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ASTHMATICS AS A SUSCEPTIBLE POPULATION IN HEALTH RISK ASSESSMENT OF AIRBORNE CHEMICALS

Mia Johansson

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Asthmatics as a Susceptible Population in Health Risk Assessment of Airborne Chemicals

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Mia Johansson

Principal Supervisor:
Associate Professor Mattias Öberg
Karolinska Institutet
Institute of Environmental Medicine
Unit of Work Environment Toxicology
Swedish Toxicology Sciences Research Center (Swetox)

Co-supervisor(s):
Professor Gunnar Johanson
Karolinska Institutet
Institute of Environmental Medicine
Unit of Work Environment Toxicology

Associate Professor Linda Schenk
Karolinska Institutet
Institute of Environmental Medicine
Unit of Work Environment Toxicology
Royal Institute of Technology
Department of Philosophy

Opponent:
Professor emeritus Len Levy
University of Cranfield
Institute of Environment and Health

Examination Board:
Associate Professor Bert-Ove Lund
Swedish Chemicals Agency
Swedish University of Agricultural Sciences

Associate Professor Anna-Carin Olin
Göteborg University
Institute of Medicine
Department of Occupational and Environmental Medicine

Associate Professor Mikael Adner
Karolinska Institutet
Institute of Environmental Medicine
Unit of Experimental Asthma and Allergy Research

To my family

ABSTRACT

Asthma is a common chronic airway disease and about 235-300 million individuals of all ages are affected by the disease around the world. A precondition of asthma has been suggested to increase individual susceptibility to acute exposure from airborne irritant chemicals. In the health risk assessments of such chemicals, acute guideline values are derived to guide in preparedness and in emergency response, and to protect the general population, including susceptible subpopulations such as asthmatics. Experimental data on susceptible subpopulations are lacking for many chemicals and default inter-individual assessment factors (AFs) have been applied in the derivation of guideline values. However, the scientific basis for these default AFs in regard to asthmatics has been inadequate.

The aims of this thesis were threefold. First, to study the extent to which experimental data regarding chemicals tested on asthmatics are included in the derivation of different sets of acute to short-term guideline values, and second, to determine whether there is a general difference in the airway response between healthy and asthmatic individuals, and whether current AFs for inter-individual variability provide sufficient protection for asthmatics. Last, to gain more knowledge on the susceptibility of asthmatics by measuring the airway response to chlorine in naïve and allergen-sensitized mice.

In Paper I, the analysis of how asthmatics are considered in the derivation of Acute Exposure Guideline Levels (AEGLs) reveals that only 14 of 250 chemicals in the support documents had been tested on asthmatic subjects in experimental studies. A comparison between the AEGL support documents and nine other sets of acute to short-term guideline values shows that all of them were incomplete with respect to experimental data on asthmatics. In Paper II, experimental studies in which both healthy and asthmatic subjects were tested under the same conditions served as the basis for evaluating the lowest observed adverse effect concentrations (LOAECs) for healthy subjects and for asthmatic subjects. The ratios of these LOAECs were calculated and presented as estimated differential response factors (EDRFs). We found evidence of higher sensitivity among asthmatics (EDRF > 1) to 8 of 19 tested chemicals, and to 3 of 11 mixtures. Thereafter, concentration–response relationships confirmed the higher sensitivity of asthmatics to sulfuric acid and sulfur dioxide, and the Benchmark concentration (BMC) analysis of sulfur dioxide indicated a nine fold higher sensitivity among asthmatics. In Paper III, the consideration of asthmatics in the Derived No-Effect Levels (DNEL), developed under the European Union (EU) registration, evaluation, authorization and restriction of chemicals (REACH) legislation revealed that only 14 registered chemicals had been tested on asthmatics in experimental studies. Many of the DNELs for acute inhalation were higher than our estimated overall LOAEC or no observed adverse effect concentrations (NOAECs) in asthmatics, indicating a low or no safety margin. The experimental asthma model in Paper IV showed that exposure to 80 ppm of chlorine in naïve (but not OVA-sensitized) mice resulted in increased airway responsiveness and elevated numbers of neutrophils in the bronchoalveolar lavage fluid. Concentration-dependent reductions in respiratory frequency were seen in both groups. These results do not support an increased susceptibility to chlorine among mice with induced eosinophilic airway inflammation. Exclusion of asthmatics in the derivation of acute to short-term guideline values may interfere with trustful and efficient health-protective actions. The use of an AF of 10 when there is a lack of experimental data on asthmatics may be adequate to protect this subpopulation from the deleterious respiratory effects of airborne chemicals.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Idag lever ungefär 235-300 miljoner individer med astma i världen och antalet har ökat de senaste årtiondena. Personer i alla åldrar och samhällsklasser drabbas och i Sverige har ungefär en av tio sjukdomen. Astma är en luftvägssjukdom som kännetecknas av inflammation i luftvägarna och episoder med svårigheter att andas, s.k. astmaattacker. Olika faktorer som fysisk ansträngning och exponering för allergener och luftburna kemikalier kan utlösa en astmaattack.

Exponering för luftburna irriterande kemikalier kan ske på arbetsplatser, men allmänheten kan också exponeras i samband med transport- och industriolyckor. Hälsoriskbedömningar genomförs för att veta när man behöver vidta säkerhetsåtgärder i samband med exponering för kemikalier. Som ett led i riskbedömningen kan riktvärden beräknas. Riktvärden motsvarar den maximala kemikaliekoncentration vid vilken inga negativa hälsoeffekter uppstår hos individer, och de kan beräknas för olika exponeringsvägar, exponeringstider och för olika grupper i samhället. När det saknas information om hur en kemikalie påverkar astmatiker använder man data från friska försökspersoner eller försöksdjur. För att kompensera för den osäkerhet som detta innebär används en extra säkerhetsmarginal, en s.k. bedömningsfaktor eller säkerhetsfaktor.

Syftet med den här avhandlingen var; 1) att undersöka om astmatiker är skyddade från akut exponering för luftburna kemikalier både på och utanför arbetsplatsen, 2) att studera hur stor skillnaden är i känslighet mellan astmatiker och friska individer när de exponeras akut för luftburna kemikalier, och, 3) att få mer kunskap om astmatikers känslighet genom att experimentellt studera hälsoeffekter från kemikalieexponering i en astmamodell på möss.

För att ta reda på hur myndigheter och organisationer i olika länder och på EU-nivå har tagit hänsyn till astmatiker i framtagandet av hälsobaserade akuta riktvärden analyserades hälsoriskbedömningar i delarbete I och III. I delarbete II analyserades data från tidigare experimentella studier, gjorda på astmatiska och friska individer, för att ta reda på hur stor skillnaden är i känslighet mellan grupperna. I den experimentella modellen (delarbete IV) exponerades både möss med allergen-inducerad luftvägsinflammation och friska möss för klorgas i olika koncentrationer. Effekter på andningsfrekvens och lungfunktion samt inflammationssvar och lungskador analyserades.

Resultaten visar att man inte tar tillräcklig hänsyn till astmatiker vid framtagandet av akuta riktvärden och att riskbedömare inte använder sig av alla tillgängliga experimentella data på astmatiker som underlag i hälsoriskbedömningar. Astmatiker visade sig vara känsligare än friska för 11 av 30 irriterande kemikalier och blandningar och de var upp till nio gånger känsligare än friska vid exponering för svaveldioxid. Den använda djurmodellen visar inte någon ökad känslighet mot klorgas hos djur med allergisk luftvägsinflammation. Expertgrupper och riskbedömare bör systematiskt ta astmatikers eventuella känslighet i beaktande i framtagandet av akuta riktvärden för luftburna ämnen. Utifrån våra data föreslår vi användandet av en säkerhetsfaktor på 10 för att skydda astmatiker när experimentella data fattas för denna grupp.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following articles. They will be referred to as **Paper I-IV**.

I. **Johansson M**, Johanson G, Öberg M. 2012. How are asthmatics included in the derivation of short-term values for emergency planning and response? *Regul. Toxicol. Pharmacol.* 63:461-470

II. **Johansson M**, Johanson G, Öberg M. Evaluation of the experimental basis for assessment factors to protect individuals with asthma from health effects during short-term exposure to airborne chemicals. *Crit. Rev. Toxicol.* *(E-pub ahead of print http://dx.doi.org/10.3109/10408444.2015.1092498)*

III. **Johansson M**, Johanson G, Öberg M, Schenk, L. Does industry take the susceptible subpopulation of asthmatic individuals into consideration when setting Derived No-Effect Levels (DNELs)? *(Submitted for publication)*

IV. **Johansson M**, Gustafsson Å, Johanson G, Öberg M. Comparison of airway response in naïve and ovalbumin sensitized mice during short-term inhalation exposure to chlorine. *(Manuscript)*

CONTENT

LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AEGL	Acute Exposure Guideline Levels
AF	Assessment factor
AHR	Airway hyperresponsiveness
AIHA	American Industrial Hygiene Association
ATSDR	U.S. Agency for Toxic Substances and Disease Registry
BALF	Bronchoalveolar lavage fluid
BMC	Benchmark concentration
C_{RS}	Respiratory compliance
CLP	Classification, Labelling and Packaging Regulation
DECOS	Dutch Expert Committee on Occupational Standards
DFG	German Research Foundation
DNEL	Derived No-Effect Levels
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
EDRF	Estimated differential response factor
ERPG	Emergency Response Planning Guidelines
FEV1	Forced expiratory volume in one second
FVC	Forced vital capacity
G	Peripheral tissue resistance
H	Peripheral tissue elastance
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
INERIS	French National Institute for Industrial Environment and Risks
LOAEC	Lowest observed adverse effect concentration
MAK	Maximum Concentration at the Workplace
MCh	Methacholine
MRL	Minimal Risk Levels
NOAEC	No observed adverse effect concentration
NRC	US National Research Council
OA	Occupational asthma
OEHHA	California Office of Environmental Health Hazard Assessment
OEL	Occupational exposure limits

OVA	Ovalbumin
POD	Point of departure
RD_{50}	Exposure concentration that induces a 50% depression in respiratory frequency
REACH	Registration Evaluation and Authorization of Chemicals
REL	Reference Exposure Levels
R_n	Newtonian resistance
R_{RS}	Respiratory resistance
SCOEL	Scientific Committee on Occupational Exposure Limits
SE-OEL	Swedish Occupational Exposure Limits
SRaw	Specific airway resistance
STEL	Short-term exposure limits
SWEA	Swedish Work Environment Authority
TD	Toxicodynamic
TK	Toxicokinetic
TLV®	Threshold Limit Values
TSD	Technical support document
U.S. EPA	U.S. Environmental Protection Agency
VSTAF	French Acute Toxicity Threshold Values
WEA	Work exacerbated asthma
WOE	Weight of evidence

1 INTRODUCTION

The focus of this doctoral thesis is to study asthmatics as a susceptible population in health risk assessment of airborne hazardous chemicals.

Acute exposure to such chemicals can be linked to fires, accidental releases in workplaces, or to transportation accidents. Additional examples are natural disasters, chemical warfare, and terrorist attacks. The effects of acute exposure on the airways in humans may vary from mild discomfort to severe medical conditions or lethality. Several national and international organizations (e.g., the United States Environmental Protection Agency, which appoints the National Advisory Committee on Acute Exposure Guideline Values, NAC/AEGL) have developed health-based guideline values for acute to short-term exposure with the aim of protecting the general population, including susceptible subpopulations, against the adverse effects from acute exposure to irritant chemicals.

When guideline values are determined, assessment factors (AFs, also called uncertainty factors or safety factors) are often used to compensate for the variation in susceptibility between species and between individuals. It has previously been found that there is a lack of knowledge regarding the consideration of asthmatics as a susceptible population in health risk assessments and that the scientific basis for AFs is inadequate. In addition, the number of experimental studies testing the effects of acute chemical exposure on asthmatic individuals is limited due to ethical and practical difficulties. Additional experimental studies are therefore needed to gain more information on the susceptibility of asthmatics.

This thesis includes investigations on how asthmatics are included in the derivation of different sets of acute to short-term guideline values (Papers I and III), an extensive evaluation of the scientific basis for AFs to protect asthmatics (Paper II), and an experimental asthma model to investigate airway response following chlorine administration in mice (Paper IV).

2 BACKGROUND

2.1 ASTHMA

Asthma is a chronic inflammatory airway disease with increased airway hyper-responsiveness (AHR). The disease is often associated with a history of respiratory symptoms (e.g., wheezing, chest tightness, a shortness of breath, and coughing) and expiratory airflow limitation. Triggers such as exercise, allergen or irritant exposure, or poor adherence to medication may cause episodes that represent a change in symptoms and lung function from the patient's usual status, i.e., asthma exacerbations. Severe exacerbations may be life threatening (Global Initiatives for Asthma (GINA) 2015).

2.1.1 Prevalence

According to the World Health Organization (WHO) and GINA, there are approximately 235–300 million asthmatic individuals in the world and asthma is currently one of the most common chronic diseases (GINA 2015; WHO 2015). Increased urbanization will likely lead to an increase in asthmatics worldwide, but the nature of this relationship is, as of yet, unclear (Croisant 2014; Riedler et al. 2000; WHO 2015).

The prevalence of asthma ranges from 1% to 18% in different countries and has increased among both children and adults over the past few decades. The increased prevalence has been

associated with that for other allergic diseases such as eczema and rhinitis (Masoli et al. 2004); a trend observed over the past 40 years (Croisant 2014). Asthma accounts for 1 in 250 deaths worldwide, with many of them due to suboptimal long-term medical care and a delay in obtaining help during an asthma attack, and these deaths are often preventable (Masoli et al. 2004).

The economic cost of asthma is considerable both in terms of direct medical costs (hospital admissions and pharmaceuticals) and indirect costs (time lost from work, school, and premature death) (Bahadori et al. 2009; GINA 2015; Masoli et al. 2004). The costs are correlated with comorbidities, age, disease severity, and the level of asthma control (Bahadori et al. 2009).

2.1.2 Causes and phenotypes

The causes of asthma are not completely understood. Nevertheless, the strongest risk factors for developing the disease are a combination of a genetic predisposition with exposure to inhaled substances and particles that may irritate the airways or provoke allergic reactions. Examples of such substances and particles are indoor and outdoor allergens (e.g., house dust mites, pollution, pet dander, pollens, and molds), environmental tobacco smoke, chemical irritants in the workplace, as well as air pollution. Additional triggers are cold air, extreme emotional arousal (e.g., anger or fear), physical exercise, and certain medications such as beta-blockers, aspirin, and other non-steroidal anti-inflammatory drugs (WHO 2015). One of many barriers to reducing the burden of asthma involves environmental factors such as indoor and outdoor air pollution, smoking, and occupational exposures. These factors have to be identified and properly addressed (GINA 2015; Masoli et al. 2004).

Asthma is a heterogeneous disease that is often divided into different phenotypes. More information on the most common phenotypes is presented in Table 1 (GINA 2015). There are several additional adult phenotypes of asthma. Some are related to triggers such as aspirin or non-steroidal anti-inflammatory drugs, environmental allergens, occupational allergens or irritants, menses, or exercise (Wenzel 2006). The severity level of asthma may change over months or years but the levels are often classified as mild intermittent, mild persistent, moderate persistent, or severe persistent disease (GINA 2015; Masoli et al. 2004).

Table 1. The most common phenotypes of asthma (GINA 2015).

Phenotype	Description
Allergic asthma	Most common phenotype. Eosinophilic airway inflammation that often starts in childhood.
Non-allergic asthma	Can be present in adults. Sputum sometimes includes neutrophilic or eosinophilic cells.
Late-onset asthma	Non-allergic. Adults, especially women.
Asthma with fixed airflow limitation	Patients with long-term disease. Probably due to airway wall remodeling.
Asthma with obesity	Little eosinophilic airway inflammation but prominent respiratory symptoms.

Most cases of asthma start in childhood from viral infections in the respiratory tract or exposure to cigarette smoke and other airborne pollutants that lead to a lymphocyte T-helper type 2 (T_H2) immune response (Fahy 2013). T_H2 secretes cytokines (interleukin (Il)-3, Il-4, Il-5, Il-9, Il-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF)), which initiate a cascade of allergic inflammation such as T_H2 cell survival, B-cell isotype switching to immunoglobulin E (IgE)-synthesis, mast cell differentiation and maturation, eosinophil

maturation and survival, and basophil recruitment (Holgate 2012). From this cascade, children with a pre-existing atopy, or specific genetic risk factors for T_H2 inflammation regulators, or those children who have other less well-understood susceptibility will become asthmatic. Why T_H2 immune responses are initiated during childhood and become persistent is not well understood (Fahy 2013).

About 50% of adults with asthma and almost all asthmatic children have allergic asthma. In allergic asthmatics, IgE antibodies are present in the serum and/or skin-prick tests are positive for common allergens (Kudo et al. 2013). The numbers of CD4+ cells, producing Il-4 and Il-5, are also increased in asthmatics with allergic asthma (Lambrecht and Hammad 2015). Il-13 plays an important role in regulating airway inflammation. The T_H17 and T_H9 cell subtypes are also known to contribute to the inflammation, or to enhancing smooth muscle contraction, or stimulating mast cells (Kudo et al. 2013). Airway remodeling concerns the structural changes in the airway walls, which is an important cause of irreversible airway narrowing and airflow limitation. Airway remodeling is caused by repeated cycles of injury and repair and is a result of long-term inflammation. Studies also suggest that mechanical stresses from bronchoconstriction may lead to remodeling (Hirota et al. 2013).

Non-allergic asthma usually develops in adulthood and neither T_H2 cells nor IgE antibodies are present (Lambrecht and Hammad 2015). Non-allergic neutrophilic asthma is related to the T_H17 immune response (Pelaia et al. 2015).

It has been proposed that the term “endotype” should be used instead of “phenotype” to highlight the molecular heterogeneity of asthma and to target treatment at the molecular mechanism (Fahy 2013). The presence of the cytokines Il-4, Il-5, and Il-13, as well as eosinophils in the blood and tissue, determines if the endotype is defined as T_H2-high or T_H2-low asthma (Lambrecht and Hammad 2015). However, the mechanisms involved in asthma endotypes other than the T_H2-high endotype are not well understood (Fahy 2013).

The cause of about 17% of all adult-onset asthma in the working population is thought to be due to occupational exposures (Toren and Bland 2009). Baur and colleagues (2012) conclude that the prevalence of irritant-induced occupational asthma (OA) is underreported. Brooks et al. (1985) identified the acute onset of hyperreactivity of the airways as being associated with exposure to strong irritants and named the illness “reactive airways dysfunction syndrome” (RADS) (Brooks et al. 1985). Baur et al. (2012) concluded that 47 different agents (e.g., chlorine, cleaning agents, and isocyanates) might cause RADS. However, the susceptibility of individuals with OA will not be investigated in this thesis.

2.1.3 Diagnosis

An asthma diagnosis is made by identifying the typical respiratory symptoms (e.g., wheezing, chest tightness, a shortness of breath, and coughing) and by the individual having a variable expiratory airflow limitation (GINA 2015). Ward and colleagues (2004) suggest, based on a questionnaire study, that there is an 89.4% chance that an asthma diagnosis is accurate.

Physiological lung function tests (i.e., spirometry) measure how an individual inhales or exhales volumes of air as a function of time and the tests are applied when diagnosing asthma (Miller et al. 2005a). Some of the important spirometry tests are the forced vital capacity test (or FVC, the volume delivered during an expiration made as forcefully and completely as possible starting from a full inspiration), the forced expiratory volume in 1 second (or FEV_1, the volume delivered during the first second of the FVC maneuver), and the peak expiratory

flow test (or PEF, the maximum speed of expiration). Measuring FEV_1 is considered as a more reliable method for diagnosing asthma as compared to PEF because the results may differ between different PEF meters. The FEV_1/FVC ratio is normally within the range of > 0.75–0.80 in adults and > 0.90 in children. Values lower than these suggest airflow limitation and can be identified from flow meters with age-specific predicted values (GINA 2015).

Reversibility testing with drug administration is commonly used to determine how the patient's lung function can be improved with treatment (Miller et al. 2005a). Moreover, variations in improvement and/or a deterioration in the symptoms and lung function (during one day, day-to-day, visit-to-visit, seasonally) can be measured to confirm the diagnosis of asthma (GINA 2015). Repeated spirometry testing should be performed by the same examiner and the time of day should be within 2 h of previous test times (Miller et al. 2005b). The minimal important difference for an improvement or worsening of the FEV_1 result based on the asthmatic patient's perception of change is about 10%. Generally, in adults, an increase or decrease in the FEV_1 result of > 12% or a change in the PEF reading of at least 20% is considered as asthma (GINA 2015).

The diagnosis of allergic asthma (or atopic status) can be determined by skin-prick testing or by measuring the level of antigen-specific IgE in the serum (GINA 2015). In addition, nitric oxide can be used as a marker to diagnose asthma since atopy is associated with increased levels of nitric oxide in exhaled breath. This method can also be used to verify the response and adherence to treatment and to predict asthma exacerbations (ATS 2005).

Inhalation challenge testing induces airway obstruction, e.g., bronchoconstriction or AHR, and a positive challenge test can confirm the diagnosis of airflow limitation. The substance inhaled is usually histamine or methacholine (MCh) and they act directly and predominantly on the airway's smooth muscle (Sterk et al. 1993).

The patient can be challenged with exercise to make the diagnosis of exercise-induced bronchoconstriction (EIB). A bicycle ergometer or treadmill is often used (ATS 2000). An additional challenge test involves isocapnic hyperventilation with cold and/or dry air. The advantage of this method compared to exercise is the ability to investigate a dose–response relationship. Furthermore, it is moderately similar to the bronchoconstriction seen following inhaled MCh and histamine in asthmatic individuals (Sterk et al. 1993).

2.1.4 Treatment and control

At present, there is no cure for asthma but there are treatments available to control symptoms and the future risk of adverse outcomes. The treatments are mainly bronchodilators or anti-inflammatory medications. Many individuals in the world do not have access to asthma medications or medical care. Ideally, better healthcare could be provided if we increased the economic wealth and improved the distribution of resources between and within countries (Croisant 2014).

Asthma medications can be divided into three categories: controller medications, reliever (rescue) medications, and add-on therapies for patients with severe asthma. Asthma severity is assessed based on the level of treatment needed to control the symptoms and exacerbations. The asthma medications and asthma severity are described further in Table 2. The use of low-dose inhaled corticosteroids (ICS) is very effective in reducing asthma symptoms and in reducing the risk of asthma-related exacerbations, hospitalization, and death (GINA 2015).

Table 2. Asthma medications and severity of the disease (GINA 2015).

Severity of asthma	**Controller medications** Regular use to reduce inflammation, symptoms, the risks of exacerbations, and changes in lung function.	**Reliever (rescue) medications** As-needed relief of symptoms, (e.g., worsening asthma or exacerbations), or as short-term prevention for exercise-induced bronchoconstriction (EIB)	**Add-on therapies** In case of persistant symptoms and/or exacerbations, despite use of high-dose controller medications
Mild asthma (intermittent or persistent)	Low-doseinhaled corticosteroids (ICS), leukotriene receptor antagonists, or chromones.	Short-acting beta$_2$-agonists (SABA)	
Moderate asthma (persistent)	Low-dose ICS/long-acting beta$_2$-agonist (LABA).	SABA	
Severe asthma (persistent)		SABA	High-dose ICS/LABA to prevent the risk of uncontrolled disease

The European Respiratory Society/American Thoracic Society Task Force on Severe Asthma consider that only individuals with refractory asthma and those whom do not respond to treatment of comorbidities are considered "severe" asthmatics (Chung et al. 2014). In addition, there is a category called "untreated severe asthma" that applies to individuals from low-resource countries who do not have access to ICS and other medications (i.e., uncontrolled asthma with no access to controlled medication) (GINA 2015).

The level of asthma control in an individual can be determined by the applied treatment, the disease processes, the genetic background, the environment or psychosocial factors. Poor symptom control is strongly associated with an increased risk of asthma exacerbations. Both symptom control (i.e., regular questioning) and the future risk of adverse outcomes (i.e., regular lung function assessments) are important and should be taken into account when choosing an asthma treatment and reviewing the patient's treatment response (GINA 2015).

It has been reported that approximately 50% of adults and children on long-term asthma treatment fail to take medications at least part of the time (Boulet et al. 2012). Treatment non-adherence can be due to, for example, misunderstanding instructions, forgetfulness, cost, a perception that treatment is not necessary, or side effects. In addition, the patient may have difficulties using the inhaler (GINA 2015).

Several studies have confirmed that it is possible to control the asthma disease in most patients regardless of the level of severity. Consequently, in recent years, some asthma guidelines include recommendations related to the level of asthma control rather than the level of severity (Papaioannou et al. 2015).

2.1.5 Work exacerbated asthma

The prevalence of asthma among workers may be similar to that among the general population (or somewhat lower, due to the hindrance that asthma presents to work). Among 474 workers in Maine, 13.5% reported having asthma (either as diagnosed by a physician or on the basis of reported symptoms characteristic of this disease; Henneberger et al. 2003), a value that is relatively similar to the 10.9% reported by Masoli and colleagues (2004) for the entire population of the United States.

Asthma-related exposures in the work environment are less frequently addressed than in the home environment because the employer may or may not accept their responsibility or have the ability to control exposures (Wagner and Henneberger 2006). Several conditions at work can exacerbate asthma symptoms, for example, exposure to irritant chemicals, second-hand smoke, common allergens or dusts. Additional examples are emotional stress, temperature, and physical activity (Henneberger et al. 2011).

Work-exacerbated asthma (WEA) has received less attention than OA, which is caused by work (Henneberger et al. 2011). The incidence of WEA among employed adults with asthma ranges from 8% to 25%, depending on the epidemiologic criteria utilized (Henneberger et al. 2002; Saarinen et al. 2003; Tarlo et al. 2000). Individuals with WEA report more days with symptoms, seek more medical care, and have a lower quality of life as compared with adults with asthma unrelated to work (Henneberger et al. 2011). One consequence of WEA is leaving work either temporarily or permanently (Wagner and Henneberger 2006).

Henneberger and colleagues (2015) showed that irritating agents have the strongest association with WEA and especially exposure to environmental tobacco smoke and inorganic dusts. An evaluation of workers' claimed time lost at work during a 5-year period reveals that 72% of the asthma claims fulfilled the WEA criteria (Lim et al. 2014). The agents that caused WEA differed between the investigated industry groups, implying that both common and less common triggers should be targeted to reduce the duration and frequency of WEA.

2.1.6 Animal models

Due to ethical reasons, not all parameters can be investigated in experimental studies on humans and studies on the progression of diseases are complicated. Animals have similarities to humans and can be used in asthma research at a relatively low cost.

Most animals, with the exception of cats (Reinero 2010; Venema and Patterson 2010) and horses (Herzberg et al. 2006), do not develop asthma naturally. However, several species such as mice, rats, guinea pigs, and rabbits can be used in experimental models to study asthma. However, a model is just a model, and it can never fully mimic human asthma. It can only be used to study what we already know, or to examine one asthma phenotype at the time (Persson 2002; Wenzel and Holgate 2006).

Systemic and airway inflammation can be induced experimentally by exposure to allergens (e.g., the egg white protein ovalbumin (OVA), house dust mites, *Aspergillus*, cockroaches, or pollens). Chronic challenge protocols usually include repeated inhalation exposure to low-level allergens for weeks or months (Kumar et al. 2008; Lloyd 2007), while acute allergen challenge protocols generally take place over several days. A chronic challenge involving allergens leads to airway remodeling, which is not present after acute challenges (Lloyd 2007). Airway responsiveness is often studied in models with the use of a challenge with, for example, MCh, or carbachol (Säfholm et al. 2011).

Currently, mice are the preferred animal species used as experimental models of allergic airway disease. The use of mice includes advantages such as a well-characterized genome and immune system, the availability of inbred and transgenic strains, short breeding periods, and relatively low maintenance costs (Glaab et al. 2007).

The most common mouse asthma model is the use of repeated intra-peritoneal injections of OVA with a T_H2 adjuvant (e.g., aluminum hydroxide) to initiate immune activation followed by several challenges with an OVA aerosol to induce airway inflammation (Kips et al. 2003). A systemic CD4+ (T_H2) immune response and eosinophilic airway inflammation can be experimentally induced in the BALB\c mice strain. This strain has an increased sensitivity to OVA, and sensitization leads to the formation of IgE antibodies in the serum, and increased numbers of eosinophils in the lungs (Fulkersson et al. 2005). In addition, AHR and airway remodeling can be studied in BALB\c mice (Lloyd 2007).

The C57BL/6 mice strain can also be used in allergy models. BALB\c is often preferred since C57BL/6 mice do not develop AHR, and they exhibit a T_H1-response instead of the T_H2-dependent allergic inflammation that is seen in humans. In addition, the inflammatory responses of the airway smooth muscle are higher in BALB\c mice compared to C57BL/6 mice (Säfholm et al. 2011).

Another common animal species used in asthma models is the guinea pig. The guinea pig is more similar to humans in regards to the anatomy of the lung (dichotomous), the anatomy and function of the airway smooth muscle, and mast cell distribution. Stimuli can induce a cough similar to that seen in humans. In addition, the release of histamine in the mast cells enables an early allergic reaction due to sensitization. Conversely, guinea pigs are not inbred, only a few strains are available, they are not as well studied as mice, and they have an axon reflex influencing the airways that is not present in humans (Canning and Chou 2008).

OVA has been widely used as an allergen in animal models. This model mimics many specific features of allergic asthma but it has some drawbacks. OVA does not induce asthma in humans, sensitization via the respiratory tract is not possible, and chronic OVA exposure leads to tolerance, limiting studies of airway remodeling and of other features of chronic asthma (Stevenson and Birell 2011).

The use of house dust mites instead of OVA has some advantages. Many human asthmatics have house dust mite specific IgE antibodies and the mechanisms behind sensitization via the respiratory mucosa can be studied. In addition, long-term exposure to house dust mites in animals can model chronic asthma and airway remodeling (Stevenson and Birell 2011).

2.2 ACUTE EXPOSURE TO AIRBORNE CHEMICALS

2.2.1 Health effects in humans

The Chemical Abstracts Service Registry^{SM} database includes more than 104 million unique chemical substances and about 15,000 more are added each day (CAS 2015). Individuals can be acutely exposed to hazardous airborne chemicals from accidental releases in workplaces, from fires, or from transportation accidents. Similarly, acute exposure can occur from deliberate terrorist actions or chemical warfare. The negative health effects from acute exposure to airborne chemicals vary from mild discomfort to severe medical conditions or lethality.

The subjective effects from exposure to airborne chemicals are often first apparent in the eye. Douglas and Coe (1987) measured the relative sensitivity of the human eye and lung by testing eight different gases and the threshold concentration was on average 1.5 times lower in the eye compared to the lung.

The sensory irritation pathway starts when local irritants interact with receptors of the nervous system (i.e., trigeminal nerve endings). A cascade of reflexes and defense mechanisms (i.e., eye blinks, coughing) follows and it involves local toxicity in the eyes and in the upper respiratory tract (nasal cavity, larynx/pharynx). Exposure at high concentrations or for long duration can lead to neurogenic inflammation and, eventually, to tissue damage. On the contrary, the tissue irritation pathway starts with the interaction of the local irritant with the epithelial cell layers of the eyes and the upper respiratory tract. The first response is adaptive changes and these are followed by inflammation and irreversible damage (Brüning et al. 2014).

The use of nasal or oral breathing may influence the first contact site in the respiratory tract. Local effects in the upper respiratory tract are linked to sensory irritation and appear faster than those in the lower respiratory tract do, e.g., lung edema and inflammation (Brüning et al. 2014). The nose is protective of the lower respiratory tract since it filters, humidifies, and heats inhaled air. In addition, the nose protects the gas-exchange regions of the lung by trapping inhaled particles, metabolizing airborne xenobiotics and absorbing water-soluble and reactive vapors and gases (Harkema et al. 2011).

The route of exposure (e.g., inhalation), transport (e.g., nasal mucous, lipid membranes), and the target site (e.g., lower respiratory tract) are determined by the physiochemical properties of the chemical. Examples of such properties are volatility, reactivity, water solubility, and lipophilicity. In addition, the molecular structure and size (expressed as the mass median aerodynamic diameter (MMAD)) of the chemical are important. Aerosols of different sizes can be inhaled (< 100 µm), can enter the smaller airways (< 10 µm), and reach the alveoli (< 4 µm) (Brüning et al. 2014). Low water-soluble chemicals affect the alveoli, while high water-soluble chemicals mainly cause an effect in the upper respiratory tract. However, very high concentrations of water-soluble chemicals will also produce effects in the alveoli (Nowak et al. 2002). The locations of the effects from different chemicals in relation to water solubility are presented in Figure 1.

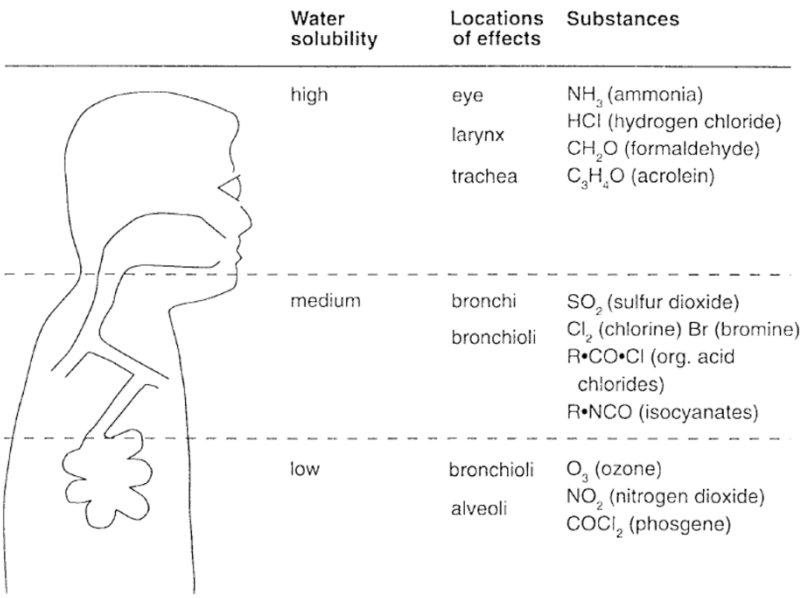


Figure 1. Locations of the effects from different substances in relation to water solubility. From Nowak and Hoeppe, Acute exposure to toxic agents. In Grassi C, et al.: Pulmonary diseases, ©1999, Reproduced with the kind permission of McGraw-Hill Education. All rights reserved.

2.2.2 Experimental studies with human individuals

Exposure data that are relevant for health risk assessment of airborne chemicals often include epidemiological and/or inhalation experiments using animals and/or human subjects. Epidemiological studies represent real–life scenarios but include confounding factors–such as socio–economic status, smoking, additional chemical exposures and spirometry cannot be measured at a high frequency. Inhalation experiments on humans are performed in an environmentally controlled setting, often with the use of an exposure chamber (Figure 2).

Figure 2: Human exposure chamber. Photo by Mia Johansson.

Exposure–response relationships can be investigated in experimental studies by the use of spirometry measurements and subjective symptom questionnaires before, during, and after the exposure. Lower-, upper-, and non-respiratory symptoms are scored according to severity. Variations in airway response following changes in temperature, humidity, and exercise level (bicycle or treadmill) can be investigated. Subjects of different ages and subpopulations can be studied (Utell and Samet 1992).

Subjects are exposed to the chemical while breathing in a mouthpiece, head dome, face mask, or in a chamber with or without a nose clip. In addition, tidal breathing can be applied, which includes an inhalation challenge with an aerosol either through a facemask or via a mouthpiece. Quiet tidal breathing at a spontaneous frequency is undertaken for 2 min using a nose clip. FEV_1 is measured before the test and at 30 and 90 s after each inhalation. The maximal bronchoconstriction at a certain dose corresponds to the lowest value on FEV_1 and the test ends when the FEV_1 has decreased by 20% or more from baseline. The results are presented as the concentration of the aerosol (usually MCh or histamine) causing a 20% fall in FEV_1 (PC_{20}) (Sterk et al. 1993). Mouth-only inhalation in experimental studies can be considered as somewhat artificial because the natural human scrubbing system for substances in the nasal airways is bypassed (Proctor 1981).

The use of human subjects in experimental settings has ethical considerations, especially in the case of susceptible subpopulations and/or children. For obvious reasons, humans cannot be harmed physically, psychologically, or emotionally. Informed consent must be given by all subjects and they should be allowed to stop their participation whenever they want to. The study group is often small and the exposure levels are low. In addition, the exposure duration is short, which is relevant to acute exposure scenarios but difficult to extrapolate to chronic exposure scenarios (Utell and Samet 1992).

Exposure to some chemicals may be complicated. For example, in studies on particle pollution, the variability in particle size affects the site of deposition (Utell et al. 1985). An additional example is that exposure to sulfuric acid in individuals with high levels of naturally occurring ammonia in their airways shows smaller effects compared to those with low levels of ammonia (Larson et al. 1977; Mariglio et al. 1983).

2.2.3 Experimental studies with small animals

Many risk assessment documents for airborne chemicals lack data from controlled experimental studies on asthmatic individuals. The number of studies is limited due to ethical and practical difficulties. In addition, the applied temperature, humidity, and workload in these studies may not represent real-life situations of humans. As a result, experimental studies must be supplemented with epidemiological and mechanistic studies including cells, tissues, or animal models (Bylin 1987).

Environmentally controlled exposure conditions, concentration, and duration, together with biochemical, physiological, and histological examinations are possible to perform in rodents. However, unrealistic experimental designs will make the extrapolation between animal and human difficult (Utell and Samet 1992). Inhalation studies on rodents enable the use of higher exposure levels and more invasive methods for analyzing inflammatory and pulmonary effects from exposure to airborne irritants. Several risk assessment documents are based on data from inhalation studies on rodents. However, those data are mainly based on the response in healthy animals.

Sensory nerves such as neuropeptide-rich C fibers are extensively innervated in both human and rodent airways (Nielsen 1991; Nielsen et al. 2007) and stimulation of the nasal trigeminal sensory nerves initiates a sensory irritation response in rodents. This is characterized by a decreased respiratory frequency due to a prolonged pause at the beginning of the expiration (Nielsen 1991; Vijayaraghavan et al. 1993). The concentration of a chemical irritant that induces a 50% depression in respiratory frequency, the RD_{50}, is an important toxicological parameter, since it generally correlates with observations in humans (Alarie 1973). It can be used for the categorization of chemicals as irritants, and as a tool in the derivation of guideline values and occupational exposure limits (Bessac et al. 2008). Kuwabara and colleagues (2007) found a relationship between the RD_{50} in mice and the LOAEL for human sensory irritation based on studies of 26 chemicals, which supports the use of the RD_{50} measure in the derivation of guideline values for the general population.

Ventilation, lung volume, airflow, and gas exchange are common in humans and in most mammals. Changes in breathing patterns can be measured via non-invasive techniques with conscious animals. The least invasive technique, unrestrained barometric plethysmography, in which the parameter enhanced pause (Penh) can be measured, is the least precise method, and the use of this method has been questioned. There are other non-invasive techniques with restrained animals such as head-out plethysmography, double chamber plethysmography, and the forced oscillation technique. These techniques enable repeated exposures in many animals, and nearly natural breathing patterns, but only the airflow parameters can be studied. A calibrated pneumotachograph is connected to the head-out plethysmograph and respiratory flow can be measured. Disadvantages with this technique include the induction of stress, and that inhalation exposure results in both nasal and gastrointestinal uptake (Hoymann 2007).

Changes in lung mechanics can be measured via invasive techniques with anaesthetized animals. Invasive plethysmography in spontaneously breathing anaesthetized animals can either be repetitive (orotracheally intubated animals) or non-repetitive (tracheotomized intubated animals). Orotracheal intubation means that there is no stress, and the measurement of valuable parameters of resistance and compliance can be undertaken, with the inhalation exposure focused on the lungs, using sophisticated exposure apparatus. On the other hand, this method takes more time, is technically challenging, and it decreases the respiration.

The use of the non-repetitive forced oscillation technique is the most invasive technique but it is also precise. The animal is anaesthesized, tracheotomized, and paralyzed, and placed in a small animal ventilator (flexiVent™ SCIREQ®). The animal does not experience stress, the inhalation exposure is focused on the lungs, and valuable parameters such as resistance and compliance can be measured both in the central and peripheral lung (Hoymann 2007).

2.2.4 Species differences in lung anatomy and physiology

Mice have a proportionally larger nasal surface area compared to humans and they are obligate nose breathers (Mizgerd and Skerrett 2008). The nose and upper respiratory tract have a scrubbing effect for particles and gas, which is bypassed in oral breathing. In addition, the upper respiratory tract differs in terms of epithelial cell types and numbers.

The mouse lung consists of one left lung lobe and four right lung lobes, while humans have two on the left and three on the right. The lengths and diameters of the airways differ and one of the most important differences is the branching of the bronchi. It is asymmetric (monopodial) in mice, which leads to direct airflow to the lower respiratory tract. The symmetric (dichotomous) pattern in humans leads to increased susceptibility to depositions in the areas where branching occurs. In humans, and not in mice, bronchioles and alveolar ducts are present in the distal part of the airways. Mice have rapid clearance from the lung and more particles go to the gastrointestinal tract than to the lung lymph nodes, which is the where the slow clearance of particles occurs in humans (Warheit 1989).

The ventilation rate (163 breaths/min) and heart rate (624 beats/min) are higher in the mouse compared to humans (12 breaths/min, 65 beats/min), which leads to differences in tidal volume and perfusion rates, as well as tissue volumes, and deposition and uptake (Davies and Morris 1993).

2.3 HEALTH RISK ASSESSMENT

2.3.1 The risk assessment process

According to the WHO International Programme on Chemical Safety, risk assessment is *"a process intended to calculate or estimate the risk to a given target organism, system, or (sub)population, including the identification of attendant uncertainties following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system"* (IPCS 2004, p. 14).

The risk assessment process can be divided into four stages: hazard identification (HI), hazard characterization (HC) (or dose–response assessment), exposure assessment, and risk characterization (Figure 3). HI and HC taken together represent the hazard assessment in which possible adverse effects from exposure to a chemical are investigated. The outcome of the four stages in risk assessment is evaluated in the risk management stage where decisions related to risk reduction are made. Environmental health, economic, social, and political aspects are evaluated, as well as regulatory options (IPCS 2004; NRC 1994).

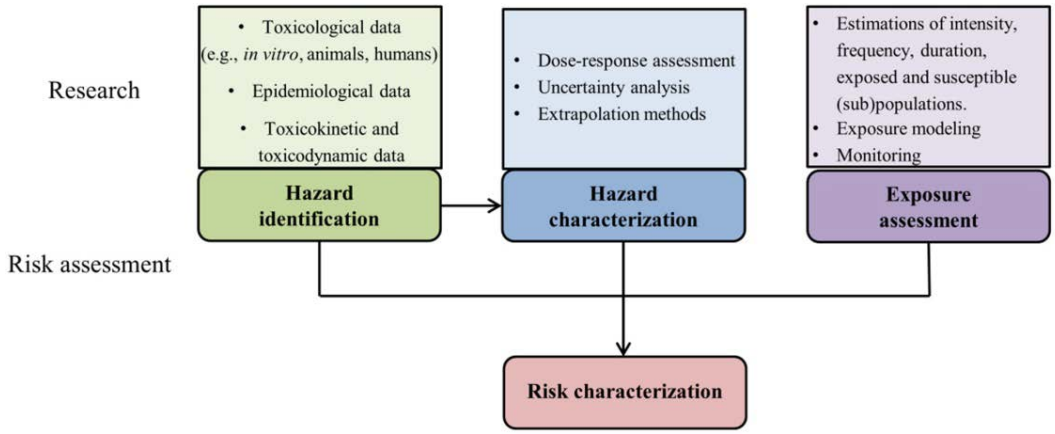


Figure 3. A simplified illustration of the risk assessment process including research which serves as basis for risk assessment (NRC 1994).

2.3.1.1 Hazard identification

Chemicals with the potential to cause adverse effects in an organism, system, or subpopulation are identified here. The circumstances behind exposure to these chemicals and the relevant exposure concentrations are evaluated and the link between negative effects and the chemical is characterized. Toxicological information on toxicokinetics (i.e., how the body absorbs, distributes, metabolizes, and eliminates chemicals) and toxicodynamics (i.e., the effects that chemicals have on the human body) are important, but data from monitoring, epidemiological and other experiments are also applied (IPCS 2004; NRC 1994; U.S. EPA 2014).

2.3.1.2 Hazard characterization

HC involves the qualitative, and wherever possible, quantitative characterization of the relationship between exposure to a chemical and the severity of the adverse effect. A dose–response (referred to as concentration–response in this thesis) assessment should be included and it describes how the likelihood and severity of responses are connected to the exposure concentration of a chemical. Uncertainties connected with the concentration–response relationships are, e.g., the intensity and pattern of exposure, and differences in susceptibility such as age, lifestyle, pregnancy, or pre-existing diseases. Extrapolation from the effects in animals to human health effects and of high-dose responses to low-dose responses are also addressed here (IPCS 2004; NRC 1994).

The most sensitive adverse health effect is identified: the so–called point of departure (POD). The no observed adverse effect concentration (NOAEC) has traditionally been used as the POD in the derivation of guideline values in health risk assessment. However, the NOAEC approach has been seriously criticized and an alternative mathematical model, the Benchmark dose approach (referred to as the Benchmark concentration in this thesis, BMC), was therefore proposed as preferable by Crump (1984). Toxicological findings reported as quantal or continuous data can be applied to the BMC approach. A critical effect size (CES) of 5–10% is usually applied to the BMC analysis of quantal data. The CES can be modified for continuous data to the percentage change in response relative to background or to a range of responses (Sand et al. 2008).

2.3.1.3 Exposure assessment

Exposure assessment is the estimation and measurement of hypothetical or actual exposures to chemicals in humans. The intensity, frequency, and duration of the exposure are determined. The assessment ideally includes sources, routes, and uncertainties related to the exposure, as well as real and potential pathways. Scenarios can be created to estimate exposure and modeling is often applied. Exposed populations and susceptible populations are also characterized (IPCS 2004; NRC 1994).

2.3.1.4 Risk characterization

Here, the outcome of the risk assessment and a discussion of the uncertainties associated with estimates of risk for the studied population should be presented in a transparent and consistent way. Information from the previous three stages is implemented in a qualitative or quantitative estimate of the risk of negative health effects in individuals exposed to the chemical of concern. The results should be expressed clearly, assumptions and uncertainties are stated, reasonable alternative interpretations are identified, and scientific conclusions should be separated from policy judgements (IPCS 2004; NRC 1994; U.S. EPA 2014).

2.3.2 Acute to short-term guideline values for inhalation exposure

2.3.2.1 Guideline values aimed at the protection of the general population in emergency settings

The worst industrial accident to date occurred in Bhopal, India, on December 3, 1984. Methylisocyanate leaked out from a pesticide plant and killed more than 2000 individuals. Up to 200,000 people were injured or disabled (Jasanoff 1988; first presented in Lepkowski 1985). A need for guideline values to define safe exposure levels became apparent. The American Industrial Hygiene Association (AIHA) derived the guideline values Emergency Response Planning Guidelines (ERPGs). The U.S. National Research Council and the U.S. Environmental Protection Agency (U.S. EPA) later developed the Acute Exposure Guideline Levels (AEGL) (Rusch et al. 2000).

Both sets of values are used for emergency response planning, preparedness, and as guidance to those responsible for risk management decisions in cases of accidents with a sudden release of hazardous airborne chemicals. They target the general population, including susceptible subpopulations. The AEGL program includes the derivation of three threshold levels above which the following toxic effects are predicted: AEGL-1: mild and reversible, such as discomfort, irritation, or asymptomatic non-sensory effects; AEGL-2: irreversible, long lasting, or escape impairing; and AEGL-3: life threatening to health or death. Similar threshold levels have been developed for the ERPG program (i.e., ERPG-1, ERPG-2, ERPG-3) with the exception that odor is included as a threshold effect for ERPG-1. The AEGL values are derived for five different exposure durations (10 min, 30 min, 1 h, 4 h, and 8 h) while the ERPG values are derived for 1 h of exposure (AIHA 2008; NRC 2001).

Additional examples of values for emergency response are the Dutch intervention values for dangerous substances, the U.S. Department of Energy's Temporary Emergency Exposure Levels (TEEL) and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC's) Emergency Exposure Indices (EEIs) (Craig et al. 2000; ECETOC 1991; Wood et al. 2006). In addition, the EU-funded project ACUTEX developed a methodology for deriving Acute Exposure Threshold Levels (AETLs) in 2006 with the aim of them being implemented in the Seveso II Directive and constituting a complement to other acute guideline values (Wood et al. 2006).

2.3.2.2 Guideline values aimed at the protection of workers in occupational settings

Occupational exposure limits (OELs) are derived to protect healthy workers from negative health effects related to exposures during their working lifetime (8 h per day, 40 h per week). Similarly, 15-min short-term exposure limits (STELs) or other acute to short-term guideline values—representing peaks, ceilings, or time-weighted averages of exposures—are set to protect workers. These values are generally not aimed at determining how to protect individuals in emergency situations but rather to protect them during normal workdays.

The American Conference of Governmental Industrial Hygienists (ACGIH) developed the first set of OELs called "maximum allowable concentrations" in the 1940s, today known as the Threshold Limit Values (TLV®) (ACGIH 2015). Many countries developed their own OELs in the 1970s and the European Commission Member States started adopting proposed values from the Scientific Committee on Occupational Exposure Limits (SCOEL) in 1991 (SCOEL 2013). Some OELs are legally binding (e.g., OELs in various countries) while others are indicative (e.g., ACGIH-TLV®). SCOEL has developed both legally binding and indicative OELs. Not all OELs are health-based but rather are derived from the influence of economic or technical factors (Deveau et al. 2015).

2.3.2.3 Derived No-Effect Levels (DNEL)

The European regulation REACH (EC 1907/2006), concerning the registration, evaluation, authorization, and restriction of chemicals, entails major change in the approach to the regulation of industrial chemicals. A key part of the evaluation is the determination of Derived No-Effect Levels (DNELs) (i.e., the levels of exposure below which no adverse health effects in humans are expected to occur). Registrant companies are required to provide DNELs for all substances they manufacture and/or import in quantities of more than 10 tons per year. To date, two of the three deadlines for registration have expired (the third being in 2018) and the extensive effort expended by registrants is illustrated by the 51,920 dossiers covering 13,441 unique substances that were submitted by September 15, 2015. Selected information from these dossiers, including the DNELs themselves and summaries of the toxicological data on which these are based, is available in the European Chemicals Agency (ECHA) database of registered substances (www.echa.europa.eu) (ECHA 2012a).

The procedure by which DNELs should be derived is outlined in Chapter R.8 of the ECHA guidance for REACH obligation-holders (ECHA 2012b). These values should consider relevant populations (workers, consumers, and other individuals liable to be exposed indirectly via the environment, as well as certain susceptible and/or vulnerable groups, such as pregnant women and children), the duration of exposure (long- and short-term/acute), the routes of exposure (inhalation, oral, and dermal), and the type of toxic effects (local or systemic). DNELs for acute inhalation are generally based on minutes to a few hours of exposure, with REACH specifying a routine duration of 15 min for workers (ECHA 2012b, pp. 102–104). In the case of consumers, the ECHA guidance defines a daily dose as that resulting from 1–24 h of exposure, with peak levels of exposure lasting from minutes to hours (ECHA 2012b, p. 8).

2.3.3 Assessment factors

AFs (also called uncertainty factors or safety factors) are used in health risk assessment to compensate for the variation in biological variability and uncertainty between animals and humans, and between average humans and susceptible individuals. In addition, extrapolations from short-term to long-term exposure durations and from data on LOAECs to NOAECs are

applied, and from an insufficient to a sufficient database (Dankovic et al. 2015). In addition, modifying factors are used to account for other uncertainties, e.g., exposure scenarios or from an interplay between the areas above.

2.3.3.1 Susceptible individuals

The susceptibility to chemical exposure observed in human individuals depends on age, body weight, genetic background, and health status (e.g., pre-existing diseases). Small children (especially fetuses, infants, and juveniles) are often considered more susceptible because they consume more food, water, and oxygen in relation to their body weight, and they are in the process of maturation. In addition, they spend more time outdoors compared to adults and it is known that they get specific damage from, for example, alcohol, smoking, and pharmaceuticals (Eckhardt 2014). The effects from ambient air pollutants have been shown to be most pronounced during the first year of life (Götschi et al. 2008). In the respiratory system, cellular differentiation, proliferation, and physiological function change postnatally and while the child is growing. For example, 80% of the alveoli develop postnatally. Consequently, their response to environmental exposures is most likely different from that seen in adults. However, more knowledge on the susceptibility of children is needed (Dietert et al. 2000). For obvious ethical reasons, the susceptibility to chemical irritants in children is difficult to investigate experimentally. de Pee and colleagues (1991) discovered similar airway responses between asthmatic and non-asthmatic children and adults after a challenge with MCh (de Pee et al. 1991).

2.3.3.2 Inter-species and inter-individual assessment factors

The genetic variability is large between humans and animals, which makes species extrapolation difficult. Animal data can be extrapolated to humans by the use of an allometric equation scaling factor = ($kg_{(humans)}$ / $kg_{(animal\ species)}$)^0.25 (Kalberlah and Schneider 1998b). A factor of 7 for differences between mice (30 g) and humans (70 kg) has been suggested by the ECHA (ECHA 2012b, p. 24).

Lehman and Fitzbugh (1954) introduced an AF of 100 to account for uncertainties and variabilities between animals and humans (an inter-species AF of 10) and within the human population (an inter-individual AF of 10). Many risk assessors and organizations have adopted this AF of 100 and subdivided it into two AFs of 10 to account for inter-species and inter-individual variability (Dourson and Stara 1983; Renwick 1991, 1993; U.S. EPA 1988, 1993). Dourson and Stara (1983) showed that these default values are protective against the average chemical. There are variabilities in the relationship between the exposure dose and target dose (toxicokinetics, TK), and between the target dose and adverse effect (toxicodynamics, TD), and these often represent two equal parts of the AF of 10 ($10^{0.5} \times 10^{0.5}$, i.e., 3.16 × 3.16). Renwick (1991, 1993) proposed a subdivision of the default AF of 10 into 4 (TK) and 2.5 (TD) based on observations of higher toxicokinetic than toxicodynamic differences from experimental studies. This approach is applied by, for example, the WHO (IPCS 2005). Falk-Filipsson and colleagues (2007) suggest the use of 4.5 to account for TK variability and a higher factor in the case of susceptible subpopulations. The TD for variability is suggested as 3.2, leading to a default inter-individual AF of at least 15 (4.5 × 3.2).

The applied default inter-individual AF varies between guideline values and between values for the working and general population. The European REACH legislation proposes default AF values of 5 and 10 (ECHA 2010), the Dutch TNO (the Netherlands Organization for

Applied Scientific Research) 3 and 10 (Hakkert et al. 1996), and the ECETOC 3 and 5 (ECETOC 2003). An AF for the general population of 25 was suggested in a report by the German Federal Institute for Occupational Safety and Health and the German Federal Environmental Agency (Kalberlah and Schneider 1998), while the NRC suggests a default AF of 10 in the derivation of AEGLs (NRC 2001).

2.3.3.3 Chemical-specific assessment factors for inter-individual variability

Data-derived AFs, or so-called chemical-specific assessment factors (CSAFs), have been proposed as an alternative to default AFs when sufficient data are available (Dourson et al. 1996; IPCS 1994). Physiologically based pharmacokinetic (PBPK) modeling can be used as a tool for deriving such factors. PBPK modeling can also be used in the extrapolation between different routes of exposure (ECHA 2012b) and it has been used for species-to-species extrapolation and time-scaling in the development of AEGLs (Bruckner et al. 2004). In addition, AFs that are specific for a mode of action or pathway have also been proposed to allow, e.g., human variability in Phase I metabolism. They constitute an intermediate between the chemical-specific and the default AFs (Dorne and Renwick 2005).

Slob (2002) has proposed the use of Benchmark concentration (BMC) analysis to estimate AFs for subpopulations. To estimate an AF, the critical effect concentration (CEC, or critical effect dose, CED) identified for subpopulation A can be divided by the CEC for subpopulation B. This AF estimate is more exact than measuring the difference in the NOAECs between two subpopulations, since the CEC is defined as the concentration needed for the pre-determined CES. The CES is the change in an endpoint that is considered to cause non-adverse health effects (Slob 2002).

2.3.3.4 Extrapolation between LOAEC and NOAEC data

When NOAEC data are not available as the POD for the derivation of acute to short-term guideline values for inhalation exposure, extrapolation may be needed from a LOAEC to a NOAEC value. In addition, the available LOAEC data may not be sufficient for BMC analysis and the use of an AF may be needed. Based on an extensive evaluation of LOAEC-to-NOAEC ratios, an AF of 6 has been suggested to be protective for 95% of the population, while an AF of 10 is protective for 99% (Alexeeff et al. 2002). Dourson and Stara (1983) also implied that an AF of less than 10 could be used for LOAEC-to-NOAEC extrapolations for mild effects.

2.3.3.5 Extrapolation between exposure durations

Fritz Haber and Ferdinand Flury proposed the use of the formula $c \times t = k$ (Haber's law) to describe the airway response from war gases in terms of exposure concentration and exposure time (duration) (Shusterman et al. 2006). Ten Berge and colleagues (1986) used raw data from exposures to airborne irritant chemicals to investigate the concentration–time relationship. They conclude that rather than using $c \times t = k$, the term $c^n \times t = k$ (with $n \neq 1$) is preferable for predicting mortality and that the value of n varied between 1 and 3 for 90% of the chemicals evaluated. Schusterman and colleagues (2006) evaluated the concentration–time relationship in human experimental studies on ammonia, chlorine, formaldehyde, and 1-octene. Their findings suggest that extrapolation from short to longer exposure times with the use of Haber's law may overestimate the risk of sensory irritation, while the opposite extrapolation (from long to shorter exposure times) may lead to an underestimation of risk. Both Haber's law and $c^n \times t = k$ (with $n \neq 1$) have been applied in the derivation of guideline values in health risk assessments (Shusterman 2006).

2.3.4 Asthmatics as a susceptible subpopulation

Several risk assessment programs address susceptible subpopulations such as children, the elderly, and pregnant women. In addition, individuals with genetic and metabolic disorders or pre-existing diseases are sometimes mentioned as susceptible subpopulations. The AEGLs (U.S. EPA and NRC), Reference Exposure Levels (REL, California Office of Environmental Health Hazard Assessment, OEHHA), and the Provisional Advisory Levels (PAL, U.S. EPA National Homeland Security Research Center, NHSRC) have specifically included asthmatic individuals in the definition of susceptible populations to be included in the derivation of guideline values (NRC 2001; Denton and Hickox 1999; Young et al. 2009). Young and colleagues (2009) have also investigated the difference in response between healthy and asthmatic individuals based on a few experimental studies.

The potential susceptibility of asthmatics has been investigated in relation to the derivation of National Ambient Quality Standards for the air pollutants nitrogen dioxide, ozone, and sulfur oxides (EPA 2007, 2008, 2009; Goodman et al. 2013, 2015). In addition, several experimental studies have been performed on asthmatics using an exposure chamber with the aim of investigating the susceptibility of asthmatics to air pollutants and other airborne chemicals. Several meta-analyses and evaluations including asthmatics exposed to single chemicals are available (Folinsbee 1981; Johns et al. 2010; Hesterberg et al. 2009; Goodman et al. 2009; Utell 1985; Johns and Linn 2011).

There is an awareness of the potential susceptibility of asthmatics, but the inclusion of this subpopulation in the derivation of acute to short-term guideline values has not been investigated previously, and a recommendation of an AF for asthmatics has not been available. In addition, the difference in susceptibility between healthy and asthmatic individuals has not been investigated by extensively analyzing controlled experimental studies on chemical exposure.

3 AIMS

The overall aim of this thesis was to provide knowledge that can ensure adequate protection of the general population, including susceptible subpopulations such as asthmatics, during sudden releases of hazardous airborne chemicals via a contribution to scientifically sound and internationally harmonized guideline values.

The specific aims of the studies were:

I. To investigate if and how asthmatics are included in the derivation of acute to short-term guideline values and to what extent experimental data including asthmatic subjects are applied to the derivation. In addition, to identify data gaps and inadequacies and to present a database of experimental studies that can constitute a key to supporting the selection of inter-individual AFs for chemicals with irritant properties.

II. To evaluate the experimental basis for AFs that are intended to take inter-individual variability in susceptibility to irritant chemicals into account. First, we attempted to identify any general differences between healthy and asthmatic individuals with respect to airway response to short-term exposure, and, thereafter, to determine whether the AFs currently employed for inter-individual variability are adequate for asthmatics by using the information available on four chemicals that have been examined extensively.

III. To investigate the extent to which experimental observations on asthmatic subjects are taken into consideration in connection with the registration process under the EU REACH regulation, and to determine whether asthmatics are provided with adequate protection by the DNELs for acute inhalation exposure.

IV. To explore the use of OVA-sensitized mice as a model for asthmatics and to investigate whether such mice are more susceptible to inhaled chlorine in terms of effects on their respiratory frequency, lung mechanics, and inflammatory markers.

4 METHODS

This is a summary of the methods used in this thesis. For further detailed information, the reader is referred to the method sections in **Papers I–IV**.

4.1 IDENTIFICATION OF HEALTH RISK ASSESSMENT DOCUMENTS AND GUIDELINE VALUES

The basis for choosing AEGL values to analyze in Paper I was due to their specific aim of protecting the general population, including susceptible subpopulations, such as asthmatics, from mild effects following acute exposure to hazardous chemicals (NRC 2001). In addition, the full technical support documents (TSDs) were publicly available online, which facilitated the evaluation of the inclusion of asthmatics in the derivation of AEGL values. Nine additional sets of acute to short-term guideline values were chosen based on the target population (four aimed at the general population, five aimed at the working population) and accessibility to the support documents (publicly available online, as books, or as loose-leaf documents).

In Paper III, the DNELs for local effects from acute/short-term inhalation exposure were of interest, since such effects are observed in asthmatics. The full dossiers (i.e., support documents) of the DNEL values were neither publicly available on the ECHA website (http://echa.europa.eu) nor upon request from the ECHA, which compromised our analysis of how the DNELs were derived. However, the ECHA database of registered substances contained DNEL values and toxicological information concerning their derivation (the AFs applied, references to all experimental studies) and the harmonized and notified classifications for each substance were listed in the ECHA classification and labeling (C&L) inventory.

4.2 COMPILATION OF DATABASES ON HUMAN EXPERIMENTAL STUDIES

The compiled databases of experimental studies in Papers II–III are based on references found in risk assessment documents in Paper I and on hits from computerized searches in scientific literature databases (Figure 4). In addition, complementary searches in Google scholar of specific articles that were not accessed through the scientific databases were useful. The names of specific authors were applied as search terms. These, together with the use of risk assessment documents, were good complements to the use of medical subject heading (MeSH) terms and key words when searching for experimental data.

Only human experimental studies involving environmentally controlled exposure to a single substance (and mixtures in Paper II) via a mouthpiece, head dome, face mask, or in a chamber with or without a nose clip were identified. Epidemiological studies on air pollution or studies in occupational settings where exposure was not adequately defined were excluded. Furthermore, investigations in which subjects with OA were exposed to the sensitizing chemical were also excluded.

Additional criteria for reliability and quality were applied to the identified experimental studies. These included well-documented and scientifically sound methods and data published in peer-reviewed journals. The same data published in two separate papers was regarded as a single study. The environment had to fulfill the criteria of having a normal room temperature (18–25ºC) and normal humidity. The subjects were not allowed to be current smokers, have pre-existing diseases other than asthma, have severe asthma, or have an occupational or other history of heavy exposure to irritating or sensitizing air pollutants.

All of the identified experimental studies were compiled into different databases that were categorized according to the different aims of the papers (Figure 4). The chemical involved and its concentration and duration of exposure, together with the experimental design and exercise conditions were recorded. The number of subjects, the definition of asthma, age, gender, smoking habits, and eventual use of corticosteroids were also noted.

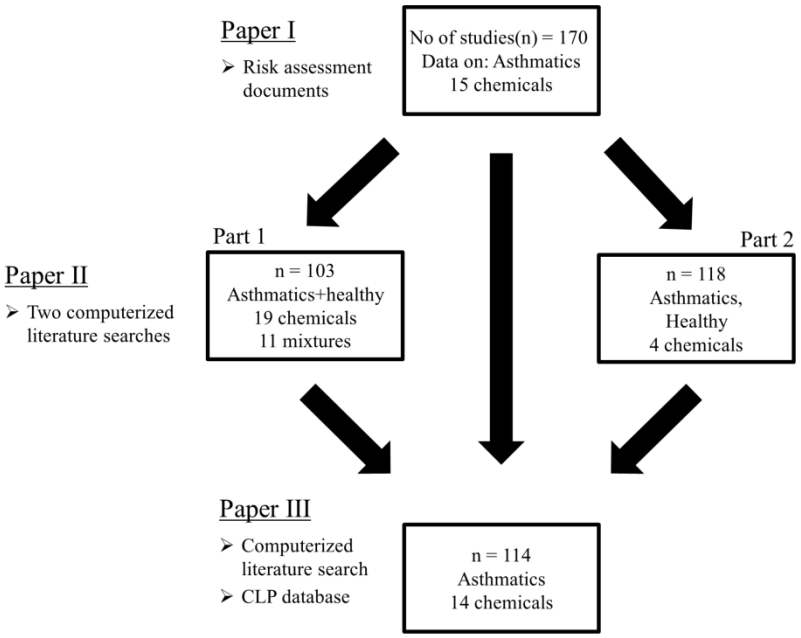


Figure 4. The overall strategy applied for collecting experimental studies including asthmatic individuals (and healthy individuals) and the development of the databases in Papers I–III. Arrows symbolize the use of experimental studies that were identified in previous papers. The number of studies is indicated with an "n". The statuses of the subjects (asthmatic/healthy) investigated in each database are presented together with the number of chemicals.

4.3 INVESTIGATING THE CONSIDERATION OF ASTHMATICS IN RISK ASSESSMENT

The AEGL TSDs (n = 176, corresponding to 250 chemicals) were categorized into: 1) documents with experimental data, 2) documents with explicit statements on asthmatics, and 3) documents including no explicit statements on asthmatics. In the second part, 15 chemicals from category 1 (experimental data on asthmatics) were analyzed in 9 additional sets of acute to short-term guideline values. The support documents were further classified by the inclusion of experimental data on asthmatics: 1) used as key studies, 2) cited in the conclusion, 3) included in the reference list, and 4) no inclusion of studies. A database with all of the identified experimental studies on asthmatics in Paper I was created (Figure 4).

The chemicals in Papers I and II that had been tested on asthmatics (Figure 4) were searched for in the ECHA database of registered substances and the inclusion of such data was investigated in more detail in the toxicological information that was provided on the ECHA website (http://echa.europa.eu).

To identify additional studies on asthmatic subjects, our previous databases in Papers I and II were complemented with a computerized literature search and an examination of substances with a harmonized classification of H335: a respiratory irritant under the Classification, Labelling, and Packaging (CLP) Regulation (http://echa.europa.eu).

Asthma studies found in the ECHA database of registered substances were compared to this new compilation of studies. The DNEL values were compared to the overall NOAECs and the overall LOAECs that were estimated for each chemical. In addition, the DNEL values for local effects following acute/short-term exposure of both workers and the general population were compared to the ten health-based guidelines described in Paper I.

4.4 ESTIMATION OF LOAECS, NOAECS AND DIFFERENTIAL RESPONSE FACTORS

The term "weight of evidence" (WOE) has been applied to health risk assessments for more than 60 years (Weed 2005). Klimisch et al. (1997) developed quality scores for each experimental study (e.g., acceptable, unacceptable, adequate, limited), which have been used by risk assessors, such as the REACH registrants, in the derivation of the DNELs in the ECHA database of registered substances (www.echa.europa.eu).

Recommendations for improving WOE evaluations in risk assessments were developed at a workshop and presented by Rhomberg and colleagues (2013). The recommendations are based on an evaluation of 50 different frameworks and are divided into four different phases related to: 1) causal question definition and data selection, 2) a review of individual studies, 3) data integration and evaluation, and 4) drawing conclusions based on interferences (for more information, see Rhomberg et al. 2013).

Several of these recommendations were applied to our WOE approach in Papers II–III. In Part 1 of Paper II, a LOAEC for each endpoint included in an experimental study was separately estimated for healthy and asthmatic individuals. Estimation of LOAECs and NOAECs for asthmatic subjects, only, was made in Paper III, in a similar manner to that in Paper II.

The factors that the WOE approach was applied to in Papers II–III were the number of studies, the duration of exposure (very short exposures were considered inconclusive), the type of outcome, and the number of subjects (a higher weight was given to studies involving more subjects). In addition, the definition of asthma and the documentation of asthma therapy were considered. The effects induced by exercise were controlled for by considering the response. The type of outcome for the LOAEC included statistically significant changes in pulmonary function, levels of inflammatory markers, and/or symptom scores. The pulmonary function parameters of FEV_1 and specific airway resistance (SRaw) were considered more relevant than other parameters were if the LOAECs (Papers II and III) or NOAECs (Paper III) from different studies were in disagreement.

In Part 1 of Paper II, an estimated differential response factor (EDRF) was calculated as the ratio between the LOAEC in healthy individuals and the LOAEC in asthmatics for each endpoint. Then an overall EDRF was estimated for each chemical tested, taking all endpoints and different studies into account. In cases where a LOAEC could not be identified in healthy subjects, the ratio between the NOAEC in healthy subjects and the LOAEC in asthmatics were calculated and noted with a greater-than sign. There is a certain amount of subjectivity involved in combining all studies on a chemical to derive an overall EDRF. Overall EDRFs were considered inconclusive if the study did not meet our criteria or if the LOAECs were not determined for either asthmatic or healthy subjects.

4.5 CONCENTRATION-RESPONSE AND BENCHMARK CONCENTRATION ANALYSES

In Part 2 of Paper II, four chemicals (nitrogen dioxide, ozone, sulfuric acid, and sulfur dioxide) were selected for concentration–response relationship analyses based on studies in which healthy and asthmatic subjects were tested either under the same conditions or in separate studies. These chemicals were chosen because of the large number of human studies that have been performed with them. Group-wise data on FEV_1 and SRaw were extracted from the identified studies, since individual details were usually lacking. The percentage change in FEV_1 and SRaw was determined by deriving the values after exposure from the baseline values.

A Benchmark concentration (BMC) analysis was performed on SRaw data from five studies involving exposure to sulfur dioxide (breathing at rest from the mouth only). The concentration–response curves were fitted to the data by employing subject status (asthmatic or healthy) as a covariant. A CES of 20% SRaw was used, according to the definition of a "small" but clear response (U.S. EPA 2007, 2008b, 2009). Here, we considered observations on the pulmonary function of each individual subject exposed to sulfur dioxide (at rest while wearing a nose clip) rather than group data. The BMC curve fitting was carried out with a nested set of exponential and Hill models (Slob 2002) using the R package PROAST version 32.2 (Slob and Cotton 2012) in the R version 2.15.2 (R Core Team 2012).

4.6 AIRWAY RESPONSE TO CHLORINE IN NAÏVE AND SENSITIZED MICE

Fifty-six BALB/C OlaHsd female mice were divided into four naïve groups and four OVA-sensitized groups. One of four concentrations of chlorine was applied: 0, 5, 30, and 80 ppm, for 15 min at day 16. All mice were weighed prior to any treatment (i.e., at day 0, 7, 13, 14, 15, 16, and 17). The experimental design is presented in Figure 5. Ethical approval for the study presented in Paper IV was received from the Regional Ethics Committee on animal experiments in Umeå, Sweden.

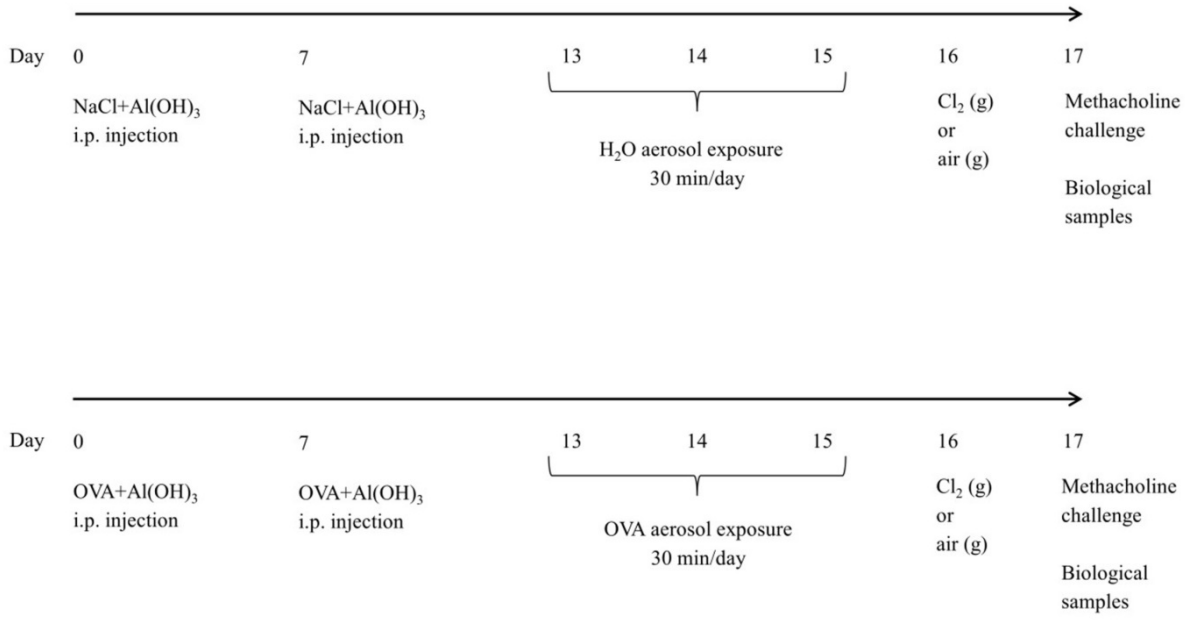


Figure 5. Experimental design for naïve (top) and OVA-sensitized mice (bottom).

The OVA aerosol challenges were performed in a Battelle exposure tower with regular nose-only tubes, while the nose-only tubes during chlorine exposures were connected to a pneumotograph to enable the online recording of respiratory frequency (head-out plethysmography) (Figures 6A-B). Prior to the chlorine exposure, each mouse was guided gently into the tube through a latex rubber collar that was placed around the neck to hold the body in position and to produce a tight system that facilitated the recording of the respiratory frequency.

Twenty-four hours after chlorine exposure, the mice were anaesthetized by pentobarbital sodium (i.p.) and paralyzed by pancuronium to avoid spontaneous breathing. AHR was measured by inhalation of MCh at increasing doses of 0, 25, 50, and 100 mg/ml via a small animal ventilator (flexiVent™, SCIREQ®) apparatus (Figure 6C). Respiratory resistance (R_{RS}), respiratory compliance (C_{RS}), Newtonian resistance (R_n), peripheral tissue resistance (G), and peripheral tissue elastance (H) were obtained.

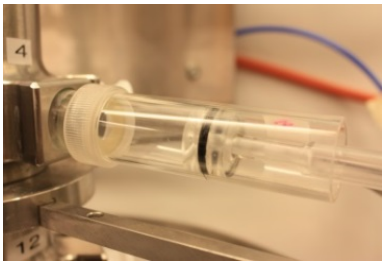
A)  B)  C) 

Figure 6. Experimental methods for inhalation exposure used in Paper IV. A Battelle exposure tower with nose-only tubes for A) the ovalbumin challenge and B) chlorine exposures (plethysmography). C) Small animal ventilator (flexiVent™, SCIREQ®) for lung-mechanic measurements. Photos by Mia Johansson.

In order to confirm the allergic immune sensitization in the mice, OVA-specific IgE antibodies were measured in the serum. Blood was sampled by orbital sinus venipuncture on anaesthetized mice. The total number of leukocytes in the bronchoalveolar lavage fluid (BALF) was counted manually, and differential cell counts of pulmonary cells (macrophages, neutrophils, lymphocytes, and eosinophils) were made in duplicate using standard morphological criteria, and by counting 300 cells per cytospin preparation in a blinded manner.

Ten cytokines and chemokines were analyzed: CCL11 (Eotaxin), CXCL1 (KC), CXCL9 (MIG), CXCL10 (IP-10), CCL3 (MIP-1α), IL-1b, IL-6, IL-10, TNF-α, and TGF-β. In addition, the right lung lobe from lavaged mice was stored in 4% formaldehyde prior to embedding the samples in paraffin. Lung sections were stained with haematoxylin and eosin for histopathological examination.

The results in Paper IV are presented as the arithmetic mean ± standard error mean (SEM), and there were 7 animals in each group, except in the OVA-sensitized group that was exposed to 0-ppm chlorine (6 animals). Graphpad Prism (version 5.0 Graphpad Software Inc., San Diego, CA, US.) was used. Statistical significance was defined as $p < 0.05$.

When recording the respiratory frequency during chlorine exposure, we experienced noise in the data, which yielded abnormally high values. Barrow and colleagues (1977) describe how a short period of increased respiratory frequency and some body movements can be expected at the start of chlorine exposure (each lasting 10 to 20 s), and that additional peaks will be seen during the exposure due to transient body movements. The noise was filtered out by the use of cut-off values (lower = 1 breath/min, upper = 400 breaths/min) (Figure 7). For values within the cut-off-range, we calculated the median respiratory frequency during air exposure (0 to 10 min) and during chlorine exposure (11 to 25 min) separately. The first minute of chlorine exposure (minute 10–11) was excluded due to body movements and the delay in response to chlorine. The percentage change following chlorine exposure compared to air exposure was calculated: (medianCl_2/medianair-1)*100.

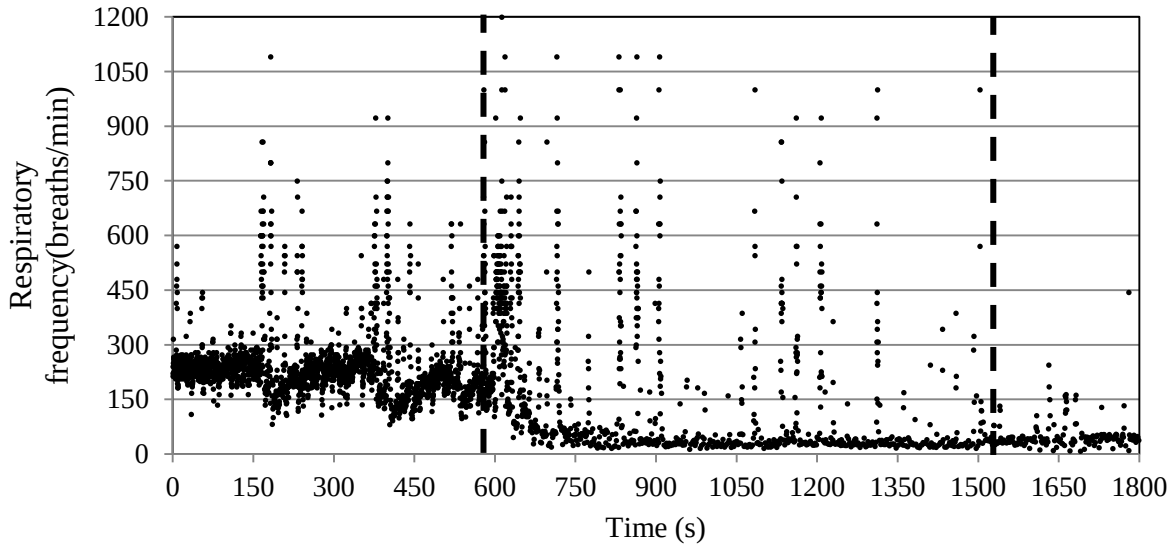


Figure 7. Example: A recording of respiratory frequency in a naïve mouse. Exposures consisted of baseline air (0–600 s), 30-ppm chlorine (600–1500 s), and air (1500–1800 s). We applied cut-off values of 1–400 breaths/min.

5 RESULTS AND DISCUSSION

Further detailed results and discussion sections are included in **Papers I–IV**.

5.1 IDENTIFICATION OF ASTHMATICS IN HEALTH RISK ASSESSMENT

5.1.1 Policy statements

The inclusion of asthmatics as a susceptible subpopulation in the derivation of acute to short-term guideline values was investigated in Papers I and III. The standing operating procedures (SOPs) for AEGL clearly includes asthmatics as a susceptible population (Table 3). The methodology for the French Acute Toxicity Threshold Values (VSTAFs) clearly states that hypersensitive populations such as asthmatics are not included in their target group (INERIS 2007, 2009). The inclusion of asthmatics in the methodology of guideline values aimed at the general population is presented in Table 3.

Table 3. Acute to short-term values for the general population in emergency settings.

Guideline values	Organization	Target population
Acute Exposure Guideline Levels (AEGL)	US National Research Council and Environmental Protection Agency (NRC/EPA)	*"[G]eneral public, **including** susceptible subpopulations, such as infants, children, the elderly, **persons with asthma**, and those with other illnesses"* (NRC 2001, p. 3).
Emergency Response Planning Guidelines (ERPG)	American Industrial Hygiene Association (AIHA)	*"[S]hould not be expected to protect everyone, but should be applicable to **most individuals in the general population**. In all populations, there are hypersensitive individuals who will show adverse responses at exposure concentrations far below levels at which most individuals normally would respond"* (AIHA 2008, p. 14).
Minimal Risk Levels (MRL)	US Agency for Toxic Substances and Disease Registry (ATSDR)	*"Conditions that may enhance susceptibility to adverse health effects include age, sex, genetic composition, nutritional status, and **pre-existing disease** conditions. **UFs of 10 are usually used to derive MRLs protective of these sensitive subpopulations**"* (Pohl and Abadin 1995).
Reference Exposure Levels (REL)	California Office of Environmental Health Hazard Assessment (OEHHA)	*"[B]oth individuals at low risk for chemical injury as well as identifiable **sensitive subpopulations** (highly susceptible or sensitive individuals) […] **asthmatics are frequently identified as a sensitive group**"* (Denton and Hickox 1999, p. 13).
French Acute Toxicity Threshold Values (VSTAF)	French National Institute for Industrial Environment and Risks (INERIS)	*"[G]eneral population **excluding susceptible and hypersusceptible individuals**"* (INERIS 2009, p. 14).

No information concerning asthmatics was included in any of the reviewed methodologies of the occupational short-term values and limits presented in Table 4. However, the DFG states that variations in the potential sensitivity of individual employees should be considered. On the other hand, the SCOEL and ACGIH specifically state that their values concern healthy workers, i.e., asthmatics are presumably excluded.

Table 4. Acute to short-term values aimed at protection in occupational settings.

Guideline values	Organization	Target population
Dutch Expert Committee on Occupational Standards (DECOS)	Health Council of the Netherlands	*"[H]ealth-based recommended occupational exposure limit for the concentration of the substance in workroom air"* (DECOS 2000, p. 4). No further details are included.
Maximum Concentration at the Workplace (MAK)	German Research Foundation (DFG)	*"The **diverse sensitivities of individual employees** (as determined by age, constitution, nutrition, climate, etc.) **are taken into consideration** in the establishment of MAK values"* (DFG 2014, p. 14).
Scientific Committee on Occupational Exposure Limits (SCOEL)	European Commission	*"Groups at higher risk in relation to a specific compound will be identified in the corresponding recommendation and available information provided, but **the OELs are established for healthy workers**"* (SCOEL 2013, p. 16).
Swedish Occupational Exposure Limits (SE-OELs)	Swedish Work Environment Authority (SWEA)	Workers. No further details are included in AFS (2011).
Threshold Limit Values (TLV®)	American Conference of Governmental Industrial Hygienists (ACGIH)	*"**[W]orkers who are normal, healthy adults"*** (ACGIH 2015).

The inclusion of asthmatic individuals in the derivation of DNELs is indirectly implied in the ECHA guidance: *"it may be necessary to identify different DNELs for each relevant human population (e.g. workers, consumers and humans liable to exposure indirectly via the environment) and possibly for certain vulnerable subpopulations (e.g. children, pregnant women)"* (ECHA 2012b, p. 2). *"The AFs for DNELs may deviate from the default values. For example, susceptible subpopulations may be assigned a higher AF than the default value, and conversely, if the point of departure is based on a study population including susceptible individuals (e.g., asthmatics), a reduced intra-species AF may be appropriate"* (ECHA 2012b, p. 160).

5.1.2 Other potentially susceptible subpopulations

There are additional subpopulations, besides asthmatics, with potential susceptibility to irritant chemicals. Several experimental studies were identified in Papers I–III and these are presented in Table 5. The focus of this thesis is on asthmatic individuals, and subjects of all ages were investigated, even though children and the elderly are often thought to be more susceptible. Data from a few experimental studies with such individuals (> 8 and < 89 years of age) were included in Papers II and III, since the observed effects from studies performed on these groups did not differ from those in young to middle-aged adults. However, the potential age-related susceptibility as well as other susceptible subpopulations have to be investigated in more detail to derive appropriate inter-individual AFs.

Table 5. Experimental studies with potentially susceptible subpopulations found during the development of Papers I–III.

Potentially susceptible subpopulation	References
Healthy elderly	Bedi et al. 1988, 1989; Dreschsler-Parks 1987, 1989, 1990, 1995; Linn et al. 1982; Koenig et al. 1993; Reisenauer 1988; Gong 1997; Bauer et al. 1988; McManus et al. 1989; Rondinelli et al. 1987; Morrow et al. 1992; Gong et al. 2005
Patients with chronic obstructive pulmonary disease (COPD)	Linn et al. 1982, 1983, 1985a, 1985b; Kerr et al. 1978, 1979; Vagaggini et al. 1994, 1996; Morrow et al. 1989; Kulle et al. 1982; von Nieding et al. 1973, 1980; Gong et al. 2005; Morrow et al. 1992; Bauer et al. 1988; Solic et al. 1982; Kehrl et al. 1985; Gong et al. 1997
Allergic adults without asthma	Linn et al. 1987; Sheppard et al. 1980; Tam et al. 1988; Koenig et al. 1982a, 1987; Jörres et al. 1996; Holtzman et al. 1979; McDonnell 1987
Hypertensive subjects	Gong et al.1998
Children: healthy, asthmatic, or allergic	Avol et al. 1985, 1987, 1989, 1990; McDonnel 1985; Koenig et al. 1980, 1981, 1982b, 1985, 1987a, 1987b, 1988a, 1988b, 1988c, 1989, 1990; Magnusson et al. 1992, 1993; Oldigs et al. 1991; Salome et al. 1996; Linn et al. 1995, 1997
Ozone-sensitive but otherwise healthy	Schelegle et al. 1989; Folinsbee and Hazucha 2000; Frampton et al. 1997; 1993; Torres et al. 1997
Healthy smokers	Kerr et al.1975; Helleday et al. 1994
Tobacco smoke-sensitive atopic asthmatics	Menon et al. 1991, 1992; Stankus et al. 1988
Gene polymorphisms	Yang et al. 2005

5.2 CONSIDERATION OF ASTHMATICS IN HEALTH RISK ASSESSMENT

In Paper I, about 85% of all chemicals in the AEGL program were defined as respiratory or eye irritants and asthmatics might be considered as a susceptible subpopulation in the derivation of these values. About 70% of the AEGL TSDs lack any explicit statement concerning asthmatic individuals (Figure 8). The reason for not including statements on asthmatics in the TSDs is not obvious. Either no information was found on experimental studies involving this subpopulation or asthmatics are not expected to be more susceptible due to, e.g., non-irritating chemicals (only 15% of the chemicals). A statement related to the

interpretation of asthmatics as potentially susceptible or not would have increased the transparency of the AEGL derivations.

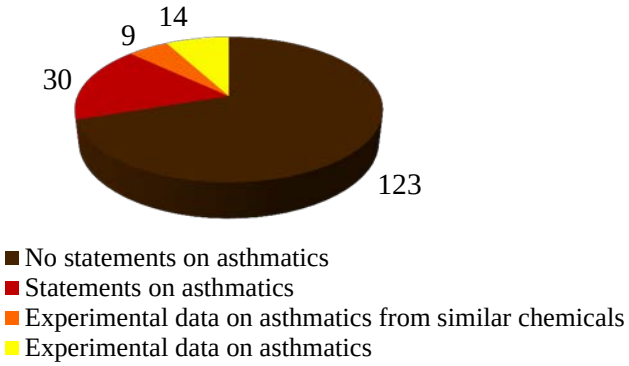


Figure 8. Inclusion of asthmatics in the AEGL technical support documents (n = 176) in Paper I.

It is stated in the AEGL SOP that an inter-individual AF (i.e., intra-species uncertainty factor) of 10 should be applied in cases where data are not available on the most susceptible population. It is also stated that an AF of 3 can be used if the inter-individual variability is expected to be small (NRC 2001). In Paper I, we found that an AF of 3 was applied in a few cases, but not in all, to compensate for a lack of data on asthmatics. A default AF is needed, e.g., for the chemicals evaluated by the AEGL committee that had irritation as the critical effect, but no data on asthmatics.

Only 15 different chemicals in the AEGL program were identified as having been tested on asthmatics in experimental studies. The corresponding support documents for these chemicals were further analyzed in nine additional sets of acute to short-term guideline values. The inclusion of key data on asthmatics in the other acute to short-term guideline values for the general population varied: ERPG (7 out of 12 documents), MRL (3/5), REL (8/11), and VSTAF (0/10).

The five sets of values for occupational settings included key studies on asthmatics in some of their support documents: SCOEL (3/10), TLV® (2/13), and DECOS (1/6). The MAK and the SE-OEL programs had no key studies with data from asthmatics. It has to be noted that SE-OEL documents only provide a scientific basis for setting the OELs, but that the SWEA does not derive the actual values. It seems that persons with asthma might be included to some extent in the derivation of acute to short-term guideline values, despite the lack of explicit policy statements regarding them. The inclusion of asthmatics varies in relation to the aim of the acute to short-term values, i.e., the general population versus occupational settings.

The database of experimental studies (n = 170) in Paper I consists of those found in the AEGL program (2/3 of all studies) and those found only in the nine other programs (1/3 of all studies). These observations reveal that literature coverage in any of the programs were generally incomplete in relation to experimental data on asthmatics.

Results from both Papers I and III point to insufficient use of experimental data in the derivation of guideline values. Only 2.6% of the 269 substances identified as respiratory irritants by the harmonized classification of the CLP Regulation were found to have been tested on asthmatic subjects. The ECHA database of registered chemicals included only 14 chemicals of the 22 known by us to have been tested directly on asthmatic subjects. Nine more chemicals were identified as reaction products or structural analogues. For 10 of the 23 (14+9) chemicals, experimental findings on asthmatics were either key or, more often,

supporting data. Thus, in many cases, relevant data on asthmatics appear to have been disregarded by the registrants. In this context, the REACH registrants more closely resemble the risk assessment programs that derive STEL values than those that derive guideline values for the general population (Paper I).

The DNEL values, investigated in Paper III, were neither systematically higher nor lower than the other ten sets of acute to short-term guideline values, and the worker DNELs were even similar to the SCOEL values. Previous observations show that long-term-inhalation DNELs for workers may be either much lower or much higher than the corresponding national 8-h OELs, but that they are generally similar to the SCOEL values (Nies et al. 2013; Schenk et al. 2015).

Overall, in Paper III, guideline values for workers, including the DNELs and the five investigated sets of values, were shown to be generally higher than those for the general population. As demonstrated previously, discrepancies between values may not only arise from different target populations and availability of data, but also from differing criteria for data selection and evaluation, the methodology employed, and risk assessing experience (Öberg et al. 2010; Schenk 2010). Öberg and colleagues (2010) provide evidence for deviations between the AEGL and ERPG values that may hamper effective risk management and communication during a sudden unintentional or intentional release of chemicals (Öberg et al. 2010). In addition, analyses of the inclusion of asthmatics (Papers I and III) demonstrate that the guideline values for occupational settings are not suitable for the protection of the entire population. However, it could be questioned as to why persons with asthma are not included, given the high prevalence of asthma in the whole population, and that the exacerbation of asthma is clearly an adverse health effect. We argue that asthmatics should be taken into consideration both in emergency and occupational settings.

The DNELs for both workers and the general population were higher than the derived overall LOAEC for two chemicals and were set close to or higher than the estimated overall NOAEC for eight chemicals, indicating small safety margins. The DNEL values were close to or higher than our estimated overall LOAECs, indicating that they may not be sufficient for the protection of asthmatics.

The absence of or the very low safety margins may lead to asthmatics experiencing exacerbations during a regular workday or even in common situations as consumers. DNELs are not meant for rare exposures, but rather for exposures that are repeated during working life or a lifetime. One obvious question is whether such low safety margins are acceptable in connection with such long timeframes. Moreover, the subjects in the 114 studies investigated in Paper III were considered to have mild asthma, so the LOAEC and NOAEC values may be inappropriate for individuals with severe asthma. Although Paper III covered few chemicals it should be noted it included all chemicals with asthma data registered in the ECHA.

Mielke and colleagues (2005) discuss the inclusion of susceptible subpopulations in the derivation of AEGL values. No single subpopulation is expected to be susceptible to all chemicals, but these have to be identified, and the risks have to be quantified (e.g., by the use of an AF). Further, they discuss how large proportion of the whole population should be protected (i.e., 95% or 99%). In this thesis, none of the guideline values discussed in Papers I and III are intended to protect hyper-susceptible groups. In this context, hyper-susceptible responders are characterized as those individuals exhibiting very rare and therefore unpredictable effects that are generally discontinuous with the known range of expected

responses. The inclusion of 99% of the population in the derivation of acute to short-term guideline values may not be a realistic or desirable goal and perhaps a coverage of about 95% is preferable.

5.3 QUANTIFICATION OF THE DIFFERENCE IN SUCEPTIBILITY BETWEEN HEALTHY AND ASTHMATICS

5.3.1 Estimated overall LOAEC and overall NOAEC values

LOAEC values were estimated for 103 experimental studies in Paper II, and both LOAEC and NOAEC values were estimated for 114 studies in Paper III. However, many chemicals did not have data that were relevant for LOAECs or NOAECs. A failure to establish either of them often resulted from exposure to too narrow a range of concentrations (which may all elicit or fail to elicit a response) and/or to a lack of statistical power due to a limited number of subjects. It was not possible to establish a LOAEC for several of the studies, and the only or the highest concentration tested was defined as the NOAEC.

The studies included the endpoints of sensory irritation, the impairment of pulmonary function, or hyper-reactivity from bronchial challenge testing. Sensory irritation is often used as the critical effect for setting AEGL-1 values. The impairment of pulmonary function is considered more severe and serves as a basis for AEGL-2 values. Both sensory irritation and the impairment of pulmonary function were most important for our determination of LOAECs and the overall EDRF.

5.3.2 Estimated differential response factors

In Part 1 of Paper II, the estimated LOAEC values for the 103 identified experimental studies, including 19 chemicals and 11 mixtures/combined exposures, were used to estimate the overall EDRFs. Furthermore, there is substantial uncertainty involved in the estimation of EDRFs based on LOAECs, which are strongly influenced by both the number of subjects and test concentrations. LOAEC-based EDRFs are often uncertain or inconclusive, mainly due to a lack of data, the inclusion of only a few subjects in the available studies, and extensive inter-individual variability.

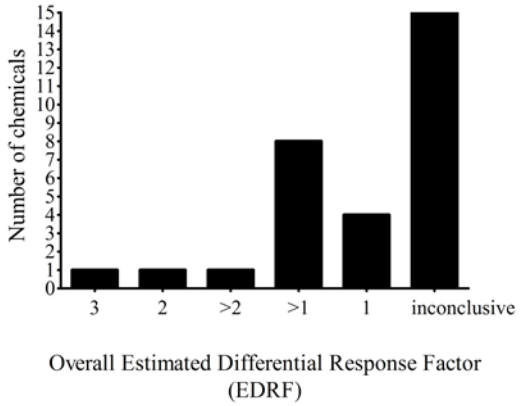


Figure 9. Chemicals classified by the overall EDRF, which expresses the relative response in healthy and asthmatic individuals.

Sixteen of the 30 chemicals/mixtures were each investigated in one study only. The overall EDRFs (i.e., derived for each chemical) are presented in Figure 9. If a single study was judged to be of high quality, it was used as the basis for the overall EDRF, but otherwise, the overall EDRF was regarded as “inconclusive.” Half of the chemicals and mixtures evoked no response at any concentration that was tested and the overall EDRFs were therefore

"inconclusive." On the other hand, the highest difference between healthy and asthmatic individuals was seen in an experimental study with sulfur dioxide, where asthmatics were 5 times more susceptible (Sheppard et al. 1980). Young et al. (2009) derived EDRFs for six substances (i.e., EDRF 1–5), which are similar to ours, although these investigators selected a single key study (or two) for each chemical, involving healthy and/or asthmatic individuals, as the basis for their calculations.

5.3.3 Concentration-response and Benchmark concentration analyses

Nitrogen dioxide, ozone, sulfuric acid, and sulfur dioxide were included in 61 of the 103 studies in Paper II, Part 1, and were selected, based on these numerous data, for the comparative analysis of concentration–response relationships in Part 2. In total, 476 separate sets of data on these chemicals were retrieved, following additional computerized literature searches, and the comparative analysis was based on the data sets that included FEV_1 and/or SRaw (n = 262).

All concentration-response relationships were based on exposures performed during exercise. It was evident that the response in asthmatics was higher than in healthy individuals following exposure to sulfur dioxide and sulfuric acid. On the contrary, no differences were observed between the groups following ozone exposure. Exposure to nitrogen dioxide resulted in responses in asthmatics, but similar effects were seen already under control exposure to filtered air, suggesting that the effects were induced by exercise rather than by the chemical.

LOAEC values are highly sensitive to the concentrations tested. Pronounced inter-individual variability at any concentration will result in higher LOAEC values, since these are based on pairwise comparisons with control data. For that reason, the BMC approach to comparative analysis of concentration–response relationships is preferred. No such analysis of experimental findings on healthy and asthmatic subjects has, to the author's knowledge, been reported previously. The BMC analysis of sulfur dioxide resulted in an EDRF of 9 (Figure 10), which is higher than an EDRF of 3–4 as suggested by Young et al. (2009).

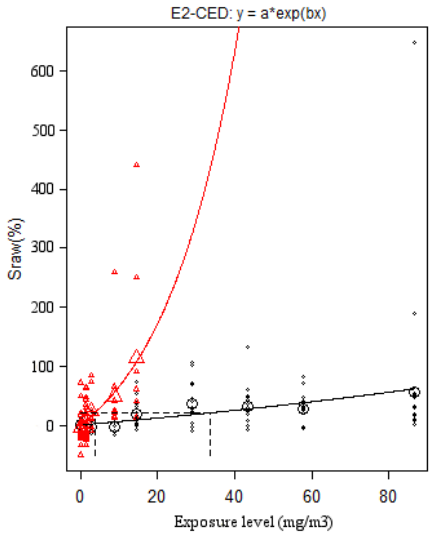


Figure 10. Benchmark concentration analysis of the specific airway resistance response in asthmatic (triangles) and healthy (circles) individuals following acute exposure to sulfur dioxide in a chamber at rest (Adopted from Paper II, ©The authors).

5.3.4 Adjustment for variability in experimental data

Human experimental studies involving a controlled environment and study population reduce confounding factors. Despite the advantage of controlled conditions, the studies are not performed according to good laboratory practice (GLP) or other guidelines, and there will always be variability in the experimental design, within each individual and within the asthmatic or healthy group. We adjusted for several factors when selecting experimental studies to reduce this variability and retrieve comparable data of high quality in Papers II and III. However, the mentioned factors will still contribute to uncertainty in the EDRF estimations and concentration–response analyses in Paper II, and in the LOAEC and NOAEC estimations in Paper III.

Humans, as compared to rodents, are not inbred, and they differ in biological aspects such as their genetics and hormonal levels. In addition, there might be a risk of bias in terms of diet, socio-economic status, or lifestyle—factors that were not controlled for in Papers II-III. The American Thoracic Society (2000) reports that bronchial responsiveness from MCh or histamine is increased by exposure to environmental antigens, occupational sensitizers, air pollutants, cigarette smoke, chemical irritants, or by having a respiratory infection. Bronchial responsiveness is decreased through the intake of medications, chocolate, or caffeine-containing drinks (ATS 2000). Pulmonary function data from the chemical exposures in Papers II–III are likely also affected by these factors.

The personnel and experimental set-up differ both between and sometimes within the studies. In addition, the studies were published within a range of almost 60 years (1952–2011), which may result in variability in the asthma diagnoses, ethical considerations during the exposures, and the methods used.

Nasal breathing is present in most adults (80–90%) at rest, but shifts to oronasal breathing during exercise, when the minute ventilation rises to between 30 and 40 L/min (Kleinman 1984). Asthmatics are more likely than healthy subjects are to perform oronasal breathing even at rest (Chadha et al. 1987). Between 70–80% of all asthmatic individuals will have an exacerbation from exercise provocation, although it is not very sensitive in clinical situations (Sterk et al. 1993). In Part 2, Paper II, separate concentration–response relationships were made for exercising subjects (bicycle or treadmill) breathing oronasally and through the mouth only, but the outcome did not differ. The level of exercise workload (i.e., breathing ventilation of 20–70 L/min) was not differentiated and this variation may have affected the results. To adjust for exercise-induced effects we included exposure to filtered air as a control.

The lung size in women is generally smaller than in men and children have an even smaller size, which may affect their response following the inhalation of chemicals. Most studies included adult men and women between 18 and 54 years of age. A few studies with 8–17-year-old children or the elderly aged 60–89 were included in Papers II–III since their pattern of effects did not differ from the available experimental findings on younger and middle-aged adults.

The variability in the duration of exposure may influence the response to chemicals and it varied between 5 min and 8 h in the identified studies. However, replacement of the factor “concentration” by “concentration*time” in the concentration–response graphs in Paper II produced results that were similar to those for concentration–response. However, according to the SOP for the AEGL values, *“With respect to mild sensory effects, they are generally not*

cumulative over a range of exposures of 10 min to 8 h. Hence, the same AEGL-1 value may be assigned to all AEGL-specified exposure periods. […] In the case of certain sensory irritants, the AEGL values may be constant across all AEGL time periods, because this endpoint is considered a threshold effect, and prolonged exposure will not result in an enhanced response. In fact, individuals may adapt or become inured to sensory irritation provoked by exposure to these chemicals over these exposure periods such that the warning properties are reduced" (NRC 2001, p. 108). In addition, the AEGL support document for sulfur dioxide states that most of the bronchoconstriction caused by this substance occurs within 10 min and both the AEGL-1 and -2 values are therefore held constant across the exposure durations (NRC 2010).

Another limitation with human experimental studies is the difficulty in obtaining a large sample of subjects, which is due to technical and economic reasons. This leads to low statistical power and thus the variability among the subjects may be high. The small group of subjects may not represent the whole population or subpopulation (Bylin 1987). In our databases of experimental data, the number of subjects varied from 4 to 66, except for five studies involving 135–786 healthy individuals.

We tried to minimize differences between the asthmatic and healthy groups by excluding studies performed during pollen season (not always stated), outside of room temperature and normal humidity, and by separating studies performed at rest from those including exercise. The exclusion of studies outside of normal room temperature (18–25ºC) was based on a study by Bethel et al. (1984) where asthmatics had a significantly larger response in lung function following exposure to cold temperatures than at normal room temperature.

The AF applied to acute to short-term guideline values is not intended to protect hypersensitive subpopulations, and studies on individuals with severe asthma were therefore excluded. At the same time, studies without a stated severity level were included and there might be variability in the definition of "mild" asthma between studies. On the contrary, Linn et al. (1987) show that there were no significant differences between mild and moderate/severe asthmatics following exposure to sulfur dioxide at different concentrations, which implies that the severity level of asthma may not contribute much to the variability.

Variability in lung function and bronchial responsiveness within the group of asthmatics is apparent, both over time within one individual, and between individuals at any point in time (Bylin 1987). Corticosteroid treatment has long-term effects that might underestimate the sensitivity among asthmatics. In most studies, but not all, asthmatics were not allowed to use this treatment for weeks or months prior to the experimental exposure. According to GINA (2015), the inclusion of subjects with mild asthma in studies is based on the assumption of appropriate asthma treatment usage. At the same time, studies require subjects to restrain from the use of treatment prior to the exposure, which leads to uncontrolled symptoms. For this reason, it would have been preferable to have categorized subjects by the treatment used, and not by the severity level of their asthma (GINA 2015).

Additional limitations with human subjects in experimental studies are erroneous self-reporting of treatment use, smoking habits, and their history of asthma. In addition, the subject may not be aware of existing exercise-induced asthma, allergies, or other pre-existing diseases.

5.1 AIRWAY RESPONSE TO CHLORINE IN NAÏVE AND SENSITIZED MICE

In Paper IV, all mice that had been immunized and challenged with OVA had an increased number of eosinophils in their BALF, increased levels of IgE in their serum, and clear signs of lung injury from the histopathological examination. These effects were not seen in naïve mice.

It can be argued that OVA is not an allergen to which humans naturally become sensitized and that the inflammation is too high as compared to humans. The percentage of eosinophils in BALF is 40–80% in OVA-sensitized mice and 1–3% in human individuals (Fulkersson et al. 2005). Chronic allergen challenges with low levels of house dust mites, *Aspergillus*, cockroaches, or pollens have been suggested to be preferable, since these allergens are more physiologically relevant than OVA, and since chronic, and not acute, challenges lead to airway remodeling that is somewhat similar to that seen in human asthmatics (Kumar et al. 2008; Lloyd 2007). In spite of this, one must remember that models are just models, and even though mouse models do not fully mimic asthma in humans, they are still able to provide more knowledge on the disease (Persson 2002; Wenzel and Holgate 2006). Every model has its strengths and weaknesses (Kips et al. 2003) and the advantages with OVA are its availability, the controlled administration dosage, and that it is relatively well defined (Kumar et al. 2008). Swedin and colleagues (2010) showed that an intranasal challenge with OVA in BALB\c mice caused more AHR and inflammation (mainly eosinophils and macrophages) as compared to an inhalation challenge to OVA aerosol. Therefore, the use of intranasal challenges in Paper IV would probably have resulted in even higher levels of eosinophils in the OVA sensitized animals.

The changes in respiratory frequency following exposures to chlorine in concentrations of 0, 5, 30, and 80 ppm were similar between naïve and OVA-sensitized mice. The RD_{50} values were slightly above 5 ppm, and this has—to the best of our knowledge—not been determined in BALB/c mice previously. The size of the RD_{50} values for chlorine observed for other mouse strains are quite similar to 5 ppm, indicating a low inter-strain variability in terms of irritation (Barrow et al. 1977; Gagnaire et al. 1994; Morris et al. 2005).

For mice, it has been empirically observed that, on average, 0.03 times the RD_{50} in mice corresponds to the 8-h TLV®-TWA for substances with sensory irritation properties (Alarie 1981). It may be of interest to note that 0.03 times the approximate RD_{50} value of 5 ppm identified in Paper IV, i.e., 0.15 ppm, is in line with the NOAEC in asthmatic human subjects of 0.4 ppm (D'Alessandro et al. 1996) as well as the AEGL-1 value of 0.5 ppm (NRC, 2004). The applied method to filter out the noise in the respiratory frequency data (described in more detail in Methods, Chapter 4.6) may have affected the percentage change following chlorine exposure. However, the abnormal responses obtained in the unfiltered data are not physiologically possible in mice and some kind of filter is therefore needed. It is perhaps worth discussing if the chosen upper cut-off value of 400 breaths/min is accurate, since applying a lower cut-off value will result in a larger change between chlorine and air exposure, and the opposite will be true after applying a higher cut-off value. The RD_{50} of about 5 ppm in Paper IV is therefore more of an estimation with considerable uncertainty. In addition, the acclimatization period of 10 min applied to all mice prior to recording the air (baseline) exposure might have been too short.

A significant decrease in body weight during the first 24 h was observed in both naïve (5.7%) and OVA-sensitized (2%) mice after exposure to 80-ppm chlorine. The decrease in naïve

mice was a clear concentration-dependent effect. However, the variation in weight in mice may be high due to the impact of food and water intake, as well as excretion through urine and feces. Therefore, small effects in terms of weight should be addressed with caution.

Interestingly, a clear increase in AHR and in the cellular infiltration of neutrophils (Figure 11) was seen in the naïve, but not in the OVA-sensitized mice, after exposure to 80-ppm chlorine. These differences between groups indicate that individuals with a neutrophilic asthma phenotype might be more sensitive to chlorine exposure compared to individuals with an eosinophilic asthma phenotype, but additional studies are needed to confirm this hypothesis.

Bogaert and colleagues (2011) observed that OVA sensitization in C57BL/6 mice with the adjuvant aluminum hydroxide produces a T_H2 inflammation, while Freund's adjuvant produces a T_H1 and T_H17 inflammation, which is more similar to neutrophilic allergic asthma in humans. The use of Freund's adjuvant for OVA sensitization in C57BL/6 mice may have been a preferred approach with which to study the inflammatory response to chlorine in Paper IV. However, as stated previously, C57BL/6 mice do not develop AHR and do not show as high an inflammatory response in their airway smooth muscle as BALB\c mice do (Säfholm et al. 2011). The use of Freund's adjuvant in OVA sensitization of BALB\c mice may not result in neutrophilic asthma since this strain develops a T_H2 inflammatory response.

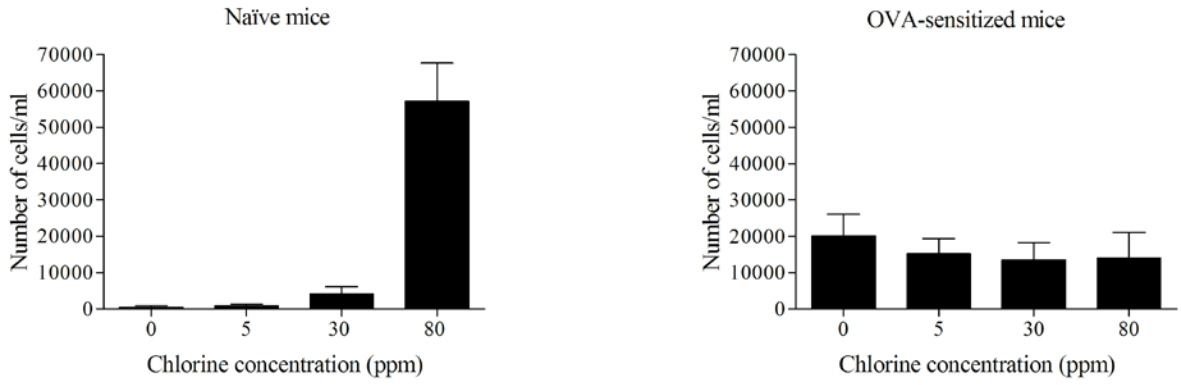


Figure 11. Analysis of neutrophils in bronchoalveolar lavage fluid from naïve and OVA-sensitized mice following exposure to various concentrations of chlorine. (Arithmetic mean ± SEM, n = 7, except for OVA-sensitized mice exposed to 0-ppm chlorine, n = 6).

The AHR was investigated in a ventilator (flexiVent™ SCIREQ®) 24–30 h after exposure to chlorine and 40–46 h after the final OVA exposure. It can be argued that the applied exposure protocol is not relevant to the expected response in a real-life emergency scenario with acute exposure to chlorine. In that sense, a preferred approach would have been to measure AHR in the flexiVent™ apparatus during chlorine exposure (instead of using MCh), but this was not possible due to the corrosive damage chlorine would produce on the apparatus. In addition, AHR measurements in the flexiVent™ apparatus are an invasive method with obligatory euthanization post-procedure, and the analyses of inflammatory cells and cytokines would not have been possible, since these responses are delayed.

The recordings of breathing frequency during chlorine exposure became a valuable complementary method to the AHR measurements in regards to evaluating the effects on lung function. No experiments have—to the best of the author's knowledge—been performed with both naïve and OVA-sensitized mice using the combination of head-out

plethysmography and MCh provocation. Inhalation studies with chlorine have been performed previously using naïve (Jonasson et al. 2013) and OVA-sensitized BALB/c mice (Hox et al. 2011). In addition, several experiments with chlorine have been performed using naïve mice from other strains (Alarie 1981; Barrow et al. 1977; Chang et al. 2012; Gagnaire et al. 1994; Hoyle et al. 2010; Morris et al. 2005; Tuck et al. 2008).

The exposure duration and chlorine concentration used varied between Paper IV (15 min at 80 ppm), Jonasson et al. (2013) (15 min at 50 and 200 ppm), and McGovern et al. (2015) (5 min at 100 ppm), but all studies reveal increases in AHR and neutrophil infiltration 24 h after chlorine inhalation in naïve BALB\c mice. McGovern and colleagues (2015) observed that after the depletion of neutrophils with antibodies directed against granulocyte receptor-1 or Ly6G, there was no recruitment of neutrophils to the airways, and the AHR was decreased during MCh challenges. Both types of antibodies caused decreases in R_{RS} and R_n, but not in G and H, and they conclude that the presence of neutrophils is a major player in terms of the oxidative burden in the airways (McGovern et al. 2015). This may explain the lack of AHR in mice with eosinophilic airway inflammation (OVA-sensitized) exposed to 80-ppm chlorine in Paper IV.

No chlorine-related increases in cytokine and chemokine levels from BALF were observed in samples from either naïve or OVA-sensitized mice. The findings of changes in cytokines and chemokines by Jonasson et al. (2013) could not be confirmed in Paper IV, indicating that the chosen time point (24 h after exposure) might have been too late to detect a transient induction.

Jonasson et al. (2013) demonstrate that injuries in naïve BALB\c mice were restricted to the larger airways following the inhalation of chlorine (200 ppm for 15 min). Hoyle and colleagues (2010) found consistent damage in the large airways in naïve FVB\N mice exposed to 100-ppm chlorine gas for 60 min, but detached epithelial cells were not visible deep into the lung. McGovern and colleagues (2015) found neutrophils in the airway lumen in male BALB\c mice 6 h and 24 h after 100-ppm chlorine gas exposure for 5 min.

The lack of chlorine-dependent findings in histopathology from both groups in Paper IV might be due to the relatively low chlorine concentration (80 ppm). Chlorine hydrolyzes upon contact with the mucus in the upper respiratory tract (nose) and in the tracheobronchiolar region (Yadav et al. 2010), indicating that only small amounts of chlorine reached the alveolar region where most of the histological sections were performed.

6 CONCLUSIONS

In conclusion, the results from **Papers I-IV** showed that:

I. More than two thirds of the AEGL TSDs lack data or even comments about asthmatics, and experimental data from asthmatics are referred to for only 15 chemicals. A comparison of the support documents for these 15 chemicals from AEGLs and 9 additional sets of acute to short-term values reveals that asthmatics are frequently disregarded in both emergency and occupational settings. Available data on asthmatics should be considered carefully in the derivation of guideline values, and if such data are lacking, this should be indicated explicitly in the case of respiratory irritants.

II. Although the available data on individual chemicals are often inconclusive, overall, the experimental findings on inter-individual variation provide support for the use of an AF of 10 to estimate the level of exposure below which respiratory symptoms and the impairment of pulmonary function are not likely to occur in the general population, including asthmatics.

III. REACH registrants do not routinely take asthmatics into consideration when setting acute/short-term DNELs, which may result in values that are inappropriately high for this subpopulation. Several of the acute/short-term inhalation DNELs investigated exceed or are close to the estimated NOAEC and/or LOAEC values for asthmatics, indicating no, or low safety margins. Updated ECHA guidance concerning susceptible subgroups in connection with the setting of DNELs may improve the derivation of these values.

IV. At 80-ppm chlorine, increases in both airway responsiveness and the number of neutrophils in BALF were evident in naïve mice, but not in OVA-sensitized mice. Concentration-dependent reductions in respiratory frequency were seen in both groups. The animal model, which represents a phenotype of eosinophilic airway inflammation, did not show any increased susceptibility following exposure to chlorine.

Overall conclusion:

Exclusion of asthmatics in the derivation of acute to short-term guideline values may interfere with trustful and efficient health-protective actions. The use of an AF of 10 when there is a lack of experimental data on asthmatics may be adequate to protect this subpopulation from the deleterious respiratory effects of airborne chemicals.

7 FUTURE RESEARCH

Further research is needed to draw safer/more definite conclusions regarding the susceptibility of asthmatics. Here are some suggestions:

- Perform high-quality experimental studies including both healthy and asthmatic subjects to obtain more precise AFs for asthmatics. Such studies should be designed to determine NOAECs and LOAECs for both healthy and asthmatic subjects and preferably be performed at rest in order to avoid exercise-induced effects.

- Gain more mechanistical information related to if and how the asthma phenotype is an important factor for increased susceptibility to irritant chemicals.

- Explore *Ex vivo* and *in vitro* methods either as a complement or as a substitute for experimental studies with human and animal subjects. For example, Guinea-Pig Precision Cut Lung Slices (PCLS) and Air Liquid Interface (ALI) might be used both as screening tools for identification of chemicals which asthmatics have an increased susceptibility to, and to provide more mechanistic knowledge about inter-individual differences.

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