

Prick and Intracutaneous Testing and IgE Testing

- Authors

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Abstract

Immediate-type allergies are frequent in specific occupational environments.

Most important is to consider the possibility of occupational factors in allergic diseases.

Depending on the substance, immunological and nonimmunological mechanisms may be relevant for symptom elicitation.

According to European Directive 2001/83/EC in vivo test allergens are medicinal products, whereas test allergens for in vitro use are medical devices following respective regulations. Standardized test preparations for in vivo and/or in vitro testing for specific IgE to occupational allergen sources are rare. False-negative as well as false-positive test reactions may occur with current test methods for detection of specific IgE to occupational allergen sources. Compared to ubiquitous inhalant allergens (cut-off ≥ 3.00 mm wheal diameter), for occupational allergens, a different cut-off (wheal diameter ≥ 1.5 mm) applies.

Skin prick testing, intracutaneous testing and in vitro investigation of specific IgE constitute one component in a stepwise assessment for diagnosis of allergen-induced occupational diseases.

High- and low-molecular-weight allergens have to be considered as occupational allergen sources in specific work environments.

Keywords

Specific igE Skin prick test Intradermal test Intracutaneous test Diagnostic algorithm Type I Allergy Contact urticaria Occupational/work-related thinitis/asthma Molecular allergy diagnostics Component-resolved diagnosis Exposures

1 Core Messages

- Immediate-type allergies are frequent in specific occupational environments.
- Most important is to consider the possibility of occupational factors in allergic diseases.
- Depending on the substance, immunological and nonimmunological mechanisms may be relevant for symptom elicitation.
- Standardized test preparations for in vivo and/or in vitro testing for specific IgE to occupational allergen sources are rare.
- False-negative as well as false-positive test reactions may occur with current test methods for detection of specific IgE to occupational allergen sources.

- For occupational allergens, a different cut-off (wheal diameter ≥ 1.5 mm) applies.
- Skin prick testing, intracutaneous testing and in vitro investigation of specific IgE constitute one component in a stepwise assessment for diagnosis of allergen-induced occupational diseases.
- High- and low-molecular-weight allergens have to be considered as occupational allergen sources in specific work environments.

2 Introduction

More than 250 occupational agents have been identified as potential sensitizers of immediate reactions, such as bronchial asthma, rhinitis, conjunctivitis, and contact urticaria (van Kampen et al. [2000](#); Tarlo et al. [2008](#); Moscato et al. [2009](#); Baur and Bakehe [2014](#); Raulf-Heimsoth et al. [2017](#)). Concomitant disease manifestation is not uncommon: In a recent study, almost half of the patients (46%) with occupational contact urticaria (CU) and protein contact dermatitis (PCD) had concomitant occupational airways disease: In 291 cases of occupational CU or PCD (in which most common causes were flour, cow dander, natural rubber latex, and acid anhydrides), concomitant occupational asthma caused by the same agent as the skin disease was detected in 21% and occupational rhinitis in 38% of patients (Helaskoski et al. [2017](#)).

About 25% of adult asthma cases may have an occupational basis including both occupational asthma as well as work-exacerbated asthma (Tarlo et al. [2008](#)). As the most frequent causes for obstructive respiratory diseases caused by allergenic substances (including rhinopathy) recognized as occupational disease in Germany (Mahler et al. [2017a](#)) in 1 year the following allergen sources were identified ($n = 565$): flour, flour products, dough, bakery, and confectionery products (61.1%); hair treatment (5.0%); fruit, vegetables, plants (5.0%); hair, bristles, feathers, horn of animals (4.4%); disinfectants (3.2%); natural rubber latex (2.5%); dust from food or animal feed (2.3%); enzymes (0.9%); molds (0.5%); and other (15.2%) (Raulf-Heimsoth et al. [2012](#)).

So far, only few allergen sources have been studied in detail (like wheat flour dust and natural rubber latex (see Sect. [4](#))), whereas most occupational sensitizers were documented only as case reports or case series.

Selecting the most appropriate diagnostic testing and management is driven by assessing whether a sensitizer is involved, and if so, identifying whether the sensitizing agent is a high-molecular-weight agent such as a protein or a low-molecular-weight (LMW) reactive chemical (Dykewicz [2009](#)).

High molecular proteins (>5 kDa), e.g., from flour, particles from foods and fodder, as well as animal danders, are the most frequent elicitors of recognized occupational airways diseases (Raulf-Heimsoth et al. [2009](#)). However, also low molecular agents (<3 kDa) such as isocyanates, hexachloroplatinate, and organic acid anhydrides may act as haptens and induce immunological processes (Grammer [2001](#)).

According to European Directive 2001/83/EC all in vivo test allergens are medicinal products (Mahler [2018](#)), whereas test allergens for in vitro use are medical devices following respective regulations (in the EU: Directive 98/79/EC to be replaced by Regulation (EU) 2017/746).

Skin prick testing, intracutaneous testing, and in vitro investigation of specific IgE constitute one component among many others in a stepwise assessment for diagnosis of allergen-induced work-related asthma, chronic cough, rhinitis, contact urticaria, or anaphylaxis (Tarlo et al. [2008](#); Moscato et al. [2012](#), [2014](#); Vandenplas et al. [2014](#); Dykewicz [2009](#); Moscato et al. [2009](#); Helaskoski et al. [2015](#), [2017](#); Siracusa et al. [2015](#)). Also the diagnosis of a protein contact dermatitis with or without preceding contact urticaria (e.g., in food handling occupations) is predominately based on positive skin prick test (SPT) results with fresh and commercial material as well as detection of specific IgE in vitro. Patch tests rarely demonstrate positive results in this disease entity (Mahler [2010](#), [2011](#)).

To set up a feasible test panel for in vivo and in vitro tests, a precise knowledge about the personal exposures at the workplace and possible allergen sources is mandatory (Alfonso et al. [2017](#)) (Table [1](#)).

Table 1

Relevant occupational Type I allergen sources. (Modified from Mahler and Drexler [2004](#))

Occupational agent	Molecular weight	Occupational field
Flour, grains	HMW	Bakeries, mills
Foods (plant-/animal-derived)	HMW	Food handling and processing
Enzymes	HMW	Bakeries, food handling and processing, animal feed industries, production of cleaning agents/detergents, pharmaceutical industries, enzyme producing industries, research laboratories, paper and textile industry
Natural rubber latex	HMW	Health care, protection glove wearing industries
Wood dusts	HMW	Wood production, wood processing, carpentry
Plant particles, pollen, tobacco leaves, tea leaves, cacao beans, castor beans, seeds, spices	HMW	Cultivation, farming, nurseries, storage
Henna	HMW	Hairdressing
Animals (laboratory/farm)	HMW	Laboratories, farming, veterinarians

Occupational agent	Molecular weight	Occupational field
Mites (house dust/storage)	HMW	Farming, bakeries, grain storage
Spider mites	HMW	Fruit farm workers
Bee mite (<i>Varroa destructor</i>)	HMW	Bee keeping
Insects, insect venoms	HMW	Laboratory workers, farm workers, contaminants in food industries, bee keepers
Parasites (e.g., Anisakis)	HMW	Contaminants in food industries
Molds	HMW	Deconstruction, cleansing, waste disposal (sorting, composting), wastewater treatment, air-conditioning (maintenance), wood and paper processing, textile industries, archiving, farming, crop growing, food handling and processing
(Di)isocyanates	LMW	Polyurethane foam preparation, thermal insulation and coatings, adhesives, sealants (e.g., in automobile industries)
Cyclic acid anhydrides	LMW	Curing agents for epoxy resins, production of polyester and alkyd resins
Metals (e.g., platinum)	LMW	Platinum refinery, catalyst production, electroplating, electronics industries, soldering
Colophony	LMW	Soldering
Drugs	LMW	Pharmaceutical industries, healthcare, nursing
Disinfectants	LMW	Healthcare
Ethylene oxide	LMW	Industrial sterilization
Ethylenediamine	LMW	Chemical industries
Persulfates	LMW	Hairdressing, chemical industries

Whereas the in vivo test methods detect cell-bound specific IgE by eliciting a localized allergic response, in vitro tests identify circulating (unbound) specific IgE. Since cell-bound and unbound specific IgE are generally in a steady state equilibrium (Metzger et al. [1984](#)), results of in vivo and in vitro tests are concordant, provided that the used allergen-extract is identical in composition and test conditions are not impaired (Table [2](#)).

Table 2

If a patient's history of occupational allergic symptoms and test results are not in concordance, several reasons – including false-negative and false-positive test

results – have to be taken into account. An overview on potential factors is given (+: factor may influence the respective test method)

(False-)negative test result due to	In vivo	In vitro
Incomplete test material (missing components)	+	+
Incorrect technique	+	–
Cut-off set to high	+	+
Allergen-specific blocking IgG-antibodies	–	+
Refractory phase (following antibody depletion in severe anaphylactic reactions)	+	+
Local IgE-production	+	+
Different immunological mechanism (e.g., late-phase reaction) or non-immunological mechanism induces occupational symptoms	+	+
Concomitant medication (systemic H1-blocker, certain H2-blockers (ranitidine), ketotifen, phenothiazine-derivatives, tricyclic antidepressants, β -adrenergic agonists, short term high dose corticosteroids, topical steroids in test site)	+	–
Skin alterations due to underlying disease (diabetic neuropathy, chronic hemodialysis)	+	–
Incomplete history concerning the culprit allergen exposure	+	+
(False-)positive test result due to	In vivo	In vitro
Contaminated test material	+	+
Test substance naturally contains histamine	+	–
Test substance is a non-specific mast cell secretagogue	+	–
Incorrect technique (distance of test sites is too low; reuse of test lancet)	+	–
Skin condition of individual is unfit for testing (eczematous skin, urticaria factitia)	+	–
Non-specific IgE-binding in individuals with total IgE >1,000 IU/ml	–	+
Carbohydrate specific IgE-binding	–	+
Clinically silent cross-reactivity to homologous allergens (different primary sensitizing allergen source)	+	+

A positive test result may indicate a Type I sensitization; however, it does not give any information on the clinical relevance of this finding, which has to be appraised together with the (occupational) exposure and clinical symptoms upon exposure. A negative test result, however, in discordance with a clear history of clinical symptoms upon exposure might not be reliable due to the quality of the used allergen source. Like all other tests, also in vivo and in vitro tests for specific IgE have a potential for false-positive and false-negative test responses (Dykewicz [2009](#)).

To this end, still a number of SPT preparations for detection of specific IgE to high-molecular-weight allergens from occupational environments are commercially available despite an increasing tendency of withdrawal of marketing authorizations by the test allergen manufacturers (Mahler [2018](#)); however, all of these materials for SPT are based on extracts of the naturally occurring allergen sources. Due to the natural variation of protein composition and allergen content, these extracts used as diagnostic material may vary concerning the allergenic components therein provided and may eventually lead to false-negative test results. To date, standardization of extracts is based exclusively on the content of major allergens and does not regard the presence of minor allergens (van Ree and CREATE Partnership [2004](#); Bonertz et al. [2018](#)). To this end, due to different use of Article 5 European Directive 2001/83/EC (exemption from marketing authorization), in the EU not all commercially available test allergens are standardized and approved by national authorities.

In contrast to environmental allergen sources (e.g., pollens), for the diagnosis of occupational immediate-type allergies, only very few diagnostic materials are available as standardized materials for in vitro or in vivo testing.

Dealing with nonstandardized test materials (e.g., from the patient's workplace) implies several obstacles for the physician in charge: (1) to obtain detailed information on the ingredients and composition of the material, (2) to provide the accurate test concentration, (3) to deal with a limited validity of the obtained test results, (4) to consider and deal with the need of recruiting control test individuals – if legally and ethically acceptable, and (5) to regard the national legal requirements (e.g., according to the national German Pharmaceuticals Act in Germany, for testing of nonstandardized agents from the patients' (occupational) environment, an official notification to the local surveillance authority has to be filed) (Mahler et al. [2017b](#)).

In case test results and the patient's history are contradictory, a different test material or additional test methods (e.g., challenge test) might be necessary.

Albeit these existing obstacles, in this chapter a succinct overview on the current state-of-the-art of skin prick and intracutaneous testing and in vitro-IgE diagnostics of relevant occupational allergen sources inducing immediate type reactions is given.

3 Test Methods

3.1 In vivo Test Methods (Skin Prick and Intracutaneous Testing)

Prior to the (in vivo) testing, a thorough history taking is necessary to define the test panel and to assess relevant influence and risk factors (e.g., previous severe reactions to a specific agent, medical preconditions, medication) (Antunes et al. [2009](#)). Prior to all in vivo test procedures, thorough patient information and written consent has been recommended (Mahler et al. [2017b](#)).

For most allergen sources, the standard in vivo test method is the skin prick test (SPT). One drop ($\approx 30 \mu\text{l}$) of test solution is applied on the intact skin of the forearm, then a disposable (25 or 26 gauge) needle is passed through the drop and inserted to the epidermal surface at a low angle which is then gently lifted in a nontraumatizing way (i.e., without inducing bleeding) (Dreborg et al. [1989](#); Antunes et al. [2009](#)). In the intracutaneous test, the same amount of test solution is injected into the dermis from a 0.5 ml tuberculin syringe through a 27-gauge needle. It is crucial to inject intracutaneously and not by mistake subcutaneously since this will lead to false-negative test results. Infectious material cannot be used for in vivo testing. Colored occupational test material should not be used for intracutaneous testing since this may leave a permanent iatrogenic tattoo.

Intracutaneous testing is the most sensitive method of skin testing, but it also induces more false-positive reactions than the SPT, especially for allergen extracts rich in histamine or having histamine-releasing activity or if pH, osmolarity, or preservatives such as glycerol or thiomersal are not controlled (Chipps et al. [1978](#)). Intracutaneous tests are more reproducible than SPT. The concentration of allergen extract required to elicit a positive reaction with intracutaneous testing is 1,000–30,000 times smaller than that necessary for a positive SPT (reviewed in: Bousquet and Michel [1993](#)).

Therefore, the intracutaneous skin tests are preferably used for the detection of low-grade sensitizations and for evaluation of allergenic extracts of low potency. Although the intracutaneous testing is a validated technique that has been extensively studied (reviewed in: Bousquet and Michel [1993](#)), the reading and interpretation is more difficult since the intracutaneously applied volume itself causes a limited wheal.

All in vivo examinations require a negative control (0.9% sodium chloride) and a positive control (e.g., 1.0% or 0.1% histamine hydrochloride for SPT and 0.01% for the intracutaneous test). Most SPT-solutions contain 50% glycerol which is nonirritating when used for SPT; however, it is irritating when injected intracutaneously (Bousquet and Michel [1993](#)). Therefore, different test solutions are required for intracutaneous testing not exceeding a glycerol concentration of 2% or preferentially stabilized by addition of 0.03% human serum albumin, instead.

Mean wheal diameter and longest wheal diameter are the two most frequently used parameters for wheal response assessment after SPT (Konstantinou et al. [2010](#)). Both mean and longest diameters correlate significantly with the wheal

surfaces. Furthermore, the longest wheal diameter alone (which is also easier and faster to measure) seems to be a better surrogate marker of the scanned and measured wheal surfaces in comparison with the mean diameter (Konstantinou et al. [2010](#)).

Wheal reactions indicative for clinically relevant allergy are usually over 3 mm in size (Bousquet and Michel [1993](#)); however, with regard to specific allergen sources (e.g., flour), it could be shown that already small wheal reactions ≥ 1.5 mm are indicative for a clinically relevant allergy in bakers (van Kampen et al. [2011](#)). In a multicenter study aiming to assess the quality, sensitivity, and specificity of different commercially available SPT solutions for selected occupational allergens relevant in the occupational field of bakers, farmers, and health care, the cut-off at ≥ 1.5 mm wheal reactions was confirmed (van Kampen et al. [2013a](#)) and is recommended for skin prick testing in the diagnosis of occupational type I allergies (van Kampen et al. [2013b](#)).

Since (rarely) anaphylactic reactions may occur under in vivo test conditions, the equipment for an intervention has to be ready for use and the personnel performing in vivo tests needs to be familiar with the treatment (Codreanu et al. [2006](#)).

3.2 In vitro IgE Testing

Of the long list of relevant occupational allergens, only a very limited number is commercially available for in vitro testing from several biomedical companies (e.g., ThermoFisher Scientific Inc.-Phadia AB, Uppsala, Sweden (ImmunoCAP-System™ (allergen in solid phase); www.phadia.com); Hycor Biomedical Inc., Garden grove, CA, USA (Ultra-Sensitive EIA System™ (allergen in solid phase); www.hycorbiomedical.com); Siemens Healthcare Diagnostics, Eschborn, Germany (Immulite-System™ (allergen in fluid phase); www.medical.siemens.com); Omega Diagnostics GmbH, Reinbek, Germany (Allergozyme® Spezifisches IgE) and Omega Diagnostics, Alva, Scotland (Allersys® Specific IgE (allergen in solid phase); www.omegadiagnostics.de and www.omegadiagnostics.com); Dr. Fooke-Achterrath Laboratorien GmbH (Allergo-O-System® (allergen in solid phase); Allergo-O-Liq® (allergen in fluid phase); www.fooke-labs.de).

All in vitro tests require an internal and external quality assurance (including control probes and interlaboratory round robin tests). The quantification of specific IgE-antibodies is based on interpolation from the WHO-standard calibration curve for total serum-IgE (Kleine-Tebbe and Jakob [2015](#)). Due to different test systems and use of different allergen extracts and reagents, the specific IgE-quantification cannot directly be compared between one test system and another (Wojtalewicz et al. [2017](#)).

Some additional allergens may be provided by these companies on special request for research purposes. If no validated test system is available, for novel allergen sources individual coupling or binding of the allergens to a solid phase may be

possible in specialized centers. For this purpose, streptavidin ImmunoCAP has been shown to be a valuable tool for sensitive and specific measurement of IgE binding to new allergens superior to previous cellulose disk-based methods (Sander et al. [2005](#); Ceska et al. [1972](#)). For research purposes, in immunoblot analysis semiquantitative specific IgE-detection can be obtained on nitrocellulose-blotted SDS-PAGE-separated extracts (Mahler et al. [2006](#)).

The diagnostic sensitivity of in vitro tests varies considerably depending on the time elapsed between allergen exposure and testing. For detecting low-grade sensitivity, the skin test may be more sensitive than the IgE antibody test (Homburgert and Katzmann [1993](#)).

Natural allergen extracts from plants (e.g., natural rubber latex, wood dusts, pollen) as well as from insect venoms used for in vitro IgE testing contain natural allergens, some of which consist exclusively of protein; some others consist of proteins with covalently bound glycan side chains. IgE binding to these cross-reactive carbohydrate determinants and cross-reactivity of occupational and nonoccupational allergen sources may hamper the diagnosis of an occupational allergy.

At least 20% of allergic patients generate specific antiglycan IgE (Altmann [2007](#)). These glycans comprise – despite some structural variation – two main motifs immunogenic for man: the xylose and the core-3-linked fucose, which form the essential part of two independent epitopes. They are the structural basis of what is known as cross-reactive carbohydrate determinants (CCDs) (van Ree et al. [2000](#); Altmann [2007](#)). Sera from patients with specific anti-CCD-IgE may exhibit positive in vitro tests to a number of cross-reactive allergen sources of occupational and nonoccupational origin containing the respective CCDs.

These cross-reactivities may involve occupational and nonoccupational allergen sources, e.g., patients with a clinically relevant insect venom allergy or pollen allergy may due to specific anti-CCD-IgE also present positive in vitro results to natural rubber latex, however, most of the time without a clinical history and without clinical relevance (Mahler et al. [2006](#)). Due to blocking antibodies or low-affinity antibody binding to CCDs in vivo, most of the time these patients present a negative SPT or very weak positive intracutaneous tests results (Mahler et al. [2006](#); Altmann [2007](#)). Recently, it has been demonstrated that even Cellulose used as a solid-phase allergen carrier can contain varying amounts of CCDs sufficient to cause false-positive test results up to 2 kUA/L with nonglycosylated recombinant allergens in patients with high levels of anti-CCD IgE antibodies (Hemmer et al. [2018](#)).

Especially in medical expertise, it is relevant to elucidate the nature and clinical relevance of specific in vitro IgE-binding to an occupational allergen source. Marker allergens to screen in vitro for the presence of specific anti-CCD-IgE, which may be primarily acquired due to a nonoccupational sensitization are the following CCD-rich allergen sources: bromelain, horseradish peroxidase, rape, and the artificial CCD MUXF3.

Since concomitant sensitizations to CCD (most frequently irrelevant) and to peptide epitopes (frequently relevant) present in one allergen source may occur at once, further investigations are necessary in vivo (SPT, provocation tests) and/or in vitro, such as (1) IgE-immunoblotting with and without periodate treatment to elucidate the CCD nature of IgE-binding (2) IgE-immunoblotting with cross-preinhibition with extracts of two possibly cross-reacting allergen sources to identify the primarily sensitizing allergen source (which after preincubation will have bound most specific IgE and therefore will prevent further IgE-binding to the secondarily cross-reacting allergen source). In contrast to the above-mentioned CCD-screening, these latter in vitro tests in general can only be provided by specialized departments and laboratories.

A limited but growing number of standardized recombinant or purified native single allergens derived from various allergen sources are commercially available for molecular allergy diagnostics (in vitro IgE testing either as singleplex or multiplex array), however not for in vivo testing. Some of the available recombinant allergens may be useful for the diagnosis of occupational allergies (Table 3). Based on current protein expression systems used, these recombinant allergens are produced as proteins without carbohydrate side chains.

Table 3

List of individual recombinant (r) or purified native (n) allergens to date *commercially* available for the in vitro diagnosis of occupational allergies (in alphabetical order of allergen source). Some of the allergens are not yet available for IgE singleplex determination, but only in a multiplex assay (microarray)

Occupational exposure	Allergen source	Recombinant (r) or purified native (n) allergen	Protein function
Latex	Natural rubber latex	rHev b 1	Rubber elongation factor
		rHev b 3	Small rubber particle protein
		rHev b 5	Acidic protein
		rHev b 6.01	Hevein
		rHev b 6.02	
		rHev b 8	Profilin
		rHev b 11	Chitinase
CCD	Pineapple	nAna c 2	Bromelin
		MUXF3 CCD	Artificial marker CCD
Enzymes	Aspergillus oryzae	nAsp o 21	Alpha-amylase

Occupational exposure	Allergen source	Recombinant (r) or purified native (n) allergen	Protein function
	Bacillus spp.	–	Alcalase
	Bacillus lichenif.	–	Maxatase
	Bacillus spp	–	Savinase
	Hen's egg (egg white)	nGal d 4	Lysozyme
	Papaya	nCar p 1	Papain
	Swine	nSus s pepsin	Pepsin
Plant foods	Apple	rMal d 1	Pathogenesis-related protein PR-10
		rMal d 3	Nonspecific lipid transfer protein (LTP)
	Brazil nut	rBer e1	2S sulfur-rich seed storage albumin
	Buckwheat	nFag e 1	2S albumin
	Cashew	rAna o 2	Legumin-like protein
		rAna o 3	2S albumin
	Celery	rApi g 1	Pathogenesis-related protein PR-10
	Hazelnut	rCor a 1.0401	Pathogenesis-related protein, PR-10
		rCor a 8	Nonspecific lipid transfer protein (LTP)
		nCor a 9	11S seed storage globulin (legumin-like)
		rCor a 14	2S albumin
	Kiwi	nAct d 1	Cysteine protease (actinidin)
		nAct d 2	Thaumatococcus-like protein
		nAct d 5	Kiwifruit

Occupational exposure	Allergen source	Recombinant (r) or purified native (n) allergen	Protein function
	Peach	rAct d 8	Pathogenesis-related protein PR-10
		rPru p 1	Pathogenesis-related protein PR-10
		rPru p 3	Nonspecific lipid transfer protein (LTP)
		rPru p 4	Profilin
	Peanut	rAra h 1	Cupin (Vicilin-type, 7S globulin)
		rAra h 2	Conglutin (2S albumin)
		rAra h 3	Cupin (Legumin-type, 11S globulin, Glycinin)
		rAra h 6	2S albumin
		rAra h 8	Pathogenesis-related protein, PR-10
		rAra h 9	Nonspecific lipid-transfer protein (LTP)
	Sesame	rSes i 1	2S albumin
	Soy bean	rGly m 4	Pathogenesis-related protein, PR-10
		nGly m 5	Beta-conglycinin (vicilin, 7S globulin)
		nGly m 6	Glycinin (legumin, 11S globulin)
	Walnut	rJug r 1	2S albumin
		nJug r 2	7S globulin
		rJug r 3	Nonspecific lipid-transfer protein (LTP)
	Wheat	rTri a 14	Nonspecific lipid-transfer protein (LTP)

Occupational exposure	Allergen source	Recombinant (r) or purified native (n) allergen	Protein function
		nGliadin	Gliadin
		rTri a 19	Omega-5 gliadin
		nTri a aA_TI	Alpha-amylase/trypsin inhibitor
Foods of animal origin	Carp	rCyp c 1	Parvalbumin
	Cod	rGad c1	Parvalbumin
	Cow's milk	nBos d 4	Alpha-lactalbumin
		nBos d 5	Beta-lactoglobulin
		nBos d 6	Serum albumin (domestic cattle)
		nBos d 8	Caseins
		nBos d Lactoferrin	Transferrin
	Hen's egg (egg white)	nGal d 1	Ovomucoid
		nGal d 2	Ovalbumin
		nGal d 3	Ovotransferrin
	Hen's egg yolk/chicken	nGal d 5	Livetin/Serum albumin (chicken)
	Shrimp	rPen a 1	Tropomyosin
	Black tiger shrimp	nPen m 1	Tropomyosin
		nPen m 2	Arginine kinase
		nPen m 4	Sarkoplasmatic calcium-binding protein
Animals	Cat	rFel d 1	Uteroglobulin (chain 1)
		nFel d 2	Serum albumin
		rFel d 4	Lipocalin
	Dog	rCan f 1	Lipocalin
		rCan f 2	Lipocalin

Occupational exposure	Allergen source	Recombinant (r) or purified native (n) allergen	Protein function
		nCan f 3	Serum albumin
		rCan f 5	Arginine esterase
	Horse	rEqu c 1	Lipocalin
		nEqu c 3	Serum albumin
	Mouse	nMus m 1	Lipocalin
	Pig	nSus s pig serum alb.	Serum albumin
Insect venoms	Honey bee	rApi m 1	Phospholipase A2
		rApi m 2	Hyaluronidase
		rApi m 3	Acid phosphatase
		nApi m 4	Mellitin
		rApi m 5	Dipeptidyl peptidase
		rApi m 10	Icarapin
	Mediterranean paper wasp	rPol d 5	Antigen 5
	Yellow jacket	rVes v 1	Phospholipase A1
		rVes v 5	Antigen 5
Mites	Blomia tropicalis	rBlo t 5	Function unknown
	Dermatophagoides pter.	rDer p 1	Cysteine protease
		rDer p 2	NPC2 family
		rDer p 10	Tropomyosin
		rDer p 23	Peritrophin-like protein
	Dermatophag. farinae	nDer f 1	Cysteine protease
		rDer f 2	NPC2 family
	Lepidoglyphus destructor	rLep de 2	NPC2 family
Molds	Aspergillus fumigat.	rAsp f 1	Mitogillin family

Occupational exposure	Allergen source	Recombinant (r) or purified native (n) allergen	Protein function
		rAsp f 2	Fibrinogen binding protein
		rAsp f 3	Peroxisomal protein
		rAsp f 4	Unknown
		rAsp f 6	Mn superoxide dismutase
Cockroach	German cockroach	rBla g 1	Involved in digestion
		rBla g 2	Aspartic protease
		rBla g 5	Glutathione S-transferase
		nBla g 7	Tropomyosin
Parasitic nematodes	Anisakis simplex	rAni s 1	Serin protease inhibitor
		rAni s 3	Tropomyosin

In contrast to a positive in vitro test result obtained with the natural whole extract of the allergen source (e.g., latex) which may be due to anti-CCD-IgE, a positive in vitro test result of specific IgE for a recombinant allergen (e.g., the recombinant latex allergens rHev b 1–rHev b11) implies that peptide epitopes are involved.

Currently, out of the list of available recombinant allergens, few recombinant allergens (rHev b 1, rHev b 3, rHev b 6.01, rHev b 6.02, rHev b 8, rHev b 11 provided from ThermoFisher Scientific Inc.- Phadia AB) are available as fusion proteins bound to maltose-binding protein (MBP) as a carrier protein. To exclude a rare specific IgE binding to MBP itself, as a negative control for recombinant allergens with the MBP carrier protein, an in vitro analysis for MBP-specific IgE is recommended.

4 Specific Allergen Sources

4.1 Flour

Type I allergies to flour are currently the most frequent causes of occupational airways disease in industrialized countries (Brant [2007](#); German Social Accident Insurance [2009](#); Jeebhay and Quirce [2007](#)). From $n = 652$ confirmed cases with allergic occupational rhinopathy and/or obstructive airways disease in Germany, 64% were allergic to flour or bakery products (compared to 4.6% allergic to

foods/fodder; 3.8% to animal dander; 3.6% to fruits, vegetables, and plants; 3.5% to hair bleach; 2.5% to natural rubber latex; and 1.7% to enzymes) (Raulf-Heimsoth et al. [2009](#)).

Wheat (*Triticum* sp.), rye (*Secale cereale*), and barley (*Hordeum vulgare*) are the most frequent allergen sources eliciting baker's asthma. Further relevant allergens in this occupational field are enzymes used as bakery improvers (e.g., α -amylase, γ -amylase (syn.: glucoamylase), cellulase, hemicellulase, xylanase, β -xylosidase (Asp n 14), and papain); allergens from amaranth, lupine, soy:

One third of 116 symptomatic bakers showed sIgE to lupine (van Kampen et al. [2015](#)). At least some of these sensitizations were based on cross-reactivity between lupine and wheat flour. However, the considerable sensitization rate could also be a sign that the use of lupine flour in bakeries may be of occupational relevance (van Kampen et al. [2015](#)). The allergens involved in occupational asthma caused by soybean in bakers are predominantly high MW proteins (present in both soybean hull and flour), whereas soy bean allergens causing asthma outbreaks (e.g., in harbor workers) are mainly low MW-proteins concentrated in the hull (Gly m 1 and Gly m 2) (Quirce et al. [2000](#)).

Also contaminations with storage mites, mold spores (e.g., from *Aspergillus* and *Alternaria* species), Mediterranean flour moths (*Ephestia kuehniella*), cockroaches, grain weevil (*Sitophilus granarius*), or the red flour beetle (*Tribolium castaneum*) have been described as relevant allergens in bakers (Raulf-Heimsoth et al. [2009](#), [2017](#)).

Allergen test preparations are not commercially available for all of these allergen sources for in vivo use or in vitro tests for specific IgE examination, making it necessary to test with poorly standardized extracts prepared from the patient's workplace materials. For the interpretation of the test results, it is important to have the limitations of those test preparations in mind.

So far, skin prick tests solutions for most flours are commercially available from a number of allergen manufacturers. However, commercially available skin prick test preparations for detection of wheat and rye flour sensitization showed a wide variation in allergen content leading to a rate of false-negative skin prick test results to up to 55% (wheat flour) or 60% (rye flour), respectively, compared to a bronchial provocation test as a gold standard (Sander et al. [2004](#)). The use of several test solutions in parallel as well as a native flour suspension may be necessary to confirm an allergy against a flour component. Additional tests (in vitro IgE and/or provocation tests) may be necessary.

Currently, 28 characterized allergens from wheat (*Triticum aestivum*) have been included in the WHO/IUIS Allergen Nomenclature database ([2018](#)) (<http://www.allergen.org/>; for essential criteria for acceptance of a protein as an allergen in the database, see (Pomés et al. [2018](#))), of which 14 are marked as food allergens: nonspecific lipid transfer protein 1 (Tri a 14), beta-amylase (Tri a 17), agglutinin isolectin 1 (Tri a 18), 1 (Tri a 14), gamma gliadin (Tri a 20), thioredoxin

(Tri a 25), high molecular weight glutenin (Tri a 26), and low-molecular-weight glutenin GluB3–23 (Tri a 36), alpha purothionin (Tri a 37), mitochondrial ubiquitin ligase activator of NF- κ B 1 (Tri a 41), hypothetical protein from cDNA (Tri a 42), hypothetical protein from cDNA (Tri a 43), endosperm transfer cell specific PR60 precursor (Tri a 44), elongation factor 1 (EIF1) (Tri a 45); the route of allergen exposure of the remaining 14 wheat allergens included in the WHO/IUIS Allergen Nomenclature database is to date unknown. Depending on the predominant route of exposure (inhalation in bakers, ingestion in food allergy), different allergens seem to be more frequently relevant. However, wheat α -amylase inhibitor (most relevant for baker's asthma) has been described as a relevant sensitizing allergen following both inhalation or ingestion (James et al. [1997](#); Pastorello et al. [2007](#)) and agglutinin isolectin 1 has also been reported as an allergen relevant in baker's asthma (Sutton et al. [1984](#)).

The IgE binding of sera from bakers with baker's asthma indicates that a diversity of allergens is involved in baker's asthma. Regional differences concerning the frequency of allergen recognition in bakers have been observed.

No major allergen for baker's asthma (recognized from at least 50% of bakers) was detected in a study with wheat flour-positive sera from 40 German bakers with work-related asthma/rhinitis examined for specific IgE to 17 recombinant wheat flour allergens expressed in *Escherichia coli* (Sander et al. [2011](#)). In this study, five subunits of the wheat α -amylase inhibitors (WTAI-CM1 (rTri a 29.0101), WTAI-CM2 (rTri a 29.0201), WTAI-CM3 (rTri a 30), WDAI-0.19 (rTri a 28), and WMAI-0.28 (rTri a 15)), thioredoxin (rTri a 25), thiol reductase (rTri a 27), 1-cys-peroxiredoxin (rTri a 32), triosephosphate-isomerase (rTri a 31), $\alpha\beta$ -gliadin (rTri a 21), serpin (rTri a 33), glyceraldehyde-3-phosphate-dehydrogenase (rTri a 34), a nonspecific lipid transfer protein (nsLTP; rTri a 14.0201), dehydrin (rTri a 35), profilin (rTri a 12), and peroxidase were used as well as ImmunoCAPs with the recombinant allergen ω 5-gliadin (rTri a 19) and two CCDs (horse radish peroxidase (HRP) and the N-glycan of bromelain (MUXF)): Thirty bakers (75%) had IgE to at least one of the 19 single allergens.

Concerning the specific IgE recognition in this group of allergic bakers, the following frequency was found: Fifteen bakers (38%) had IgE to any α -amylase inhibitor and 12 (30%) to at least one CCD. Most frequent was IgE to WDAI-0.19, HRP, and MUXF (25% each), followed by WTAI-CM1 (20%); thiol reductase (16%); WTAI-CM3 (15%); WTAI-CM2 and thioredoxin (12.5%); WMAI-28, triosephosphate-isomerase, $\alpha\beta$ -gliadin (10%); 1-cys-peroxiredoxin (7.5%); dehydrin, serpin, and glyceraldehyde-3-phosphate-dehydrogenase (5%); ω 5-gliadin, nsLTP, and profilin (2.5%). Control individuals reacted exclusively to CCDs (80%), profilin (60%), thioredoxin (30%), triosephosphate isomerase, and nsLTP (10%).

Oliveri et al. observed several sensitization profiles to specific wheat allergens to be associated with different occupational allergic phenotypes (rhinoconjunctivitis, asthma, and dermatitis) in 81 consecutive bakers reporting occupational symptoms using commercial tests (skin prick test (SPT), specific IgE,

ISAC microarray) and six additional dot-blotted wheat allergens (Tri a 39, Tri a Trx, Tri a GST, Tri a 32, Tri a 12, Tri a DH) (Olivieri et al. [2013](#)): A positive SPT to wheat was found in 29 bakers and was associated with work-related asthma ($p < 0.01$). Wheat-specific IgE was detected in 51 workers and was associated with work-related asthma ($p < 0.01$) and rhino-conjunctivitis ($p < 0.05$). ISAC Tri a 30 was positive in three workers and was associated with work-related dermatitis ($p < 0.05$). Wheat dot-blotted allergens were positive in 22 bakers. Tri a 32 and Tri a GST were positive in 13 and three bakers, respectively, and both were associated with work-related dermatitis ($p < 0.05$). This association increased ($p < 0.01$) when Tri a 32, Tri a GST and Tri a 30 were analyzed together ($p < 0.01$). Wheat IgE levels were associated with work-related dermatitis ($p < 0.01$).

Further wheat allergens described to be relevant in baker's asthma are acyl-CoA oxidase (Posch et al. [1995](#)), fructose-bisphosphatase aldolase (Weiss et al. [1997](#)), peroxidase (Sanchez-Monge et al. [1997](#)), wheat gliadins (Bittner et al. [2008](#)), and wheat serine proteinase inhibitor (Constantin et al. [2008](#)).

In a more recent study, 101 bakers with wheat flour allergy (40 German, 37 Dutch, and 24 Spanish) and 29 pollen-sensitized control subjects with wheat-specific IgE but without occupational exposure were examined with regard to their component-resolved individual sensitization profile to 21 single allergens (i.e., 19 recombinant wheat flour proteins and two CCD-determinants) (Sander et al. [2015](#)): Eighty percent of bakers had specific IgE levels ≥ 0.35 kUA/L and 91% had specific IgE levels of ≥ 0.1 kUA/L to at least one of the 21 allergens. The highest frequencies of IgE binding were found for thiol reductase (Tri a 27) and the wheat dimeric α -amylase inhibitor 0.19 (Tri a 28). The sensitization patterns of bakers from the different countries were similar. Key results of this study with regard to diagnosing occupational allergy in bakers were:

- To diagnose *occupational* wheat flour allergy, a combination of IgE tests to 5 components, Tri a 27, Tri a 28, tetrameric α -amylase inhibitor CM2 (Tri a 29.02), serine protease inhibitor-like allergen (Tri a 39), and 1-cys-peroxiredoxin (Tri a 32) produced the best results, and, with a cut-off value of ≥ 0.12 kUA/L, the best performance measures (97% specificity and 70% sensitivity).
- Nonoccupationally acquired cross-reactivity between grass pollen allergens and wheat allergens was proven for 9 components (the 2 CCDs and seven 7 recombinant wheat allergens: profilin, nsLTP 9.1, nsLTP 9.2, ω 5-gliadin, thioredoxin, triosephosphate isomerase, and GAPDH).
- Cross-reactivity between wheat flour allergens and rye flour was proven for all components with the exception of $\alpha\beta$ -gliadin and nsLTP 9.1 = Tri a 14.1). All other tested wheat flour allergens seem to have cross-reacting homologues in rye flour.

In summary, component-resolved diagnostics help to distinguish between sensitization caused by occupational flour exposure and wheat seropositivity based on cross-reactivity to grass pollen. For routine diagnosis of baker's allergy, however, allergen-specific IgE tests with whole wheat and rye flour extracts still remain mandatory because of superior diagnostic sensitivity (Sander et al. [2015](#)).

A complete panel of recombinant wheat allergens may improve the diagnostic accuracy of wheat allergy in bakers. However, for in vitro IgE investigation so far only a very limited number of single allergenic components from wheat flour are commercially available (ThermoFisher Scientific – Phadia AB, Uppsala, Sweden): α -Amylase-/Trypsin-Inhibitor (nTri aA_TI), natural gliadins, and ω 5-gliadin (rTri a 19).

Exposure to wheat flour is usually considered the most important cause of baker's asthma. However, other flours frequently used in bakeries may play a role as relevant allergen source causing occupational asthma, too: e.g., rye flour allergens (enzymatic inhibitors) and allergens from barley (Letrán et al. [2008](#)). Because of the close relation of Triticeae, homologous groups of proteins exist in wheat, rye, and barley (Sander et al. [2015](#)).

Currently, 4 characterized allergens from rye (thereof one food allergen (gamma-secalin (Sec c 20)) have been included in the WHO/IUIS Allergen Nomenclature database (<http://www.allergen.org/>); the route of allergen exposure of the remaining allergens is to date unknown. An α -amylase/trypsin inhibitor (Sec c 1 from rye (*Secale cereale*)) was reported to provoke positive skin-specific IgE responses in 15/21 (71%) patients with baker's asthma (García-Casado et al. [1995](#)).

To date, from barley 7 allergens (thereof five food allergens: profilin (Hor v 12), alpha-amylase inhibitor BMAI-1 precursor (Hor v 15), alpha-amylase (Hor v 16), beta-amylase (Hor v 17), gamma-hordein 3 (Hor v 20)) have been included in the WHO/IUIS Allergen Nomenclature database (<http://www.allergen.org/>); the route of allergen exposure of the remaining allergens is still unknown. None of these is currently commercially available.

Further characterization of commercially available marker allergens within component-resolved diagnosis associated with specific disease manifestations for these frequent allergen sources in bakers is preferable.

Cross-reactivities may be relevant in highly pollen sensitized individuals, since subjects with pronounced sensitizations to grass or tree pollen, but without prior occupational exposure to flours, may experience both sensitization and allergic asthmatic reactions to flours due to cross-reactive grass and tree pollen allergens (Merget et al. [2011](#)). None of the single rye allergens is commercially available for in vitro tests, yet.

High concentrations of wheat and rye flour-specific IgE and clear skin prick results in symptomatic bakers are good predictors for a positive inhalation challenge test which currently constitutes the gold standard in the diagnosis of baker's asthma (van Kampen et al. [2008](#)). Whereas challenge tests may be decisive in individuals with a history of occupational symptoms and negative or weakly positive test results, challenge tests with flours may be avoided in strongly sensitized bakers (van Kampen et al. [2008](#)).

4.2 Foods

In the food handling and processing industries, occupational rhinitis and asthma have been frequently observed in the commercial fish and seafood industries (Jeebhay and Cartier [2010](#); Moscato et al. [2009](#)). Occupational asthma is more commonly associated with shellfish (4–36%) than with bony fish (2–8%) (Jeebhay and Cartier [2010](#)). Molecular studies of their allergens suggest that aside from parvalbumin and tropomyosin, other as yet uncharacterized allergens are important (Jeebhay and Cartier [2010](#)). Also allergens from parasites (e.g., sea squirt and *Anisakis* spp) have to be considered. In fish breeders and aquarium hobbyists, also insects used as provender for fish may cause occupation-related allergic respiratory diseases: e.g., Hemoglobin of chironomid larvae (Chironomidae) could be identified as a potent inhalable allergen (Raulf-Heimsoth et al. [2017](#)).

Sensitization to crustaceans in the seafood-processing industry usually occurs via inhalation (Lopata and Jeebhay [2013](#)). Tropomyosin and arginine kinases from Crustaceans are incriminated as most important elicitors of airborne disease. Recently, exposure to airborne proteins, tropomyosin, endotoxins, trypsin, and N-acetyl- β -d-glucosaminidase in their breathing zone could be demonstrated in Norwegian crab processing workers at varied levels between worker groups and factories (Thomassen et al. [2016](#)). Several food allergens have been characterized on a molecular level for many different Crustaceans and have been included in the WHO/IUIS Allergen Nomenclature database (<http://www.allergen.org/>): Arginine kinase (e.g., from North Sea shrimp: Cra c 2), Sarcoplasmic calcium-binding protein (e.g., Cra c 4), Myosin, light chain 1 (e.g., Cra c 5), Troponin C (e.g., Cra c 6), Triosephosphate isomerase (e.g., Cra c 8).

Of these, for in vitro IgE-diagnostics, representative marker allergens for tropomyosin (rPen a 1 and nPen m 1), Arginine kinase (nPen m 2), and Sarkoplasmatic calcium-binding protein (nPen m 4) are commercially available.

Muscle protein parvalbumin is the major allergen from bony fish being most commonly implicated in ingestion-related sensitization; however, this allergen also seems to be involved in aerosolized sensitization to fish: A highly cross-reactive allergenic isoform of pilchard parvalbumin was demonstrated to be one of the airborne allergens responsible for allergic respiratory disease in fish-processing workers, however, in contrast to (ingested) food allergic patients, who recognize mainly the monomeric form of parvalbumin (12 kDa), occupationally sensitized workers appear to recognize higher molecular weight isoforms, including dimers and oligomers of parvalbumin. Further fish proteins seem to be involved in airborne allergy, yet, have still to be fully characterized on a molecular level (Lopata and Jeebhay [2013](#)).

To this end, beta-parvalbumins have been characterized as cross reactive food allergens on a molecular level from many fish species (Kuehn et al. [2014](#)), which have been included in the WHO/IUIS Allergen Nomenclature database (<http://www.allergen.org/>): Clu h 1 (Atlantic herring), Cyp c 1 (Common carp), Gad c 1 (Baltic cod), Gad m 1 (Atlantic Cod), Lat c 1 (Barramundi), Lep w 1 (Turbot fish), Onc m1 (Rainbow trout), Ras k 1 (Indian mackerel), Sal s 1 (Atlantic

salmon), Sar sa 1 (Pacific pilchard), Sco 1 (Atlantic mackerel), Seb m 1 (Snapper), Thu a 1 (Yellowfin tuna), Xip g 1 (Swordfish).

For in vitro determination of specific IgE against the cross-reactive major fish allergen parvalbumin, rGad c 1 and rCyp c 1 are commercially available.

As further cross-reactive fish allergens, present in various fish species, Beta-enolase (Gad c 2 from cod, Sal s 2 from salmon, Thu a 2 from tuna) and Aldolase A (Gad c 3 from cod, Sal s 3 from salmon, Thu a 3 from tuna) have been characterized as food allergens (Kuehn et al. [2014](#)) and included in the WHO/IUIS Allergen Nomenclature database, which, however, are not commercially available, yet, for in vitro diagnostics. Species-specific (noncrossreactive) fish allergens have been reported, yet, await further characterization.

Also proteins of hen's egg have been reported as inducers of occupational asthma (Dykewicz [2009](#)).

Food handlers, workers in the food processing industries, and chefs belong to the occupations at risk for occupational contact urticaria (OCU) and protein contact dermatitis (PCD) (Mahler [2010](#)). In OCU, two different mechanisms have to be distinguished: immunological (IgE mediated, e.g., due to foods) or nonimmunological (NICU) due to a variety of low-molecular-weight substances such as preservatives, fragrances, and foodstuffs containing a high content of endogenous histamine (Mahler [2010](#)). An extensive list of foods has been described as occupational allergens inducing OCU at corresponding workplaces (Amaro and Goossens [2008](#)). Reports and case series of OCU in the food industry have been recently reviewed and summarized in a systematic review: OCU has been reported in many different occupations, mostly in individuals dealing with seafood, meat, vegetables, and fruits, such as chefs, cooks, bakers, butchers, slaughterhouse workers, and fish-factory workers; foodstuffs that commonly induce occupational PCD include fish, seafood, meats, vegetables, and fruits (Lukács et al. [2016](#)).

The ever-expanding list of occupational protein sources inducing OCU and PCD (of which many are attributable to the food industries) has been divided into four groups: group 1 – plant-derived proteins (fruits, vegetables, spices, plants, woods); group 2 – animal-derived proteins (e.g., epithelia, meat and body organs, various body fluids, dairy products, seafood, etc.); group 3 – grains (e.g., rye, wheat, barley, oat, cornstarch); and group 4 – enzymes (e.g., α -amylase, glucoamylase, cellulase, xylanase, and protease) (see below) (Amaro and Goossens [2008](#); Mahler [2010](#)).

A number of food extracts are commercially available for in vivo and in vitro IgE detection; however, the allergen content and standardization is frequently not satisfactory. Therefore, skin prick-to-prick testing with fresh material may be required. Additionally, several well-characterized single (recombinant or purified native) allergens from foods are available from biomedical companies (most from

ThermoFisher Scientific Inc.-Phadia AB, Uppsala, Sweden) for in vitro IgE examination (Table [3](#)).

4.3 Enzymes

The industrial use of fungal and bacterial enzymes mainly of hydrolytic nature (e.g., proteases, glycosidases, esterhydrolases involved in the cleavage of polymers) is widespread (Baur et al. [2000](#)). Sensitization occurs by inhalation of a number of enzymes, and sensitizing potential has been also demonstrated for enzymes not yet labeled as sensitizers (Baur et al. [2000](#)). An increasing use of genetically engineered enzymes in the synthesis of flavorings, fragrances, and other applications has been reported (Budnik et al. ([2017](#))). These data confirm the previous findings showing that genetically engineered enzymes are potent allergens eliciting immediate-type sensitization (Budnik et al. ([2017](#))). Owing to lack of commercial diagnostic tests, few of those exposed receive regular surveillance including biomonitoring with relevant specific IgE assays (Budnik et al. ([2017](#))).

In the production as well as in the processing of flour, enzyme exposure (e.g., α -amylase, glucoamylase, cellulase, hemicellulase, xylanase, β -xylosidase, and papain) represents a potent allergen source for Type I sensitization (Vanhanen et al. [1996](#); Sander et al. [1998](#)).

Further occupational fields with exposure to sensitizing enzymes are as follows:

1. 1. Food processing industries (e.g., fungal enzymes: α -amylase, glucoamylase, xylanase, cellulase, β -glucanase, pectinase, lactase, lipase, glucoseoxidase; bacterial enzymes: α -amylase, maltogenic amylase, proteases; lysozym from egg white, pepsin A from swine; papain from papaya, bromelin from pineapple (Baur et al. [2000](#); Bindslev-Jensen et al. [2006](#); Sen et al. [1998](#); Casper et al. [2008](#)))
2. 2. Animal feed industries (e.g., α -amylases, glucoamylases, cellulases, hemicellulases, xylanases, β -glucanases, proteases, lipases, phytases (Baur et al. [2000](#); Vanhanen et al. [2001](#)))
3. 3. Production of cleaning agents and detergents (e.g., serine proteases from *Bacillus subtilis*, i.e., subtilisins (maxatase, alcalase, esperase, savinase), α -amylases, cellulases, lipases, (Vanhanen et al. [2000](#); Baur et al. [2000](#)))
4. 4. Pharmaceutical industries, enzyme producing industries, and research laboratories (e.g., fungal and bacterial α -amylases, proteases, fungal cellulase, xylanase, lactase, lipase, phytase, glucose oxidase; hyaluronidase, lysozyme, pepsin A, papain (Baur et al. [2000](#); Vanhanen et al. [1997](#)))
5. 5. Paper industries (e.g., amylases, cellulases, hemicellulases, xylanases, pectinases (Baur et al. [2000](#); Torres et al. [2011](#)))
6. 6.

Textile industries (e.g., amylases, cellulases, xylanases, proteases, lipases, catalases (Baur et al. [2000](#); Schumacher et al. [2001](#)))

Licensed skin prick test solutions for enzymes are currently not available in most EU member states and test systems for in vitro detection of enzyme-specific IgE are scarce (Table [3](#)). False-positive skin prick test results may occur in individuals with negative bronchial provocation (Moneo et al. [1995](#)). Two previously investigated SPT solutions for α -amylase reached a sensitivity of 100% and a specificity of 80% (van Kampen et al. [2011](#)).

Specific IgE testing in vitro is commercially available for the following enzymes (e.g., from e.g., ThermoFisher Scientific Inc.-Phadia AB, Uppsala, Sweden): alcalase and savinase (*Bacillus* spp), maxatase (*Bacillus licheniformis*), bromelain (nAna c 2 from pineapple), α -amylase (nAsp o 1 (also referred to as Asp o 2) from *Aspergillus oryzae*), papain (nCar p 1 from papaya), lysozyme (nGal d 4 from egg white), and pepsin (nSus s from swine). Some specialized centers for occupational medicine may provide well-characterized in-house preparations of further enzymes (Moneo et al. [1995](#); Sander et al. [1998](#)). Skin prick test with enzyme extracts self-prepared from enzymes (purchased from life science companies) at protein concentrations from 50 to 100 μ g/ml (Vanhanen et al. [1996](#), [2000](#), [2001](#)) to 100 mg/ml (Merget et al. [1993](#)) has been performed. In the latter study, a protein concentration of 10 mg/ml showed best sensitivity and specificity. When using self-prepared test materials in vivo, the national legal requirements and restrictions imposed by national pharmaceuticals acts have to be considered.

4.4 Natural Rubber Latex

The incidence of occupational Type I allergy to latex varies in different countries according to present exposure levels in disposable natural rubber latex gloves (Allmers et al. [2002](#), [2004](#); Vandenplas et al. [2009](#); Williams et al. [2008](#); Reunala et al. [2004](#); Bensefa-Colas et al. [2015](#)). Currently, in most EU member states, licensed skin prick test solutions for natural rubber latex are scarce or not available at all. Due to the inhomogeneity of the composition of the natural rubber latex sap from *Hevea brasiliensis* even depending on the hour of its harvest, the test preparations based on natural extracts used for in vivo as well as in vitro testing may not provide all relevant latex allergens.

Therefore, for the in vivo testing, the use of several test preparations of natural rubber latex in parallel has been suggested (Ruëff and Przybilla [1999](#)). Up to date, fifteen allergens (Hev b 1–Hev b 15) have been characterized and included in the WHO/IUIS Allergen Nomenclature database (<http://www.allergen.org/>). Of these, Hev b 2, 5, 6.01, and 13 were identified as the major Hev b allergens in the health care setting, whereas Hev b 1 and 3 are of minor relevance since < 20% of the health care workers have positive IgE antibody responses to these allergens (Raulf-Heimsoth et al. [2007](#)). They should be present in standardized latex extracts and in extract-based in vitro allergosorbents. Spiking the natural latex extract with the recombinant major allergen Hev b 5 increased the sensitivity of

one of the in vitro tests (Raulf-Heimsoth et al. [2007](#)). Currently, only 7 of the known latex allergens are available as recombinant single components for in vitro IgE-diagnostics (rHev b 1, rHev b 3, rHev b 5, rHev b 6.01, rHev b 6.02, rHev b 8, and rHev b 11).

The most recently characterized latex-allergen (Hev b 15), a Serine protease inhibitor, has been shown to be recognized by specific IgE of one third of tested health care workers with allergic symptoms to natural rubber latex (Rihs et al. [2015](#)). Monosensitizations to Hev b 15 were not found: all Hev b 15-positive sera revealed concomitant specific IgE to rHev b 6.01 and – with 1 exception – also to rHev b 5 (Rihs et al. [2015](#)).

Taken together, a negative in vitro *or* in vivo test does not completely rule out an existing occupational Type I allergy to latex since a relevant allergenic component may be missing in the extract. And vice versa, a positive in vitro test to a natural rubber latex extract does not confirm unequivocally occupational latex allergy since the in vitro IgE binding may be either caused by CCD-specific IgE binding which is mostly without clinical significance or the IgE binding may be due to cross-reaction of homologous allergens contained in latex (Hev b 8, a profilin) and a different primary sensitizing allergen source (e.g., tree pollen-derived allergen Bet v 2, another profilin).

Recently, most likely due to modified exposure levels, a shift in presentation of clinically manifest latex sensitization has been observed: Specific IgE levels to latex used to be significantly higher in former patients (diagnosed 20 years ago) with suspected latex allergy ($p < 0.001$) than in formerly sensitized individuals without clinically manifest latex allergy. However, this significant difference was lost in current allergic versus asymptotically sensitized patients with positive skin tests (Saleh et al. [2019](#)).

In case of doubt, a provocation by a glove use test has been suggested (Ruëff and Przybilla [1999](#)). However, it has to be kept in mind that – depending on national regulations and allowed remaining low protein content in disposable gloves – a use test with current latex gloves may not provide positive test results despite an earlier acquired occupational latex allergy. In the absence of high allergen gloves, balloons have been used instead for the provocation.

(1) A history of allergic symptoms at the workplace when in contact with latex and (2) a positive prick test to one of the latex test preparations and/or positive in vitro test (e.g., to recombinant latex allergens Hev b 2, 5, 6.01, and 13) support the diagnosis of an occupational latex allergy (Reunala et al. [2004](#); Raulf-Heimsoth et al. [2007](#)).

4.5 Wood Dusts

Proteins contained in fresh wood dusts constitute potent inhalant sensitizers in the wood production and wood processing industries. A meta-analysis on the risk of respiratory diseases caused by exposure to wood dust demonstrated that

workers exposed to wood dust had a significantly increased risk to develop respiratory diseases (Pérez-Ríos et al. [2010](#)).

Occupational asthma and rhinitis both of allergic and nonallergic origin due to exposure to wood dusts have been described, as well as IgE-mediated contact urticaria and irritant and allergic contact dermatitis (Jacobsen et al. [2010a, b](#); Kanerva et al. [1998](#)).

The panel of woods responsible for Type I allergic reactions is manifold and includes tropical trees like obeche (*Triplochiton scleroxylon*), antiaris (*Antiaris toxicaria*), tali (*Erythrophleum guineense*), jatoba (*Hymenaea* sp), and Pau marfin (*Balfourodendron riedelianum*), as well as trees growing in the temperate zones like ash (*Fraxinus excelsior*), oak (*Quercus robur*), beech (*Fagus sylvatica*), Robinia (*Robinia pseudoacacia*), pine (*Pinus sylvestris*), spindle tree (*Euonymus europaeus*), and western red cedar (*Thuja plicata*) (Kespohl et al. [2005, 2006](#)). In contrast to many other types of wood, which mainly induce allergic skin reactions, the tropical abachi wood (= obeche (*Triplochiton scleroxylon*)) has a very high protein content that makes it an effective airway sensitizer for Type I reactions (Raulf-Heimsoth et al. [2017](#)). Two major allergens from obeche wood have been identified, of which Trip s 1, a class 1 chitinase, has been shown to be a strongly cross-reactive allergen sharing some, but not all IgE-binding epitopes with rHev b 6.02 from natural rubber latex (Kespohl et al. [2005](#)). Another allergologically relevant wood is black locust (*Robinia pseudoacacia*): This wood is used, for example, as a substitute for teak and thus is frequently used in outdoor areas. Occupational exposure to black locust wood leads to clinically relevant IgE-mediated sensitizations; the IgE-binding proteins were identified to be 27 kD- and 47 kD-sized molecules (Raulf-Heimsoth et al. [2017](#)). Although 96% of their examined wood-sensitized workers were atopic, Kespohl et al. found no significant correlation between wood sensitization and sIgE to beech and birch pollen (Kespohl et al. [2010](#)).

No “universal” wood allergen exists that could be used as a screening tool for wood dust sensitization. Individual wood allergen extracts have to be prepared and tested for each wood species (Kespohl et al. [2010](#)). Allergen contents decrease during wood storage due to degradation.

Besides proteinogenic epitopes, wood dusts may also contain CCD-epitopes leading to false-positive in vitro test results. In beech and pine wood-sensitized workers, the prevalence of CCD sensitization was high (73%) (Kespohl et al. [2010](#)). Specifying IgE epitopes demonstrated that sera of workers reporting allergic symptoms recognized proteinogenic IgE epitopes of wood allergens, whereas workers without allergic symptoms had primarily specific IgE raised against glycogenic structures. In western red cedar, an association between specific IgE to plicatic acid (a nonproteinogenic, low-molecular-weight (LMW) substance of 440 Da) and occupational asthma was shown (Vedal et al. [1986](#); Grammer [2001](#)).

Licensed skin prick test solutions or intradermal test solutions for wood dust are not available in most EU member states, and also allergens for in vitro diagnostics

of wood dust-specific IgE are scarce (commercially available for in vitro IgE-detection: obecke (ThermoFisher Scientific Inc.-Phadia AB, Uppsala, Sweden). In parallel to in vitro testing for specific IgE antibodies to wood dust allergens, a screening for CCD-specific IgE antibodies with CCD-marker allergens (e.g., bromelain, horseradish peroxidase, MUXF3) should be done.

In conclusion, wood-dust specific IgE screening in vitro and/or in vivo with commercial extracts within a feasible diagnostic algorithm prior to individual provocation tests is currently very limited. Few specialized centers only may be able to provide well-characterized in-house preparations.

4.6 Further Plant-Derived Allergens

Dusts from further plant material have been described to induce occupational rhinitis and asthma in the respective producing industries (e.g., tobacco, tea, coffee, cocoa, dried fruit, hot pepper, saffron, cotton seeds, sunflower seed, and ficus species) (Moscato et al. [2009](#); Tarlo et al. [2008](#)).

Gardeners, fruit growers, and workers in greenhouses are extensively exposed to pollen from flowers, fruits, and vegetables, which do not affect the general population due to the nature of their pollination, which in contrast to grasses, trees, and weeds is not wind- but insect-pollination (Raulf-Heimsoth et al. [2017](#)): Allergic diseases like rhinitis, conjunctivitis, and bronchial asthma were described for people working with tulips (petals, stems, pollen, bulbs). Also freesia, gerbera, and tuberous begonia led to allergic symptoms in exposed gardeners (pollen extraction, pollination of flowers) and specific IgE against chrysanthemum pollen was detected in 20% of the workers in Dutch greenhouses of a chrysanthemum growing company. Pollen of Brassica oleracea (cauliflower and broccoli) and strawberry pollen have been described as new important occupational allergens in exposed personnel (Raulf-Heimsoth et al. [2017](#)).

Psyllium (syn. Ispaghula) from *Plantago psyllium* is used as dietary fiber (laxative and dietary supplement) as well as thickener in foods. Occupational rhinitis and asthma have been described in the production of Psyllium as well as in nursing (Moscato et al. [2009](#)).

Protein allergens from vegetable gums (arabic, guar, tragacanth) may induce allergic symptoms in food handlers, printing/bookbinders, and carpet manufacturing, and henna dye in hairdressers (Moscato et al. [2009](#); Tarlo et al. [2008](#)).

Whereas to date only very few skin prick test preparations for these occupational allergen sources are commercially available (e.g., for chrysanthemum pollen, marguerite pollen, aster pollen), for several of them (e.g., green coffee bean, castor bean, cotton seeds, sunflower seed, ficus species and Psyllium (syn. Ispaghula)) in vitro tests for detection of IgE are provided from one or the other biomedical company (e.g., ThermoFisher Scientific Inc.-Phadia AB, Uppsala, Sweden).

4.7 Laboratory and Farm Animals

The most studied and well-described rodent allergens from laboratory animals are mice (*Mus musculus*) and rat (*Rattus norvegicus*) allergens (Phipatanakul [2002](#)). Three mouse allergens have been identified, of which Mus m 1 has been included in the WHO/IUIS Allergen Nomenclature database (<http://www.allergen.org/>) and is commercially available for specific IgE-diagnostics: Mus m 1 (syn.: mouse urinary protein, MUP) is found in the rodents' urine as well as in hair follicles and dander. Its gene expression is testosterone dependent and therefore the allergen content in male mice is four times higher than in females (reviewed in Phipatanakul [2002](#)). A second allergen, Mus m 2, is a glycoprotein found in hair and dander, but not in urine; the third allergen is albumin, which is allergenic in about 30% of mice-sensitive individuals (Phipatanakul [2002](#)).

Previously, two rat allergens had been identified in urine, saliva, hair, and dander: rat n 1A and rat n 1B. Moreover, rat fur and saliva have been further studied: rat fur contains five major allergens of >22 kDa, while salivary glands demonstrate at least five other major allergens with a molecular weight of <22 kDa. Further minor or intermediate allergens have been identified, although not well characterized (Phipatanakul [2002](#)). So far, only Rat n 1 has been included in the WHO/IUIS Allergen Nomenclature database (<http://www.allergen.org/>), however, is not yet commercially available for specific IgE-diagnostics.

Different job descriptions are associated with different exposures to animal allergens: The highest exposure typically occurs in handlers who are responsible for cage cleaning and feeding of the animals. Persons involved in daily experimental use of the animals (technicians, students, investigators) have intermittent contact with the animals and therefore lower levels of exposure. Symptomatic inflammatory responses in sensitized workers correlate with airborne allergen concentrations and active maneuvering of the cages rather than with quiet activity (Phipatanakul [2002](#)).

Licensed skin prick test solutions are available in some EU member states for mouse, rat, rabbit, hamster, and guinea pig from several allergen manufactures; however, little is known about their standardization, sensitivity, and specificity. In vitro IgE-diagnostics are not only available for the same allergen sources listed under the SPT solutions but are available for few more allergen sources (e.g., gerbil, ferret, chinchilla, and mink). Further tests such as pulmonary function tests in correlation with exposure, bronchial, nasal, or conjunctival provocation challenges may be needed to establish the diagnosis of clinically manifest allergy (Phipatanakul [2002](#)). However, licensed provocation test solutions are currently not available in most EU member states.

Besides animal dander, personal exposures in farmers are multiple and include fungal spores, actinomycete spores, endotoxins, bacteria, storage mites, (1 → 3)-β-d-glucans, fungal antigens, organic dust, inorganic dust, silica, and ammonia (Eduard et al. [2009](#)). Airways diseases of either immunologic or nonimmunologic origin may result (Eduard et al. [2009](#); Dykewicz [2009](#)). Exposure to the highly

toxic gas hydrogen sulfide can cause sudden death if presented under anaerobic circumstances, but the gas represents hardly any risk as an irritant to the lungs in concentrations that might be measured in animal houses in modern farming under aerobic circumstances (Omland [2002](#)). Concerning Type I allergens from the farm environment, data from France, Sweden, Denmark, the UK, and Finland have revealed a high prevalence of sensitization to mites (see below). Challenges with any one of the storage mite species were positive in 18% and with cow dander in 20% of farmers with allergic rhinitis (Terho et al. [1985](#)). Except for findings from Finland (Terho et al. [1987](#)), sensitization to animal dander, molds and pollen are less prevalent (Omland [2002](#)). In $n = 664$ French farmers with no sign of bronchial hyperresponsiveness, the prevalence of sensitization was 15.4% to ≥ 1 (out of 6) mites, 11.6% to ≥ 1 cereal dusts (6 in all), 5.3% to ≥ 1 (out of 5) pollen, 5.3% to ≥ 1 (out of 6) animal danders, and 2.6% to 1 (out of 3) molds. In $n = 77$ subjects with bronchial hyperresponsiveness, the prevalence was 24.7% to storage mites, 21.3% to cereal dust, 9.1% to pollen, 7.8% to animal dander, and 5.2% to molds (Omland [2002](#)).

In dairy farmers, the prevalence of IgE-dependent sensitization to cow dander was found to be 14% in Germany (Hinze et al. [1997](#)). In a recent study on bovine allergen exposure levels in a ruminant clinic and dairy barns, personal bovine allergen exposure levels in the ruminant clinic ranged from 0.10 to 24.8 $\mu\text{g}/\text{m}^3$ (geometric mean (GM) 1.34 $\mu\text{g}/\text{m}^3$) and exposure levels varied dependent on job titles (Samadi et al. [2018](#)). Personal exposure levels in dairy barns are ranged from 0.10 to 46.8 $\mu\text{g}/\text{m}^3$ (GM 1.47 $\mu\text{g}/\text{m}^3$). Type of bedding materials in the barns appeared to be a significant determinant of bovine allergen levels: Compost bedding, particularly, increased allergen levels (Samadi et al. [2018](#)).

A correlation of cow hair allergen Bos d 2 content in house dust in the farmers' living quarter with sensitization in farmers with cow hair asthma has been found (Hinze et al. [1997](#)). Consequently, avoiding the barn is not a sufficient avoidance measure for cow hair asthmatics if the partner continues cattle farming (Hinze et al. [1997](#)).

In bird breeders, feathers and serum allergens may induce occupational asthma. Compared to other farming areas, exposure to organic dusts was found highest in poultry housings as a predisposing factor for extrinsic allergic alveolitis (Omland [2002](#)).

For in vitro testing, a long list of allergen extracts and a number of single allergens (Table [3](#)) from vertebrate dander, epithelia, feathers, serum and urinary proteins, and droppings are commercially available from several biomedical companies (e.g., from ThermoFisher Scientific Inc.-Phadia AB; Hycor Biomedical Inc., Garden grove, CA, USA; Siemens Healthcare Diagnostics, Eschborn, Germany). A number of commercial allergen extracts of varying quality specific for occupational exposures from the farming environment for skin prick testing are currently available in some EU member states from several manufacturers (e.g., epithelia and/or hair of cattle, goat, horse, rabbit, swine; feathers of chicken,

ducks, geese). For intracutaneous testing (horse) and nasal provocation (cow, horse, rabbit), only very few licensed test allergens have remained on the market. As reasons for withdrawal of existing marketing authorizations for occupational test allergens, manufacturers frequently have named rather costly production conditions meeting legal requirements in combination with a rather low demand and revenue.

4.8 Mites

Organic dusts in the farming environment contain storage mites (e.g., *Acarus siro*, *Acarus farris*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Blomia tjobodas*, *Blomia tropicalis*, *Blomia kulagini*, *Glycyphagus domesticus*, *Thyreophagus entomophagus*, *Cheyletus eruditus*) (Müsken et al. [2003](#)). They are found in stored hay and may cause respiratory allergy among farm workers. Since these mite species can infest grain, also grain elevator workers and bakers are occupationally exposed to storage mites and can become sensitized (Olsson and van Hage-Hamsten [2000](#)). It has been shown that sensitization to storage mites also occurs among urban populations, in both subtropical and tropical countries, as well as in temperate climates, indicating that this is a worldwide problem (Olsson and van Hage-Hamsten [2000](#); Wörner et al. [2014](#)).

In Europe, studies have revealed that sensitization to *Lepidoglyphus destructor* seems to be the most prevalent sensitizer of the storage mites. *Lepidoglyphus destructor* was the most dominant species in Swedish barns, whereas *Blomia tropicalis* is most prevalent in South America (van Hage-Hamsten et al. [1991](#); Olsson and van Hage-Hamsten [2000](#)). In the farming population, the total prevalence of a positive RAST to storage mites was 8.1% and to house dust mites only 7.3% (reviewed in Olsson and van Hage-Hamsten [2000](#)). House dust mites and storage mites contain many different allergens of which only few share cross-reactive allergens. Therefore, and as much as possible, the complete panel of mites should be tested in vitro and/or in vivo. Besides licensed test extracts for SPT, many mite extracts as well as an increasing number of single allergens are commercially available for in vitro IgE-diagnostics (Table [3](#)).

Among fruit farm workers, as well as in greenhouse workers, outdoor phytophagous spider mites (e.g., *Panonychus citri*, *Panonychus ulmi*, and *Tetranychus urticae*) appear to play a major role in the allergic manifestations observed among this group (Jeebhay et al. [2007](#); Raulf-Heimsoth et al. [2017](#)). So far, no commercial test extracts have been provided.

4.9 Molds

Molds and their spores are worldwide ubiquitously present. Due to their small size (2–20 µm), they belong to the respirable dusts since they are small enough to penetrate the nose and upper respiratory system and deep into the lungs. Indoors, molds grow on a wide variety of materials and within a wide temperature range depending on humidity which is most often linked to water damage and ventilation defects of the building (Karvala et al. [2008](#); Peden and Reed [2010](#)).

Molds growing in conjunction with moisture damages can induce occupational rhinitis. Especially during cleansing and reconstruction, high concentrations of mold spores can be found. Molds can induce infections, allergies, and irritant/toxic reactions. IgE-mediated allergy to molds was not common (Karvala et al. [2008](#)). Extrinsic allergic alveolitis and allergic bronchopulmonary aspergillosis may also occur.

Besides deconstruction and cleansing, further occupational fields with high levels of mold spores are waste disposal (sorting, composting), wastewater treatment, air-conditioning (maintenance), wood and paper processing, textile industries, archiving, farming, crop growing, and food handling and processing (Raulf-Heimsoth et al. [2009](#)).

Recently, it has been demonstrated that fungal and bacterial species found in high concentrations in personal samplers were also found in truck cabins, but in lower concentrations indicating that both fungi and bacteria are transported by the workers into the truck cabin and are subsequently aerosolized in the truck cabin (Madsen et al. [2016](#)).

In food processing, *Penicillium casei*, *Penicillium camemberti*, and *Penicillium roqueforti* are used for refining of cheeses and sausage and may cause occupational allergies (Raulf-Heimsoth et al. [2017](#)).

Not all mold allergens have been identified, yet. The quality of commercially available mold extracts for in vivo and in vitro testing frequently is not satisfactory (Mari et al. [2003](#)). Therefore, the diagnosis of mold allergy remains delicate.

4.10 Low Molecular Allergens

About 140 low-molecular-weight (LMW) substances have been described as inducers of asthma (Grammer [2001](#)), preceding rhinitis to the same LMW substance may rarely occur. Two different mechanisms can be involved:

1. 1.
A nonimmunological mechanism.
2. 2.
An immunological IgE-mediated mechanism in which the LMW substance is a hapten and a hapten-protein complex constitutes the immunogen. Occupational asthma from LMW sensitizers may be associated with specific IgE-antibodies (Dykewicz [2009](#)).

Extracts of protein-chemical conjugates are not commercially available for skin testing (Dykewicz [2009](#)). Recently, the use of self-prepared prick tests in the diagnosis of occupational contact urticaria, asthma, and rhinitis caused by chemicals undertaken at the Finnish Institute of Occupational Health was assessed and reported (Helaskoski et al. [2015](#)): The authors conclude that skin prick tests can be safely used for diagnosing contact urticaria, asthma and rhinitis caused by isocyanates, chloramine-T, persulfates, and chlorhexidine, but the results should be carefully interpreted and related to clinical symptoms and other diagnostic tests. This publication (Helaskoski et al. [2015](#)) contains helpful

practical aspects on test concentrations and preparation of SPT solutions from chemicals.

There are limited numbers of in vitro tests available for specific IgE to LMW protein-chemical conjugates. Although these tests do not typically have good sensitivity, when positive they can support the diagnosis of occupational asthma from a LMW sensitizer. However, many commercial tests have not been validated with proper homologous controls for these substances (Dykewicz [2009](#)).

Several LMW substances frequently induce occupational asthma:

1. 1.

Isocyanates are characterized by their highly reactive $N = C = O$ component(s). Industrially, certain isocyanates are relevant: the monoisocyanate methyl isocyanate (MIC) and several diisocyanates (e.g., hexamethylene diisocyanate (HDI), toluene diisocyanate (TDI), methylene diphenyl diisocyanate (MDI)). TDI is a highly produced diisocyanate second only to MDI of the global isocyanate market in 2000 (Sekizawa [2000](#)).

Occupational exposures to diisocyanates occur during processes in rigid polyurethane foam preparation for roof panels for thermal insulation or preparation of coatings, adhesives, sealants, and elastomers for spray floor coating, bridge decking primer, or particleboard (Sekizawa [2000](#)). For example, MDI is used for polyurethane elastomers (rollers, packing, rubber vibration insulators, synthetic leather, etc.), spandex fibers, and rubber shoe soles (Sekizawa [2000](#)). Polymeric MDI (PMDI) is the primary technical/commercial form of MDI which is actually a mixture that contains 25–80% monomeric 4,4'-MDI as well as oligomers containing three to six rings and other minor isomers, such as the 2,2'-isomer (the exact composition of PMDI varies with the manufacturer). It is used to make rigid and flexible foam and foundry resin sand binders and heat insulating material (Sekizawa [2000](#)). MDI is usually present in workplace air as vapor, but some aerosol may also coexist, depending on the type of operation. In such atmospheres, exposure may be to unreacted MDI or to a mixture of MDI and polyols, reactants used to convert MDI to polyurethane foams. Humoral as well as cellular immunity may be involved in the pathogenesis of hypersensitivity due to isocyanates (Sekizawa [2000](#)). Diisocyanate-specific IgE antibodies could be detected in 10–30% of symptomatic exposed individuals. Overlapping mechanisms may contribute to the diversity of clinical symptoms and may require further investigations other than testing for specific IgE (Dykewicz [2009](#)). The range of particle size will vary with the industrial application, and the method of sampling and analysis needs to be suited to the workplace requirement (Sekizawa [2000](#)). Specific IgG has been found to be a marker for exposure (Raulf-Heimsoth et al. [2009](#)).

Protein-chemical conjugates of isocyanates are not commercially available for skin testing. In vitro tests with LMW protein-chemical conjugates are commercially available for specific IgE to the following isocyanates: HDI, TDI, and MDI.

2. 2.

Cyclic acid anhydrides are synthetic, highly reactive low-molecular chemical compounds that are widely used as curing agents for epoxy resins and in the production of polyester and alkyd resins. The phthalic anhydride concentrations

during polyvinyl chloride (PVC) processing were low, but measurable (Keskinen [2004](#)). They are man-made chemicals commercially available at high purity as liquids or crystals, depending on the type of anhydride (Keskinen [2004](#)). Technical anhydride products may contain other related cyclic anhydrides as impurities or they can be mixtures of different isomers. They are not found in nature but may be found as environmental contaminants (Keskinen [2004](#)). Numerous cyclic acid anhydrides are industrially used for different purposes (Keskinen [2004](#)), e.g.:

- **Phthalic anhydride (PA)** (for manufacture of phthalate plasticizers, phthaleins, unsaturated polyester resins, alkyd resins, halogenated phthalic anhydrides, phthalocyanide dyes; for preparation of benzoic acid; as hardener in epoxy resins)
- **Trimellitic anhydride (TMA)** (for manufacture of plasticizers with high thermal resistance and of unsaturated polyester resins; as hardener in epoxy resins)
- **Maleic anhydride (MA)** (for manufacture of unsaturated polyesters, alkyd resins, lacquers, plasticizers, copolymers, lubricants, pesticides, pharmaceuticals, and permanent-press resins (textiles); for synthesis of some organic acid anhydrides; for a Diels-Alder reaction (i.e., a chemical reaction between a conjugated diene and a substituted alkene to form a substituted cyclohexene))
- **Hexahydrophthalic anhydride (HHPA)** (for manufacture of alkyd resins, plasticizers, insect repellents, and rust inhibitors; as a hardener in epoxy resins)
- **Methyl hexahydrophthalic anhydride (MHHPA)** (as a hardener in epoxy resins)
- **Methyl tetrahydrophthalic anhydride (MTHPA)** (as a hardener in epoxy resins)
- **Tetrahydrophthalic anhydride (THPA)** (for production of unsaturated polyester resins and alkyd resins with increased resistance to water and solvents; as a starting agent for light-colored alkyds, polyesters, and plasticizers used in adhesives; as an intermediate for pesticides; as a hardener of epoxy resins)
- **Tetrachlorophthalic anhydride (TCPA)** (as a flame retardant in unsaturated polyester resins, polyurethane foams and surface coatings and plasticizers; as an intermediate in the production of dyes and pharmaceuticals; as a hardener in epoxy resins)
- **Pyromellitic dianhydride (PMDA)** (for production of polyimide resins; polyimide resins are high heat-resistant polymers with good electrical and physical properties. They are used for films, fibers, molding compounds, varnishes, and wire coatings)
- **Chlorendic anhydride (CA)** (as a flame retardant in polyester resins and plasticizers)

Cyclic acid anhydrides react easily with water, and the corresponding acids are formed. The formation of acids explains the irritating effects on the skin and the mucous membranes of the eyes and the respiratory organs (Keskinen [2004](#)). The formation of protein adducts in vivo is believed to be the first step in the sensitization process. Allergic asthma, often preceded by rhinoconjunctivitis, is a well-documented disease of workers exposed to cyclic acid anhydrides (Keskinen [2004](#)). In case reports and industrial surveys, IgE-mediated sensitization has been verified by positive reactions in skin prick tests with self-

produced conjugates (according to Howe et al. [1983](#)) of the anhydrides and HSA and by specific IgE (Keskinen [2004](#); Helaskoski et al. [2009](#)). In exposed workers, bronchial hyperresponsiveness, a typical finding in asthma, has been correlated to the specific sensitization. Immediate, dual, or late bronchial reactions have been found in challenge tests with PA, MA, HHPA, MTHPA, TCPA, and PMDA (Keskinen [2004](#)). Also IgE-mediated contact urticaria due to cyclic acid anhydrides occurs: MHHPA, MTHPA, HHPA, CA, and MA have induced contact urticaria in exposed workers with specific IgE-antibodies and positive results in skin prick tests and in open tests with the anhydride (Helaskoski et al. [2009](#)). Prick testing and specific IgE can be used to identify sensitized persons (Helaskoski et al. [2009](#)). To confirm the diagnosis of contact urticaria following a positive skin prick test with self-produced conjugates (Helaskoski et al. [2009](#); Helaskoski et al. [2015](#)), an open application for 40 min with the undiluted conjugate used for the skin prick test and/or the diluted (1% and 10% dilution) as well as the undiluted product from the workplace with readings at 20 and 40 min has been suggested (Helaskoski et al. [2009](#)). It is noteworthy to mention that the open application test most often had to be performed with the undiluted anhydride product to obtain a positive test result (Helaskoski et al. [2009](#)). In vitro tests with LMW chemical-protein conjugates are commercially available (e.g., from ThermoFisher Scientific Inc.-Phadia AB; Hycor Biomedical Inc., Garden grove, CA, USA; Siemens Healthcare Diagnostics, Eschborn, Germany) for specific IgE to the following cyclic acid anhydrides: PA, MA, TMA, TCPA, and HHPA (however, not the entire panel from one company). Inhibition studies and passive transfer studies have supported the specificity of IgE antibodies. However, cross-reactivity between some acid anhydrides has been reported (Keskinen [2004](#)).

3. 3.

Also metals (e.g., platinum, iridium, nickel, cobalt, chromium, rhodium) can produce allergic respiratory symptoms (Grammer [2001](#); Bernstein and Merget [2006](#)). Hexachloroplatinate salts are immunogenic without need for protein conjugation (Dykewicz [2009](#)). Skin prick test preparations and in vitro tests are not commercially available. Self-prepared SPT preparations of 10–2 mol/l hexachloroplatinate showed a high specificity (Bernstein and Merget [2006](#)). For the platinum salts positive skin tests, in vitro tests for IgE and positive specific and nonspecific inhalation challenges have been reported. The degree of specific bronchial hyperresponsiveness to platinum is correlated with the degree of positive cutaneous tests but does not correlate with nonspecific bronchial responsiveness (Grammer [2001](#)).

4. 4.

Drugs (e.g., antibiotics (penicillins, spiramycin, isoniazid, tetracycline), methyl dopa, penicillamine) can induce immunological occupational asthma in pharmaceutical industry as well as in hospitals and nursing homes (Grammer [2001](#)).

Skin prick test preparations are not commercially available, for few drugs in vitro tests are commercially available. A comprehensive position paper on recommended skin test concentrations for self-prepared test preparations of

systemically administered drugs has been published by the ENDA/EAACI Drug Allergy Interest Group (Brockow et al. [2013](#)).

5. 5. Miscellaneous exposures in various industries may induce immunological occupational asthma: persulfate (hair bleach in beauty parlor and hair dressing: skin prick testing may be performed with a 1–5% aqueous solution prepared from ammonium persulfate (Mahler and Drexler [2004](#))); acrylates (health care, artificial nails); chloramine T, hexachlorophene, and chlorohexidine (antiseptics); ethylenediamine (is involved in the production of many industrial chemicals, e.g., as a precursor to chelating agents, polymers, pharmaceuticals, and agrichemicals); colophony (as soldering flux in electronics); ethylene oxide (used in industrial sterilization of heat-sensitive medical devices; in approximately 10% of symptomatic patients IgE antibodies to ethylene oxide can be detected; a commercial in vitro test is available); azo compounds (as reactive dyes in textile industries; diazonium in photocopy and fluorine polymers); plicatic acid (from wood dusts of western red cedar in lumber industry and carpentry) (Vedal et al. [1986](#); Grammer [2001](#)).

Recently, hidden and rare occupational allergen exposures have been reviewed (Quirce et al. [2018](#)): These include workplace-specific foods, spices, cosmetics, insects, enzymes, chemicals, drugs, preservatives, and coloring agents, among others. For example, sodium metabisulphite, a chemical preservative, has been described to cause occupational asthma in fishermen, food processors, and trawlermen (Quirce et al. [2018](#)). Skin-prick test preparations and in vitro tests for these allergens are not commercially available.

In conclusion, a thorough clinical and environmental assessment is necessary in every individual case of suspected work-related allergic disease to identify the existing exposure to all possibly relevant allergens – including unexpected and rare allergens. Based on this exposure analysis, a feasible test panel can be assembled, which may include self-made test preparations in adequate concentrations prepared by the physician or pharmacy for an individual patient to be tested, in case relevant in vivo and/or in vitro test allergens are commercially unavailable (e.g., as for chemicals: (Helaskoski et al. [2015](#)); or for drugs: (Brockow et al. [2013](#))). Thorough patient information and written consent has been recommended for all in vivo test procedures and has been considered particularly important when testing nonstandardized test preparations (Mahler et al. [2017b](#)).

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