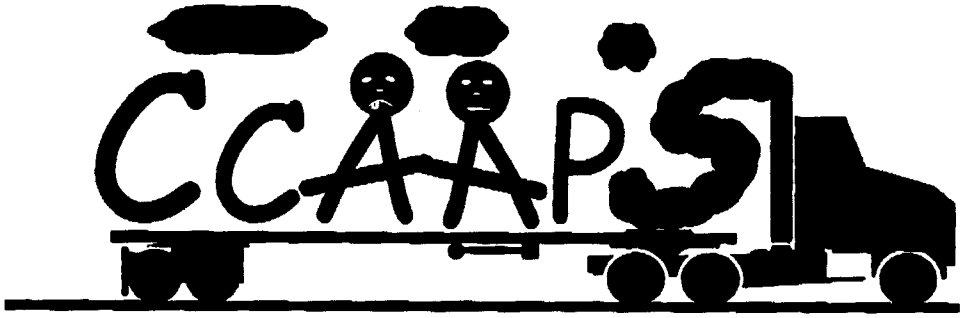


# Cincinnati Childhood Allergy and Air Pollution Study
**CCAAPS**

## October 2001 – September 2006

## Funded by the National Institute of Environmental Health Sciences (NIEHS)

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# Executive Summary

## Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS)

**Extent of the Problem:** Allergic disorders affect over 40 million children, resulting in two million missed school days and costing society more than $10 billion/year. Atopy, defined as immediate hypersensitivity to specific allergens, is the strongest risk factor for child-onset asthma. The reported increasing incidence of atopic respiratory disorders is exaggerated in urban children living in westernized countries. There is intriguing scientific evidence demonstrating that diesel exhaust particles (DEP), a constituent of truck exhaust, promote expression of Th2 cytokines and production of IgE antibodies. The concern is that these exposures enhance clinical expression of IgE mediated respiratory disorders. Hence, children residing near interstate highways are at potentially high risk for exposures to truck emissions and resultant atopic respiratory disorders.

In the Cincinnati metropolitan region, three interstate corridors intersect creating one of the busiest U.S. north/south and east/west commercial truck routes converging on a population of 1.9 million. The proposed investigation will follow two groups of children from birth through early childhood. The first group is children living within 400 m of interstate highways. This group will be matched by birth date, race and income to a second group living beyond 1 km. There are two study purposes. The first is to measure DEP exposure levels and to determine if children with higher levels of exposure are at an increased risk for atopy and atopic respiratory disorders. The second is to determine if these effects are magnified in a genetically at risk subpopulation. The proposed study is a prospective cohort and nested case control design. The cohort of newborns will be evaluated prospectively for positive skin prick tests (SPT), allergic rhinitis and asthma. Residential exposures to DEP and aeroallergens also will be characterized longitudinally. The children who develop positive SPT will be matched by race and gender to controls having negative but the same number of SPT. The case control study will evaluate potential susceptibility as measured by cytokine polymorphisms and to determine if exposure to DEP promotes the phenotypic expression of atopy and atopic respiratory disorders. This study design is optimum for determining if young children exposed to DEP have enhanced sensitization to aeroallergens and for dissecting gene-environment interactions. Results of this study may ultimately result in finding a <u>preventable</u> cause of atopic disorders in children.

**Goal of Study: To determine if infants who are exposed to DEP via truck exhaust are at an increased risk for atopy and atopic respiratory disorders and to determine if this effect is magnified in a genetically at risk population**.

**Overview of study design:** The overall investigation (Figures 1 and 2) includes both a prospective cohort study and a nested case-control study. The prospective cohort study recruits newborns (n = 792) living either within 400 m of interstate highways, hereafter referred to as exposed, or further than 1 km, hereafter referred to as unexposed and follows them to age 3 or 4. The goal of the newborn cohort study is to prospectively evaluate whether exposure to DEP increases sensitization to aeroallergens. Using birth certificate data and geocoding of addresses, parents living in exposed and unexposed areas will be identified and enrolled. Parents will be recruited and their eligibility will be determined by confirmation of atopic status via positive response to skin prick tests (SPT) to a panel of common aeroallergens. Each child's primary place of residence, including home and daycare, will receive an initial environmental evaluation by 6 months of age and yearly settled dust samples will be collected for evaluating levels of indoor aeroallergens. The child's exposure to DEP will be estimated every 4 months using a network of outdoor monitoring stations subsequent to the initial baseline visit. Children will receive annual SPT for 12 aeroallergens and a yearly medical evaluation directed at identification of allergic rhinitis and asthma symptoms. Home dust sampling and 3 outdoor monitoring stations will characterize the child's exposure relative to a skin test negative control group.

The nested case control study will include cases (n = 185) defined as children with a positive SPT. Cases will be matched by race and gender to children (n = 185) having negative but an equal number of SPT. The goal of the nested case control study is to ascertain if there is an increased risk associated with polymorphisms related to cytokine or cytokine receptor alleles. For the genetic analysis blood samples are collected from both case and control children and case parents. Individual personal air monitoring of each child's level of exposure to 6 aeroallergens also will be obtained.

**Study Location:** Ohio ranks fifth for the nation in miles of federal highways and when adjusted by square miles of land area, ranks third (BTS 1997). The study includes families residing in the Greater Cincinnati Metropolitan Region of southwestern Ohio and northern Kentucky, referred to as the Ohio and Kentucky River Valley (OKRV) Region. A 1998 investigation found that residents in the OKRV were exposed to higher levels of ozone smog more often than residents in Boston and New York (OEC 2000). Air pollution in this region is generated by 3 sources including industry, power plants and transportation, with the latter being the most important. Three major federal highways converge in the OKRV causing traffic volumes to be one of the largest with 135-150,000 vehicles and 12-16,000 trucks per 24 hours traveled on each. The I-75, I-74, I-71 radial corridors, the I-275 beltway and two lateral highway connectors, form the macro structure of the metropolitan area linking three states (OH, KY, IN) and cities, towns and villages into one continuous network, surrounding a population of 1.9 million (GCCC 1999). In 1997, the counties comprising the OKRV had higher rates for smog-related respiratory hospital admissions including asthma attacks and emphysema than these in Boston or New York City (OEC 2000). Due to a combination of traffic, topography and meteorological conditions, including high humidity, air pollutants are concentrated in this area as demonstrated by an EPA non-attainment rating for several years. In addition, this region is commonly referred to as "allergy alley" by the local medical community (OEC 2000). Therefore, the OKRV, represents an ideal location for understanding the impact that exposure to DEP and aeroallergen have on development of childhood allergy and atopic respiratory disorders.

**Subject Identification:** Monthly birth certificate records will be used to identify the cohort of newborns. Using a geographic information system (GIS) approach that utilizes ESRI's ArcView®, births will be mapped according to distance from interstate highways (English et al., 1999). Based on our prior mapping of addresses of the mother on the birth certificate, we estimated that 11% of births in OKRV occur to women residing within 400 m from federal highways. Thus, birth certificate records assist in the relatively quick identification of infants most likely to have the highest exposure to truck exhaust. Based on our preliminary investigations, we will exclude about 12.5% of the total births for those residing in the more uncertain DEP buffer areas (>400 m and <1 km) as shown in Figure 2. Other advantages of using birth certificates for cohort identification is that the cohort will be population based, the denominator known, biases related to using volunteers or clinic populations are avoided, and the cohort data base is generated in a standard unbiased manner.

**Nested Case Control Study**: We are estimating that by year 4, there will be a maximum of 251 children with a positive SPT (cases) available for the genetic analysis. Only 185 cases or 74% of 251 are needed to explore a possible genetic-exposure interaction effect, but all who agree will be included in the genetic analysis. After a child is SPT positive, each parent will be asked to sign a second informed consent for participation in the nested case control study. Each positive case will be matched to an *eligible* noncase (control) determined by season and year of birth, to allow each control the same opportunity in terms of exposure and time to become a case. Specifically, a control will be chosen at the time of case identification by sampling without replacement from the cohort of non-cases not previously included as a control.

In summary, recruited infants of atopic parents, as verified by SPT, will be prospectively followed for the development of allergic sensitization by undergoing yearly SPT. If a child becomes atopic, defined by one or more positive SPT, he/she will be identified as a case, and race and gender matched to a control who has received at least the same number of SPT as the case. These cases and controls (the nested cohort and the case parents) will be asked to participate in the genetic analysis proposed herein. Parents of the cases will be asked for a blood sample for DNA extraction for the genetic analysis, specifically, the TDT analysis. The parents of the controls will not be monitored further after their atopic status is determined, however, all homes of the participating cohort will continue to be evaluated for aeroallergens.

**Overview of the exposure assessment plan**: The exposure assessment measurements will focus on two particle size fractions: $PM_{2.5}$ for particles primarily emitted by transportation and the inhalable size fraction for aeroallergens. The $PM_{2.5}$ size fraction is of interest because DEP are primarily in this size fraction (Dockery et al., 1996), and the health effects of particles are more related to fine than to coarse particles (Krämer, et al., 2000). Furthermore, the $PM_{2.5}$ size fraction remains suspended and is transported by prevailing air currents to homes near highways (Jamriska et al., 1999; Kingham et al., 2000) where it efficiently penetrates into the interior. Both inhalable and $PM_{2.5}$ size fractions are deposited in the upper respiratory tract, the primary study focus area. For newborns to 5 years of age, 30% of 0.001-μm particles will deposit in the nasal passages, naso-oropharynx, and larynx. Particle deposition into the upper respiratory tract reaches a minimum value of 1% at 0.1 μm and a maximum value of 30% at 5 μm (ICRP 1994). Thus, both inhalable and $PM_{2.5}$ size fractions are relevant.

The sampling strategy is a multistage design as summarized in Table 1. Stages I through III will be done in all 792 homes. The first sampling stage is designed to provide surrogate

measures of potential exposure to diesel exhaust. The second stage estimates outdoor DEP at 18 sampling stations. Stage III gathers information on aeroallergens in dust to assess exposures in the child's microenvironments. Stages IV and V are conducted as part of the nested case control study and include outdoor and indoor assessment of exposures. Particle mass, particle composition (a fingerprint developed from 72 elemental species, including 15 primary signature elements) and personal aeroallergen exposure will be monitored. Since the nested case control study (n=370) includes about half the entire cohort (n=792) these data can be used to estimate exposures for the entire study population in the final data analysis. The total number of samples will be about 10,000 (plus additional 10% for field blanks and replicate analysis) for the five-year study. Of these samples, one-third will undergo elemental analysis and two-thirds of the samples will undergo analysis for aeroallergens. The results will also include morphology of particles and real-time information on the concentration and size distribution of particles in the size range of 0.02-20 μm in six homes during the four seasons.

**Table 1 Exposure Assessment Stages and Metric Values**

| Stage and Exposure Metric | Measured parameter (size range and samples) | Number of homes/stations | Number of samples/year[1] |
|---|---|---|---|
| Stage I:<br>Truck density load | - truck numbers<br>- location of the child's microenvironments in regard to the highways<br>- time spent in each microenvironment | 792 homes | Once/year |
| Stage II:<br>Outdoor monitoring: traffic-related particles (continuous)<br>Outdoor aeroallergens | - $PM_{2.5}$; analysis for mass, 15 primary and up to 72 total elemental concentrations, elemental and organic carbon. (DEP =0.1-0.3μm) ($PM_{2.5}$ = 0.01-2.5μm) | 18 stations | 324 |
| | - Pollen and fungal spores[2](2 - > 100μm), microscopic counting and identification | 3 stations | 645 |
| Stage III: Walkthrough | - walkthrough checklist<br>- dust samples; analysis for cat, dog, house dust mite, cockroach, *Aspergillus, Alternaria* | 792 | Walkthrough: Y1:528; Y2:264;<br>Dust samples: Y1: 528 ; Y2: 1056; Y3: 729; Y4 :671 |
| Stage IV:<br>Indoor monitoring of aeroallergens | - inhalable aeroallergens[2]; analysis for cat, dog, house dust mite, cockroach, pollen and fungal spores | Cumulative thru year 04 at 370 | Y1:52; Y2:364; Y3:628; Y4:740; |
| Stage V:<br>Detailed indoor monitoring | - $PM_{2.5}$; analysis for mass, 15 primary and up to 72 total elemental concentrations, elemental and organic carbon. Seasonal variation of inhalable aeroallergens[2] (1-20 μm)<br>- $PM_{0.1\text{-}0.3}$, analysis for mass, 72 elemental concentrations, elemental and organic carbon (0.1 – 0.3 μm)<br>- Dynamic measurement of fine and coarse particles[3]<br>Particle morphology (modified electrostatic sampler, total dust) | 6+ | Y2-4: PM2.5 and $PM_{0.1\text{-}0.3}$: 6 homes x 2 seasons x 5 days x 2 size fractions = 120/year;<br>Seasonal variations in aeroallergens: 6 homes x 2 seasons x 5 days = 60/year<br>Morphology:<br>6 homes x 4 samples = 24/year |

[1]The number of analyses will be 10% higher than the number of samples, because of 5% field blanks and 5% replicate analyses.
[2]Button Inhalable Aerosol Sampler (SKC, Inc.) size range 0.01->100 μm (Teflon filter with 2 μm pore size has 99.99% collection efficiency for the most penetrating particles size: approximately 0.3μm).
[3]P-track fine particle counter (TSI, Inc.), size range 0.02-1μm; Grimm optical particle counter (Grimm Technologies, Inc.) size range 0.3 – 20 μm.

**Outdoor aeroallergens:** Outdoor pollen and fungal spore counts will be continuously measured at 3 stations. Sources for pollen and fungal spores are more equally spread throughout the study area, whereas DEP sources are primarily concentrated along the highways. Therefore, fewer monitoring stations are needed for outdoor aeroallergen monitoring than for DEP. Pollen release and dispersal strongly depends on climatic factors, mainly temperature and relative humidity (Muilenberg 1995). Therefore, we propose to have one station located in the southern part of the study area (Kentucky), and one station in the northern part. These two stations will be selected

among the 18 established for the measurement of DEP. The third station will be in the air monitoring station of Hamilton County that is located 2 miles north of the Ohio River, approximately in the middle of the study area. This station is currently using a Rotorod sampler for monitoring. However, we will use the Button Personal Inhalable Aerosol Sampler for several reasons. The Rotorod sampler is non-volumetric and collects larger particles more effectively than smaller particles thus representing a semi-quantitative sampling (Crook 1995). Among the available volumetric samplers, we selected the Button Sampler for indoor sampling of aeroallergens as this device was proven to be an adequate personal monitor of inhalable aeroallergens with the sampling efficiency independent of ambient air velocity whether worn or freely suspended (Aizenberg et al., 1998, 2000). This is particularly advantageous especially for outdoor sampling. Sampling with an inhalable sampler will provide a more accurate estimate of exposure than a "total" sampler, because the inhalable sampler collects particles that can be inhaled into the human upper and lower respiratory system. By using the same sampler both indoor and outdoors, we can compare concentrations. As parallel sampling will be done with the Rotorod and Button Sampler in one of the stations, the results can be qualitatively compared.

**Walkthrough and Indoor Dust Sampling:** A walkthrough home evaluation will be conducted in all of the 792 homes included in the study to assess important characteristics and conditions of the housing, outdoor-indoor transport of traffic-related particles, and the presence of indoor sources for aeroallergens. Each home evaluation includes a check list of the following: characterization of the home (e.g., age, size and number of rooms), sources of aeroallergens (e.g., presence of pets, moisture problems), transport mechanisms of particles between the outdoor and indoor air (e.g., type of ventilation system, opening of windows), and activities of the family (e.g., hobbies, housekeeping, cooking areas). Humidity measurements in the air and on the surfaces will be performed with a thermohygrometer in each room and recorded on the checklist. The checklist for this study will be modified from existing ones (USEPA 1991; USEPA 1994; Brunekreef 1993; Macher 1999). A walkthrough home evaluation will be conducted once in each home within 6 months of the child's birth.

Dust samples will be collected by the study team in all of the 792 homes during the home evaluations. The families will be asked to repeat the dust sampling using their own vacuums during July – early September, when the humidity is highest and thus, the concentration of house dust mites and mold is expected to be highest. At time of the home evaluation, the parents will be instructed on how to do the sampling for each follow-up sampling period and will be given all materials and necessary mailing devices. For those homes that do not have a vacuum cleaner, the study team will do the dust sampling. Some variation in the results is expected, caused by the use of different kinds of vacuum cleaners owned by the families. However, by asking the families to conduct the sampling, dust measurements can be done in all 792 homes during the high humidity season avoiding the effect of seasonal variation. To test the representativeness of samples taken by the families, parallel sampling will be done by the research team in 5% randomly selected homes every year. In addition, replicate analysis will be performed on 5% of randomly selected samples. The dust sampling will be done by vacuuming into a small dust collector trap that will be inserted in the vacuum cleaner hose upstream of the vacuum cleaner bag. Each sample will be collected from the floor of the child's primary activity room by vacuuming 1 $m^2$ area for 2 minutes as described by Arlian (1999). The samples will be sieved to remove particles larger than 300 μm and the fine dust will be weighed and frozen until the immunochemical allergen analysis. Dust samples will be extracted in 2 ml of PBS/Tween and centrifuged. The

supernatants will be collected and analyzed for cat, dog, house dust mite (*Dermatophagoides farinae*), cockroach (*Blatella germanica*), and selected mold allergens (*Alternaria* and *Aspergillus)* using enzyme-linked immunosorbent assay (ELISA). The home evaluation and the allergen concentration in the dust will be used to develop a profile of each home at low or high risk for indoor and outdoor aeroallergen. For house dust mite and cat allergen, proposed threshold values (2 μg/g and 8 μg/g, respectively) will be used to classify each home at low and high category. For the other aeroallergens, distributional properties of observed mean will be used to form categories.

Endotoxins partially formed from the outer cell membrane of gram-negative bacteria are potent pro-inflammatory substances and may be responsible for modulating severity of symptoms and lung function in atopic and non-atopic asthmatics. Endotoxins have not been identified as a risk factor for the onset of atopy, and atopy does not appear to be a modifying factor in regard to endotoxin related acute peak flow changes or respiratory symptoms (Michel et al., 1996; Zock et al. 1999). Samples of household dust from each participant will be archived and stored at 7°C for potential future analysis of endotoxin concentrations if there is support in future scientific publications to pursue this factor (Douwes et al., 1995). In addition, studies have suggested that latex allergens or latex cross-reactive material in airborne and sedimented particulate matter derived from tire debris may be contributing to latex allergy. There is uncertainty as to whether the latex allergens or latex cross-reactive material in airborne samples are derived from tire dust or from other industrial sources. Latex antigens have not been FDA approved for SPT. If the incidence of atopic respiratory disorders, however, is increased in families living close to major highways, exposure to latex or latex like allergens may need to be considered as a traffic-related allergen. For this reason, indoor dust samples also will be archived from each participant's home for potential future identification of latex or latex cross-reactive material.

**Indoor Monitoring of Aeroallergens:** Indoor aeroallergen measurements will be performed in 370 homes selected for the nested case control study. The focus of the case-control study is gene-environmental interactions. To examine the relationship between atopy and atopic respiratory disorders and the exposure in genetically predisposed children, it will be important to obtain quantitative estimates of exposure. Therefore, a rigorous characterization of aeroallergen exposure, including personal air sampling, will be part of the case control study. These exposure data will be utilized for estimates of exposure for all children in the cohort study. As the homes in the case-control study will have both dust and air measurements for indoor aeroallergens, a non-linear programming estimation will be used to predict the personal exposure value from indoor allergen levels for homes that are in the cohort study but not in the case-control study. In addition, we will be able to determine the correlation of these 2 measures and what home factors may predict a high or low correlation. These data will be useful for adjusting the findings of dust measures in the larger cohort study.

The Button Personal Inhalable Aerosol Sampler will be used to collect aeroallergens inside the homes twice per year. The sampling will be done in the spring, during the tree pollen season (March 15 through June 1), and in the fall (August 15 through October 1). The fall sampling will coincide with the ragweed pollen season and with the high humidity period, when house dust mite and fungal spore concentrations are highest. In the beginning, the mother or caregiver will be asked to place the Button Sampler near the breathing zone of the child when the child is in a chair, bed, or plain area. By age 3 others have shown that children can wear and have fun wearing a personal sampling pump weighing about 2-lbs (Carmichael 1997). Then at

about age 3, the child will be asked to carry the personal sampling pump in a backpack during the waking hours. The sampling time for indoor allergens will be 48 hours. The Button Sampler will be used with a Teflon filter. The filters will be weighed to obtain the inhalable mass concentration. After that, the aeroallergens will be extracted from the filters with PBS/Tween, and the suspensions will be analyzed by ELICA for cat, dog, house dust mite, cockroach and selected mold (*Alternaria, Aspergillus*) allergens, with ELISA. The rest of the suspension will be filtered through a mixed cellulose esterase filter, and analyzed for pollen and fungal spores as described above in Stage II. One field blank will be analyzed for every 20 air-samples. Replicate analysis will be performed for 5% of randomly selected samples. Based on the time spent home indoors, each child will receive a time-weighted exposure value for each indoor aeroallergen.

**Detailed Indoor Monitoring:** A more detailed characterization of the airborne particles will be performed in selected homes during Years 2 through 4. Traffic-related particles will be measured simultaneously both indoors and outdoors for six homes to verify the (I/O)-ratios used in exposure modeling in Stage II. These homes will be selected from among those already enrolled for indoor aeroallergen sampling in Stage IV. These homes will represent "typical homes" in terms of maintenance and indoor sources for aeroallergens and typical ventilation systems. We will randomly select 3 homes near the highway and 3 in the background areas. At this time, we are considering the following three ventilation categories: 1) central air conditioning with minimum opening of windows, 2) window air conditioning units with minimum opening of windows, 3) ventilation based mainly on opening the windows (either the house does not have air conditioning units or it is not used). The final categorizing will be based on the results of the home evaluations. These houses will serve as a model for similar houses in the study. The main goals for the detailed indoor monitoring are to validate the model used for outdoor-indoor transport of diesel particles (Stage II). In addition, this will provide information on the temporal variations of indoor aeroallergens and will be used to estimate the measurement accuracy in the rest of the home and to obtain information on the temporal variations of indoor aeroallergens throughout the entire year (Stage IV).

These measurements will include $PM_{2.5}$ as described above, the mass and the elemental concentrations in the size fraction of 0.1 – 0.3 μm, particle size and concentration (obtained with direct reading instruments), and analysis of particle morphology. The size fraction of 0.1 – 0.3 μm is of interest, because particles from diesel engines range primarily in this size (Hughes et al., 1999). This size fraction will be collected with a Moudi-impactor at a flow rate of 30 l/min. The sampling time will be decided after the ongoing pilot study, but it is expected to be about 24 hours. The dynamic measurement of particle size and concentration will be performed with a Grimm optical particle sizer for the particle size range of 0.3 - 20 μm, and with the P-Trak fine the particle counter for particle size range of 0.02 - 1 μm. The dynamic measurement results will be linked with the data on the child's activities during the same 24-hour period, recorded on the time activity diary. The samples for analysis of the individual particle morphology and elemental concentrations will be collected with a modified electrostatic sampler and analyzed with a scanning electron microscope and by electron diffraction analysis. The attachment of diesel exhaust particles on aeroallergen particles (identified by morphology) will also be studied from these samples. In this analysis, the indoor and outdoor samples will be collected and characterized and their results will be compared to typical diesel combustion aerosols (generated in a controlled laboratory system) to establish similarities in morphology and other characteristics.

**Summary:** Compared to other pollutants, DEP exposure in humans appears unique in its effect on IgE production and atopy and may be particularly potent on the immature and developing child. The identification of the genetic and environmental influences on atopy and atopic respiratory disorders during early childhood will lead to greater understanding of the pathophysiology and provide more insight into the development of preventative measures for these common childhood disorders and thereby interrupts the sequela of atopic respiratory disorders.

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**Literature Cited:**


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**Figure 1. Allergic Enhancement by Diesel Exhaust Particles**

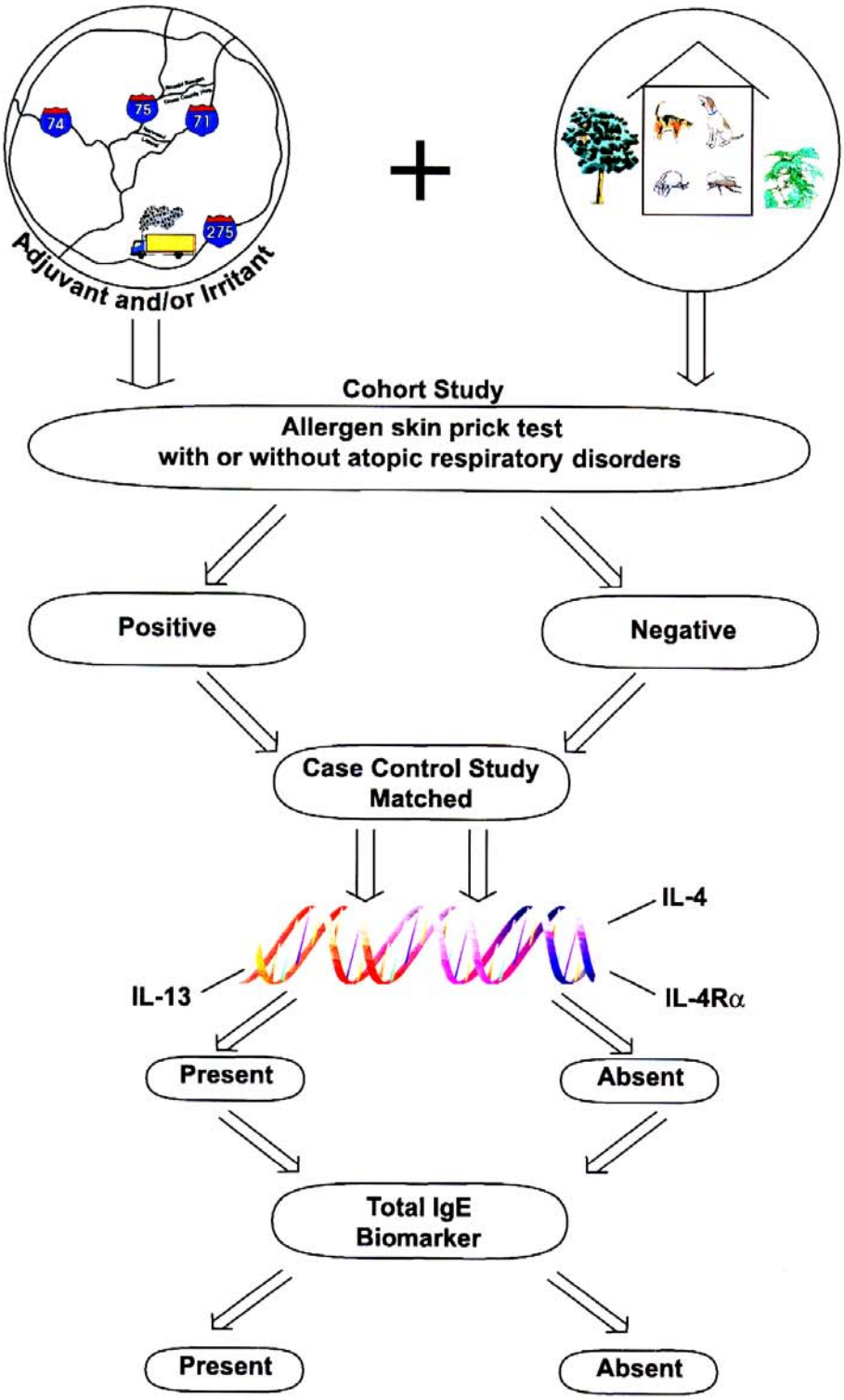

**Figure 2 Overall Study Outline of Subject Recruitment**