayed on the hands and rubbed onto the skin. It has been extensively used in agriculture, military, and civilian life.14,15 However, the impact of DEET exposure on the sinonasal cavity has not previously studied. The purpose of this pilot study is to investigate the cytotoxic effects of DEET on sinonasal epithelial cells (SNECs).

Methods
We have established a cell culture model system as previously described in the literature with modifications.16-18 Sphenoid sinus mucosal specimens were collected from patients undergoing endoscopic transsphenoidal resection of pituitary tumors without a history of sinonasal disease at the Greater Los Angeles VA according to an institutional review board–approved protocol. SNECs were expanded on tissue culture plates and transferred onto cell culture inserts, and confluent cells were cultured at the air-liquid interface.17 To determine the effect of DEET on cell growth, we performed the cell viability measurement MTT colorimetric dye assay.19 SNECs were grown in a 96-well plate and cultured for 6 days in various concentrations of DEET. After the 6-day exposure period, culture plates were treated with MTT dye and incubated overnight. Optical density (OD) were measured at 570 nm. The IncuCyte live-cell analysis system (Essen Bioscience) was used to assess cell proliferation. Live cells were monitored over an extended period of time and data presented as real-time kinetic data.20-22 SNECs were exposed to different concentrations of DEET, and wells were scanned every 2 hours from 0 hours to 6 days (144 hours) after exposure. Dosing concentrations and exposure schedules were determined based on prior published studies examining the effect of DEET on other cell types.23,24 The data are presented as fold changes in cell density from initiation (0 hours) to specific time points during the assay. Cell density was calculated with IncuCyte software and phase contrast images.

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Results

DEET Exposure Impacts Cultured Cell Growth and Viability in a Dose-Dependent Manner

When SNECs (4000/well in quadruplicates) were treated with DEET (Figure 1), a dose-response result was obtained, such that a 5-mM concentration eliminated all viable cells, and correspondingly lower concentrations (0.625-2.5 mM) had reduced effects on cell viability.

Toxicant Treatment and Live-Cell Monitoring/Analysis Using the IncuCyte Live-Cell Monitoring System

When cells were treated with DEET (4000 cells/well, in quadruplicate) (Figure 2), cell proliferation was inhibited in a concentration-dependent manner (0.625-5 mM), similar to the MTT assay. Treatment with 2.5 mM DEET was most effective for inhibition of primary cell growth.

Morphological Changes and Cytotoxic Effects

Morphological changes and cytotoxic effects were observed with DEET when SNECs were treated for 6 days. High doses of DEET (Figure 3A) disintegrated SNECs at a 5-mM concentration. At lower concentrations (Figure 3B,C; 1.25 mM and 0.625 mM, respectively), few live cells were observed. Remaining cells lost their morphology and were in the process of disintegration. These results indicate that DEET exerts deleterious effects on SNECs in a dose-dependent manner.

Discussion

The precise role of environmental pollutant and chemical exposure in the pathogenesis of CRS is not fully understood. Prolonged combined pyridostigmine bromide/DEET exposure has been shown to increase serum levels of proinflammatory cytokines in a mouse model and cause neuroinflammation.24 DEET has also been found to potentiate persistence of chronic pain in a rat model.25,26 However, the deleterious effects of DEET on sinonasal epithelia have never been previously explored.

To our knowledge, this study is the first to illustrate the cytotoxic effects of DEET on SNECs. DEET exposure significantly reduced SNEC growth and viability in a dose-dependent manner, with a 5-mM concentration eliminating all viable cells and correspondingly lower concentrations (0.625-2.5 mM) with reduced effects on cell viability. IncuCyte live-cell monitoring showed inhibition of SNEC proliferation in a concentration-dependent manner (0.625-5 mM), with 2.5 mM DEET being the most effective for inhibition of SNEC growth. SNECs exposed to DEET also exhibited morphologic changes, with lower concentrations resulting in fewer live cells and higher doses (5 mM) causing complete disintegration. A prior in vitro study demonstrated dose-dependent genotoxic effects of DEET when administered to primary nasal mucosal epithelial cells. However, no significant cytotoxic effects were observed at the concentrations studied (0.5-1 mM). This may be due to the fact that cells were only

![Figure 1](image1.png) Viability of in vitro treated sinonasal epithelial cells (SNECs) exposed to N,N-diethyl-meta-toluamide (DEET). SNECs were treated with different concentrations of DEET. Respective P values are represented with respect to control untreated cells.

![Figure 2](image2.png) Time courses (x-axis, hours) for real-time cell confluency (y-axis, fold changes in cell density) of sinonasal epithelial cells (SNECs) treated with 0.625 to 5 mM N,N-diethyl-meta-toluamide (DEET).

![Figure 3](image3.png) Dose-dependent morphological changes and cell death of sinonasal epithelial cells (SNECs) after 6 days in control (A) versus N,N-diethyl-meta-toluamide (DEET) at (B) 0.625 mM, (C) 1.25 mM, and (D) 5 mM.
exposed for 60 minutes, whereas previous studies adminis-
tered exposures over several days.27
In vivo, DEET toxicity is dependent upon the route and
duration of exposure as well as the dosage administered.
Commercially available products contain anywhere from 10% (100 mg/mL) to 100% DEET. Neurotoxicity including seizures and respiratory arrest have been reported with ingestion (ie, spraying into the oral cavity unintentionally) and excessive dermal application (25 mL of 50% DEET).28 However, minimum toxicity thresholds for upper airway toxicity from inhalant exposure have not been established. The concentrations used in this in vitro study (0-5 mM: 956 mg/mL) are significantly less than the reported dosages associated with neurotoxicity.

Cytotoxicity as a driver of inflammation has been extensively reported in the lower airway and implicated as a contributing factor in multiple pulmonary inflammatory diseases.29-31 Chemical exposures have been shown to induce lytic cell death, generation of cellular debris, and reactive oxygen species, leading to release of proinflammatory cytokines in lung tissue.30 Primary bronchial epithelial cells exposed to diesel exhaust and aldehydes demonstrate increased interleukin (IL)–8 secretion and gene expression of inflammatory (IL-6, IL-8) and oxidative stress (hemeoxygenase 1) biomarkers.30,31 Caspase 1–dependent IL-1β release has been reported as the underlying pathomechanism for pneumonia, respiratory syncytial virus bronchiolitis, and COVID-19–mediated lung injury.31,32 Similar to the lower airway, the cytotoxic effects induced by DEET exposure may lead to a proinflammatory response in sinonasal tissue, potentially contributing to CRS pathophysiology. Previous studies have also demonstrated that DEET induces CYP isoforms, adenylate kinase, and caspase 3/7, leading to cytotoxicity in human hepatocytes.23 Further study is needed to investigate this concept of chemically induced cyto-
toxicity as a potential factor in CRS.

Conclusion
This pilot study is the first to demonstrate the cytotoxic effects of the commonly used pesticide, DEET, on sinonasal epithelia. DEET exposure significantly reduced SNEC viability and proliferation in a dose-dependent manner in vitro. Additional studies are necessary to determine if such cytotoxicity is associated with chronic inflammation and establish minimum toxicity thresholds that could predict sinonasal morbidity.

Author Contributions
Jivianne T. Lee, conception and design, data acquisition, analysis and interpretation, drafting and revising work critically for intellectual content, approval, agreement of accountability; Saroj Basak, conception and design, data acquisition, analysis and interpretation, drafting and revising work critically for intellectual content, approval, agreement of accountability.

Disclosures
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