# Constructing a Biologically-based Dose Response (BBDR) Model to Refine Risk Assessment Approaches to Respiratory Effects from Inhaled Reactive Gases: Chlorine


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**LTG 1 Poster 14**

## Science Questions

Using chlorine as a prototypical irritant, we will answer the following critical questions:

- What are the roles of concentration, duration, and dosimetry factors (e.g., airflow and airway anatomy) as key determinants of the mode of action (MOA) for chlorine?
- How will integration of diverse endpoints improve the description for the pathogenesis of chlorine?
- Can information from other irritant gases be used to refine the understanding of epithelial disruption due to chlorine?
- Do different biological responses require different duration adjustment to equivalence across exposure scenarios or for extrapolation across species?

## Research Goals

- Compliment the existing rich traditional database on chlorine with mechanistic endpoints
- Characterize critical determinants of pathogenesis using a set of "C x t" studies
  - Concentration x time product is same across various experimental regimens and durations
  - Same endpoints measured in each study
- Develop a hybrid computational fluid dynamics (CFD)-physiologically-based pharmacokinetic (PBPK) model component to
  - Describe localized flux in the upper respiratory tract (URT) of rats and humans
  - Extend description to tissue phase reactions using biomarkers of dose and inflammation
- Develop a Bayesian network model that distinguishes four (4) cellular / tissue states based on degree of oxidative stress as determined with empirically measured endpoints
- Couple the dosimetry and response models to create a biologically-based dose response (BBDR) structure
- Perform human studies with parallel endpoints to provide target context verification of model predictions based on rodent data.

## Findings and Conclusions

By selecting only one endpoint on which to both base the dose-response analysis and identify the point-of-departure, current risk assessment approaches ignore critical data that can better define disease pathogenesis, characterize dose and duration dependencies, and aid interspecies extrapolation.

Evaluation of lesion correlations to preliminary simulations of chlorine flux using a computational fluid dynamics (CFD) model indicates a role for both airflow and tissue sensitivity. A hybrid CFD-PBPK model will be best to accurately address localized dosimetry in the upper respiratory tract (URT).

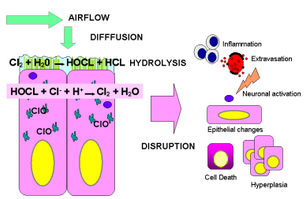
A key event in the MOA for the epithelial responses induced by inhaled chlorine is oxidative stress mediated by hypochlorous acid (HOCl) which forms in tissues by hydrolysis, with subsequent downstream biological responses.

Chlorotyrosines are stable N-chloroderivatives that result from reactions of HOCl. These have been shown to be a biomarker of chlorine exposure to specific epithelial tissues in the upper respiratory tract. (Sochaski et al., 2008). These data are being used to develop and refine the PBPK model of tissue reactions.

Distinct tissue states can be identified on the basis of the degree of epithelial perturbation and oxidative stress. A hybrid BBDR model will best address this network of effects and integrate diverse endpoints at various levels of organization (e.g., gene arrays, biochemical assays and tissue histopathology) to describe pathogenesis.

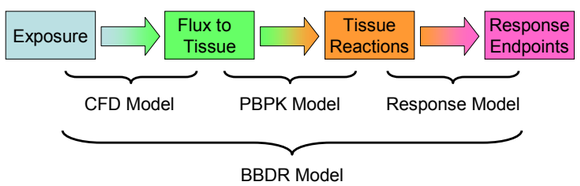
## Methods/Approach

### Conceptual MOA Model



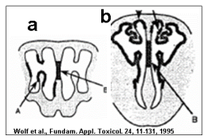

Uptake of inhaled chlorine is dictated by convective airflow and diffusion dictated by species-specific ventilation rate and airway anatomy.
Chlorine immediately hydrolyses in mucus to hypochlorous and hydrochloric acids that cause oxidative stress.
Oxidative stress results in epithelial disruption.
Disruption results in inflammation and cellular responses in tissue.

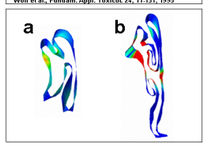
### Computational Approach



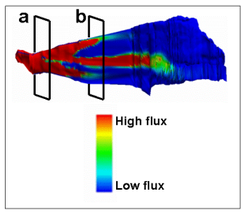

### CFD Model: Anatomy, Airflow and Flux

Uptake in the nose from both sexes of F344 rats measured to be 95% at1.0 to 2.5 ppm and100 to 400 ml/min
Air-phase of CFD model for formaldehyde modified with chlorine-specific air diffusivity to predict flux
Correlation with lesion data from bioassay suggests air flow is major determinant but also suggests a role for specific tissue susceptibility

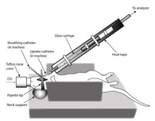



Preliminary CFD flux predictions and qualitative lesion correlation

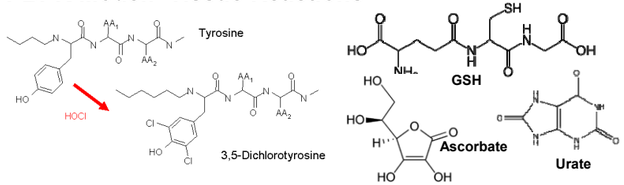



Uptake simulation

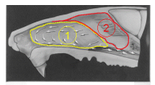


Isolated URT for gas uptake study

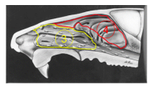
### PBPK Model: Tissue Reactions




Chlorine hydrolyzes rapidly to HOCl and HCL at pH ≥ 2
HOCL can react with aromatic protein residues to form N-chloro derivatives
Chlorotyrosine adducts serve as biomarkers for dose-response of HOCl tissue reactions
Biochemical assays of other soluble anti-oxidants also used to measure tissue reactions



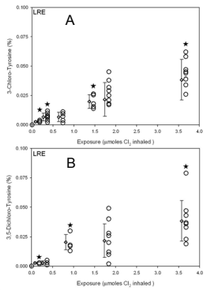
Septal Samples



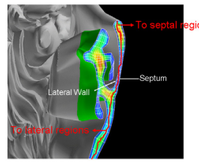
Lateral Wall Samples

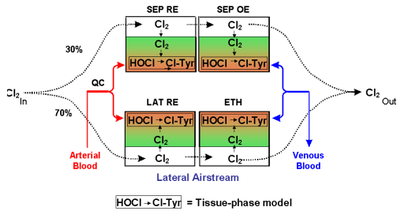
1. Septal Respiratory Epithelium (SEP RE)
2. Septal Olfactory Epithelium (SEP OE)
3. Lateral Wall Respiratory Epithelium (LAT RE)
4. Ethmoid Olfactory Epithelium (ETHMOID)

Specific sample sites to evaluate role of
  Airflow
  Tissue types
3,chloro (A) and 3,5-dichlorotyrosine (B) show dose-response (data for Lat RE shown)



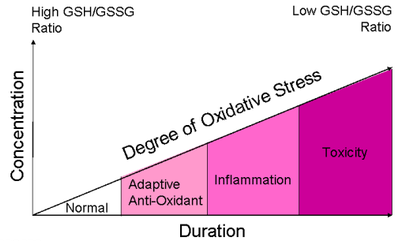

Septal Medial Airstream

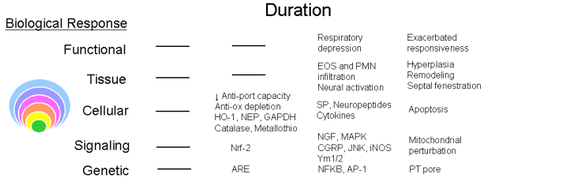


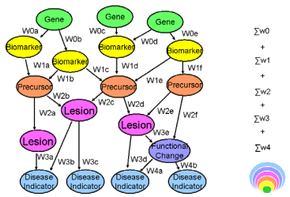

CFD mesh segmented to correspond to specific tissue sample sites
compute specific flux to different compartments
PBPK model of tissue reactions in each sample site of URT and determines boundary condition
Interface established by allocating airflow to each compartment
A hybrid CFD-PBPK model results that describes both airflow and tissue dosimetry

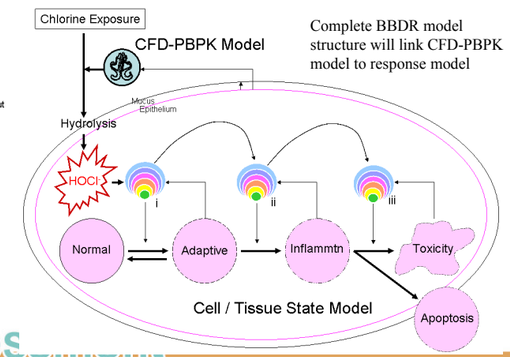
### Response Model: Endpoints and Tissue States




Different endpoint measures assigned to level of observation category
Tissue states demarcated by degree of oxidative stress
Network of endpoints used to motivate transition rates between cell/tissue states
Data will obtained in parallel between rats and humans
Human data will provide target context for testing value of information and modeling for interspecies extrapolation




Complete BBDR model structure will link CFD-PBPK model to response model

## Impact and Outcomes

The experimental and computational efforts embodied in this project are expected to provide the following contributions to NHEERL's human health program:

- Clarification of the role of oxidative stress, cytotoxicity and inflammation in epithelial disruption and pathogenesis of respiratory irritation from inhaled chlorine
- CFD-PBPK model to describe dosimetry of inhaled chlorine across species
- Response model that will differentiate between tissue states based on degree of oxidative stress, and possibly support alternative dose response modeling of adaptation and homeostasis
- BBDR model to improve duration and interspecies adjustments for inhaled chlorine and advance refinements in assessment approaches for reactive gases

## Future Directions

Application of the BBDR model to exposure scenarios of interest to NHSRC
Evaluation and generalization of extrapolation insights to other irritants
Bridge to virtual tissue models of specific reactions in the tissue phase of respiratory tract.
Link to cardiac and systemic distribution models to develop cardiopulmonary virtual tissue model

## Researchers Involved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