

Inhalation Toxicology of Azadirachtin and Neem Oil Pyrolysis Products: Derivation of a Health-Based Maximum Residue Limit (MRL_{Inhalation}) for Inhalable Crops

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Abstract

The widespread use of neem oil on crops intended for human inhalation (e.g., cannabis, hemp) poses an unquantified public health risk. Current regulatory safety standards are based on ingestion data, which fails to account for the chemical transformation (pyrolysis) that occurs during smoking or vaping. This pre-experimental proposal outlines a comprehensive, nine-phase investigation to analytically characterize the degradation products of neem oil residue (Azadirachtin and fatty acids), biologically verify their pulmonary toxicity, and derive a robust, health-protective Maximum Residue Limit (MRL_{Inhalation}). The study integrates analytical chemistry (Py-GC/MS), computational toxicology (PBPK modeling), and in vitro/in vivo lung toxicology to establish the No-Observed-Adverse-Effect-Level (NOAEL) necessary for regulatory action.

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1 Introduction and Problem Statement

The primary active compound in neem oil, **Azadirachtin**, is a complex tetranortriterpenoid used globally as an organic pesticide. When applied to inhalable crops, residual neem oil (comprising Azadirachtin and various triglycerides) is exposed to high temperatures (180°C to > 900°C) during consumption. This process, known as **pyrolysis**, fundamentally alters the chemical structure, potentially generating highly toxic, low-molecular-weight compounds (e.g., aldehydes and polycyclic aromatic hydrocarbons, PAHs). The critical deficiency is the complete absence of toxicological data for these pyrolytic products on mammalian lung tissue.

Main Research Question

What are the acute and chronic toxicological effects on human respiratory tissues from inhaling the pyrolysis products of neem oil residue (Azadirachtin and fatty acids) under simulated smoking and vaping conditions?

2 Review of Critical Gaps

Current regulatory standards (based on oral toxicity) are inapplicable. This investigation must fill the following gaps:

1. **Chemical Identification Void:** The specific chemical degradation pathways and the final toxic products of Azadirachtin and neem's fatty acids under thermal stress are unknown.
2. **Toxicological Inhalation Data Void:** No LD₅₀, NOAEL, or LOAEL exists for the inhaled products of neem pyrolysis.
3. **Regulatory Translation Gap:** A validated methodology for translating on-plant residue concentration (ppm) into a biologically relevant human dose (tissue dose) via the inhalation route is needed.

3 Methodology: Phased Approach

This proposal outlines a sequential, multi-disciplinary approach across four core phases (Phases 5–8).

3.1 Phase 5: Analytical Toxicology (Pyrolysis Characterization)

Objective: Identify and quantify all chemical species in the inhaled aerosol.

- **Sample Preparation:** In-house treatment of inert plant substrate to achieve a representative, high-end residue concentration (e.g., 50 – 100 ppm Azadirachtin).
- **Simulation & Collection:** Utilize a custom apparatus to simulate both smoking (~ 900°C) and vaping (~ 200°C). Collect the aerosolized condensate via cooled impinger traps.

- **Analysis:**

- **Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS):** Primary identification of volatile/semi-volatile products, including aldehydes (acrolein, formaldehyde), PAHs, and novel Azadirachtin fragments.
- **High-Performance Liquid Chromatography (HPLC):** Quantification of the transfer rate of intact (unpyrolyzed) Azadirachtin into the aerosol.

Deliverable: A definitive "chemical fingerprint" and a dose-per-inhaled-volume ($\mu\text{g}/\text{L}$ air) for all major pyrolytic compounds.

3.2 Phase 4: Computational Toxicology (Predictive Modeling)

Objective: Refine exposure levels and prioritize in vivo testing. (This runs concurrently with Phase 5).

- **QSAR Modeling:** Input molecular structures of identified degradation products to predict mutagenicity, carcinogenicity, and respiratory irritation potential.
- **PBPK Modeling:** Develop a lung model to calculate the delivered dose (actual concentration reaching the deep alveolar tissue) from the analytical data (Phase 5). This factor is crucial for regulatory scaling.

3.3 Phase 6: Biological Verification (In Vitro / In Vivo Toxicology)

Objective: Establish the dose-response relationship for pulmonary harm.

- **In Vitro Cellular Assays:**

- **Model:** Co-culture of Human Bronchial Epithelial (HBE) and Alveolar Macrophage (AM) cells.
- **Endpoints:** LD_{50} (Cytotoxicity), ROS (Oxidative Stress), and cytokine release (IL – 6, TNF – α) to measure inflammation.

- **In Vivo Sub-Chronic Study (NOAEL Determination):**

- **Model:** Rat/Mouse Inhalation Model (28-day exposure regimen, multiple doses).
- **Endpoints:**
 1. NOAEL (No-Observed-Adverse-Effect-Level) and LOAEL.
 2. Pulmonary Function Tests (PFT): Measure lung mechanics (resistance, compliance).
 3. Bronchoalveolar Lavage (BAL): Inflammatory cell counts and protein leakage (injury markers).
 4. Histopathology: Tissue examination for fibrosis, macrophage accumulation, and cellular damage.

3.4 Phase 7: Regulatory Formulation

Objective: Translate the NOAEL into a public health standard.

- **Reference Dose (RfD) Calculation:**

$$\text{RfD}_{\text{Inhalation}} = \frac{\text{NOAEL} \times \text{PBPK Factor}}{\text{Uncertainty Factors (UF)}}$$

- **MRL_{Inhalation} Derivation:** Using the RfD, body weight, and estimated high-end consumption rates, calculate the maximum allowable on-plant concentration in ppm.

Projected Outcome: The highly sensitive nature of the lung and the confirmed generation of irritants (Phase 5) are expected to yield an MRL_{Inhalation} that is analytically below the practical limit of quantification (LOQ). This necessitates a ****Zero-Tolerance / Non-Detect**** policy.

4 Conclusion and Policy Recommendation

Successful completion of this program will close a critical public health gap by transforming an unknown risk into an evidence-based, actionable policy. The scientific findings will provide the mandate for regulatory bodies to immediately cease the practice of using neem oil on inhalable crops during the post-vegetative stage, requiring a mandatory pivot to non-residual or biological alternatives.

5 References

References

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