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The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals

111. Industrial Enzymes

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ARBETS MILJÖ INSTITUTET
NATIONAL INSTITUTE OF OCCUPATIONAL HEALTH

A Center for Research on Occupational Health

Sweden´s National Institute of Occupational Health employs over 300 scientists in research on the work environment. The research is led by 30 professors. The Institute does mostly applied research, but some questions also require focused basic research.

The scientific competence of the Institute is organized into six areas: Physiology, Chemistry, Medicine, Psychology, Technology and Toxicology. This broad base of expertise provides solid support for the Institute's cross-disciplinary approach.

The Institute is responsible for training and educating personnel working within the occupational health services as physicians, nurses, physiotherapists, psychologists and safety and hygiene engineers.

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Preface

The Nordic Council is an intergovernmental collaborative body for the five countries, Denmark, Finland, Iceland, Norway and Sweden. One of the committees, the Nordic Senior Executive Committee for Occupational Environmental Matters, initiated a project in order to produce criteria documents to be used by the regulatory authorities in the Nordic countries as a scientific basis for the setting of national occupational exposure limits.

The management of the project is given to an expert group. At present the Nordic Expert Group consists of the following member:

Helgi Gudbergsson	Municipal Institute of Public Health, Iceland
Petter Kristensen	National Institute of Occupational Health, Norway
Per Lundberg (chairman)	National Institute of Occupational Health, Sweden
Vesa Riihimäki	Institute of Occupational Health, Finland
Adolf Schaich Fries	National Institute of Occupational Health, Denmark

For each document an author is appointed by the Expert Group and the national member acts as a referent. The author searches for literature in different data bases such as Toxline, Medline, Cancerlit and Nioshtic. Information from other sources such as WHO, NIOSH and the Dutch Expert Committee is also used as are handbooks such as Patty's Industrial Hygiene and Toxicology. Evaluation is made of all relevant scientific original literature found. In exceptional cases information from documents difficult to access are used. The draft document is discussed within the Expert Group and is finally accepted as the Group's document.

An editorial work is performed by the Group's Scientific Secretary, Brita Beije, at the National Institute of Occupational Health in Sweden.

Only literature judged as reliable and relevant for the discussion is referred to in this document. Concentrations in air are given in mg/m^3 and in biological media in mol/l. In case they are otherwise given in the original papers they are if possible recalculated and the original values are given within brackets.

The documents aim at establishing a dose-response / dose-effect relationship and defining a critical effect based only on the scientific literature. The task is not to give a proposal for a numerical occupational exposure limit value.

The evaluation of the literature and the drafting of this document on industrial enzymes was made by Jonas Brisman, M.D., Department of Occupational Medicine, Sahlgrenska hospital, S:t Sigfridsgatan 85, S-412 66 Göteborg, Sweden. The final version was accepted by the Nordic Expert Group June 13-15, 1994 as its document.

Brita Beije
Scientific Secretary

Per Lundberg
Chairman

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1. Introduction

Chemical reactions in biological systems rarely occur in the absence of a catalyst. These catalysts are proteins or glycoproteins called enzymes. Presence of enzymes can be regarded as a prerequisite for life. Enzymes are characterized by their catalytic power and specificity. They accelerate reactions by factors of at least a million. These characterstics also make them extremely useful in biotechnological processes.

This document is mainly focussed on immunologically mediated adverse effects by enzymes on the respiratory tract and the skin. The available documentation reflects the way of describing the toxic effects of environmental factors by scientists in allergy and dermatology; first comes case reports, after that cross-sectional studies. Studies with a longitudinal epidemiologic design are unfortunately rare.

2. Industrial Enzymes

Table 1. Industrial enzymes and their major applications

Acylase	pharmaceuticals
Amylase	alcoholproduction, animal feed, baking, brewing, detergents, pulp and paper, starch- and sugar production, textile industry, wine and fruit juice
Amyloglucosidase	baking, brewing
Carbohydrase	brewing
Cellulase	alcohol production, animal feed, brewing, protein industry pulp and paper, textile industry, baking
Chymotrypsine	pharmaceuticals
Glucanase	animal feed, brewing, wine and fruit juice
Glucosidase	alcohol production
Glucoseisomerase	starch- and sugar production
Glucoseoxidase	baking
Lactase	dairy
Lipase	dairy, leather production, oils and fat, starch and sugar production, wine and fruit juice
Pectinase	wine and fruit juice
Protease	alcohol production, animal feed, baking, brewing, detergents, leather porduction, meat portioning, protein industry
Pullulase	starch- and sugar production
Streptokinase	pharmaceuticals
Trypsine	pharmaceuticals
Urokinase	pharmaceuticals
Xylanase	pulp and paper

3. Occurrence, Production and Use

Man has used enzymes since prehistoric times, when he discovered that juice extracted from grapes could be fermented and turned into wine (17). By chance it was discovered that the process was dependent on airborne spores of yeast fungi falling into the juice jars. It was also observed by herdsmen, using the stomachs of slaughtered animals as canteens, that milk stored in the stomachs clotted to cheese. This "mysterious" phenomenon was later described by Homer in the Odyssey. In both examples it was in fact naturally occuring enzymes that made these reactions possible.

During the centuries, similar discoveries taught man vital processes such as how to leaven bread and brew beer. In the 19th century, scientists studying the process of fermentation discovered the existence of enzymes. In 1833 Anselm Payen and Jean Francois Persoz isolated a substance from malt which converted starch into sugar. The substance was an amylase enzyme. In 1836 Theodor Schwann isolated the active substance from the stomach wall, pepsin. In 1876 William Kühne proposed the word "enzyme". Enzyme comes from greek words "en" which means "in", and "zyme", which means "yeast".

A parallel development took place in the Far East where the koji mould was used since ancient times in the production of e.g. fermented liquors. The Japanese scientist Takamine developed an industrial production of fungal amylase by growing Aspergillus oryzae on rice or grain.

Otto Röhm, one of the pioneers of modern enzyme technology in Germany, described in 1905 how to use a preparation from pancreas for batting hides in leather tanneries. Previously hides and skins had been immersed in baths of animal dung and thereby utilizing proteolytic enzymes that catalyst the breaking down of proteins in hides. (In fact it was not enzymes from the animals that were active, but enzymes of bacterial origin that were present in the gastro-intestinal tract). The use of the proteolytic enzyme trypsin from the pancreas not only made work of the leather tanneres more agreeable, but also made batting processes more reliable.

Enzyme-containing animal bile is said to have been used in washing since ancient times and the first commercial enzyme-containing washing powder was marketed in 1913. This trypsin-containing powder was not a success. The enzyme was unstable in the alkaline environment caused by the high soda content in the powder.

During World War Two, because of a shortage of soap, the interest in using enzymes for washing rose dramatically, and this area became intense in terms of industrial research and development.

In 1959, a Swiss company marketed a washing powder containing the enzyme protease from one of the species of Bacillus subtilis. This was a major advance in enzyme technology, since the bacteria could be fermented in large tanks, thus permitting mass production without dependence upon animal organs. Proteolytic enzymes from these bacteria are called subtilisins.

Table 2. Major enzyme applications and used enzymes

Alcohol production	amylase
	cellulase
	glucosidase
	protease
Animal feed	amylase
	cellulase
	glucanase
	protease
Baking	amylase
	amyloglucosidase
	cellulase
	glucoseoxidase
	protease
Brewing	amylase
	amyloglucosidase
	carbohydrase
	cellulase
	glucanase
	protease
Dairy	lactase
	lipase
Detergents	protease
	amylase
Leather producution	lipase
	protease
Meat portioning	protease
Oils and fat	lipase
Pharmaceuticals	acylase
	chymotrypsine
	streptokinase
	trypsine
	urokinase
Protein industry	cellulase
	protease
Pulp and paper industry	amylase
	cellulase
	xylanase
Starch and sugar production	amylase
	glucoseisomerase
	lipase
	pullulase
Textile industry	amylase
	cellulase
Wine and fruit juice	amylase
	glucanase
	lipase
	pectinase

Meanwhile a Danish firm developed a subtilisin preparation stable under the conditions of heat and alkalinity existing in normal laundering. This product was introduced in 1962. The breakthrough for enzyme containing washing powders

came when the Danish firm, in collaboration with Swiss and Dutch firms, marketed their products on the European market in 1965 and in America in the following years.

The market for enzymes has an estimated turn-over of 700 million USD yearly with an annual increase of 8-12 % (23). In Scandinavia there is enzyme production in Denmark and Finland. Detailed description of enzyme production is usually confidential due to the high-technology involved.

The production is mainly based on fermentation in tanks with one out of four species of microorganisms; Bacillus, Aspergillus, Saccharomyces or Trichoderma. Extraction from plants (papain, bromelain) and animal pancreases does also occur. With the use of modern molecular biology and genetical technology more purified and effective enzymes can be produced in large quantities.

4. Occupational Exposure

Enzymes are applied today in a variety of uses and can be found in a wide range of occupational settings. Bakers are probably the largest exposed occupational group since enzyme, mainly α-amylase, is routinely added to flour in many countries (46). Major enzyme applications are listed in Table 2.

5. Sampling and Analysis of Substance at Workplace

The question of exposure to enzymes is often being reduced to the dichotomy of yes or no. This is partly due to the difficulties in sampling and analysing very low levels of enzymes in airborne dust. At first, sampling was performed with a so called "galley sampler" which was a high volume sampler used for area measurements. These measurements were essentially used for dust control and seldom published. Later on, as more sensitive analytical methods were developed, portable pumps for personal sampling with smaller sampling volymes, came into use.

Enzymes can be analysed by measuring the enzymatic activity after adding a suitable substrate. Such enzymatic assays have been reported in several exposure studies. The enzymatic activity of proteolytic enzymes such as subtilisins was originally expressed as glycine units (GU) and air-concentrations as GU per m^3 of air (18, 68). Other enzymatic assays were developed, usually with a commercial enzyme preparation with a content of 2-3 % crystalline substilisin as a standard. This permitted expressing air-concentrations in $\mu g/m^3$ (76). An assay measuring amylase activity, with a commercial amylase preparation as a standard, has also been reported (15).

Examples of more sensitive immunochemical analytical techniques are a radioimmuno assay for papain (77) and an assay for subtilisin (1).

At present, there is no single technique developed for analysing different enzymes.

In 1970 ACGIH proposed a ceiling limit TLV for **pure** subtilisin of 0.3 $\mu g/m^3$ The proposed TLV was lowered to 0.06 $\mu g/m^3$ in 1973 and its equivalent in glycine units to 2.5 GU/m^3. This TLV was adopted in 1974 (2). Since the standard for this TLV provides pure subtilisin, the TLV must be adjusted when a standard with lower subtilisin content is used.

ACGIH has published a table with TLV's adjusted according to the enzyme content of the standard (2). (See Table 3.)
As can be seen from the table, a minor inaccuracy in the enzyme content of the standard may cause a substantial difference in the calculated TLV. Obviously, this hampers comparisons between air-levels in exposure studies. It is not known whether the TLV of subtilisins is applicable to other enzymes as well.

Table 3. Some equivalent terms for the TLV in mg/m^3

Ingredient	Pure Enzyme Content %	TLV mg/m^3
Crystalline Active Pure Enzyme (CAPE)	100	0.00006
Sigma "Pure" Enzyme	60	0.0001
"As Received" Enzyme	2.4	0.002
"Pre-Mix" or "Coated Concentrate"	0.36	0.017
Finished Product (variable)	0.012	0.5

5.1. Air levels

Weill et al reported area sampling from two detergent producing factories (76). Sampling was designed to denote average and peak exposure to subtilisins for three exposure groups. In plant A, which employed dust control, the average exposure ranged <1-18 μg enzyme/m^3 and peaks reached up to 60 $\mu g/m^3$. In plant B (without dust control) average exposure was between <1 and 30 $\mu g/m^3$ and peaks up to 1000 $\mu g/m^3$. Results represent an equivalent amount of 3 % crystalline subtilisin and the equivalent ACGIH TLV is about 3 $\mu g/m^3$.

Göthe et al studied two detergent factories (37). In factory A the detergent base was mixed with enzyme powder and no dust control was in use. In factory B the enzyme was encapsulated as granules and dust control measures were undertaken. Factory A had subtilisin-levels of 5.4 GU/m^3, the other about or below 1 GU/m^3. Both stationary and personal sampling was done.

Juniper et al reported enzyme dust concentrations with stationary sampling in the packing department of a factory producing enzyme-containing detergents from 1969 to 1975 (43). In the beginning of that period levels were typically about 1 GU/m^3 and fell towards the end of the period to about 0.2 GU/m^3.

Liss and coworkers measured subtilisins in a dry bleach manufacturing process (47). Values between <0.002 to 1.5 $\mu g/m^3$ in the blending area and <0.002-0.76 $\mu g/m^3$

Table 4. List of exposure measurements of enzymes

Enzyme	Assay	Exposure group/task	Measured level ($\mu g/m^3$) average	range	Ref.
Subtilisin	E	Factory A			76
		Low	<1		
		Moderate	1-5		
		High.	3-18		
		Factory B			
		Low	<1		
		Moderate	1-5		
		High	3-30		
Subtilisin	E	*Blending*	0.64	<0.002-1.57	47
		Filling	0.49	<0.002-0.76	
Subtilisin	I	Processing			
		area	0.023	<0.008-0.031	1
		personal	<0.043	<0.004-0.021	
		Packing			
		area	0.012	0.008-0.017	
		personal	<0.036	0.004-018	
Subtilisin	I	*Quality technician*	0.53	0.450-0.60	71
		Packers 1	0.99	0.24-1.70	
		Packers 2	0.47	0.29-1.10	
		Packers 3	0.70	0.22-1.30	
Amylase	E	*Packing*	30		16
Amylase	E	*Weighing*	7.3	1.2-21.0	42
		Breadmaking	0.2	0.1-2.0	
Amylase	E	*Bakers*	0.6		19
			Measured level (GU/m^3) average	range	
Subtilisin	E	*Factory A*	5.4	2.9-10.4	38
		Factory B	1	0.2-1.7	
Subtilisin	E	*Packing*		0.2-1	43

E = enzymatic
I = immunochemical

in the filling area were reported. (In this case the TLV of 0.06 $\mu g/m^3$ was calculated to correspond to a measured level of 3.9 $\mu g/m^3$ since the used subtilisin contained only 2.6% active enzyme).

Agarwal et al also measured detergent enzyme concentrations in a dry bleach factory (1). Area measurements were on average 0.023 $\mu g/m^3$ during processing and 0.012 $\mu g/m^3$ during packing. Personal sampling showed 0.0043 µg during processing and <0.036 $\mu g/m^3$ during packing. No comparision with the TLV was done in this study.

Swanson et al measured papain concentrations in a meat portioning facility with an immunochemical method (71). Personal samples showed average concentrations of 0.47 - 0.99 $\mu g/m^3$. According to the authors, these values are overestimates for pure papain since the papain standard only contained about 14 % pure papain. Area sampling was also performed. An Andersen cascade impactor was used to obtain particle sizing; about half the airborne papain was associated with particles having an aerodynamic diameter less than 9.4 µm.

α-Amylase occurring as flour additive has been measured by an enzyme-activity method in three studies. The same commercial α-amylase product was used as standard in all three studies.

Brisman and Belin used personal sampling and measured 30 $\mu g/m^3$ during packing flour additives in a factory making bread-improvers (16). α-Amylase exposure was measured both personally and stationary in six Finnish bakeries (42).

Burdorf et al (19) performed personal measurements in 12 bakeries, sampled at random in Sweden.

6. Toxicokinetics

Enzyme exposure occurs from dust or liquid aerosols. In this respect there are no data specifically relating to enzymes, but they are considered to behave as other particulate matter. Since there are different sources from which enzyme aerosols can emerge, the size of the particles is also very variable. The particles are deposited on the skin or on the mucous membranes of the airways. The place of deposition is dependent on the size of the particle (15).

7. Biological Monitoring

No methods have been reported.

8. Mechanisms of Toxicity

When an enzyme comes into contact with the skin or the respiratory tract, antibodies to this enzyme may be formed (sensitization). When the sensitizer is encountered again, a contact dermatitis may arise if there is a cellmediated allergic reaction in the skin, or an immediate reaction if IgE-antibodies are present. Other isotypes may be formed, mainly IgG. They seem to be markers of exposure rather then mediating symptoms.

Direct enzymatic action may also cause health-effects in enzyme-exposed tissue.

9. Effects in Animals and in Vitro Studies

9.1. Irritation and sensitization

Guineapigs were sensitized by intratracheal instillation of the proteolytic enzyme papain. A second dose may cause a fatal allergic reaction (36).

9.2. Acute toxicity

A single intratracheal instillation of 1 mg of papain into guineapigs or rats produces emphysema (35). Golden hamsters which inhale an aerosol of 3 % papain during four hours also get emphysema (32).

Inhalation of either subtilisin or papain causes hemorrage in the respiratory tract of animals. Emphysema develops in animals that recover from the acute effects of papain but not in those that receive subtilisin (33).

10. Observations in Man

10.1. Acute effects by contact and systemic distribution

Skin contact with detergent enzyme can cause a dermatitis due to a primary irritant effect (52).

10.2. Effects of repeated exposure on organ systems

10.2.1. Skin

After the introduction of enzyme-containing washing powders there was much concern about possible dermatological effects among users. These effects were repeatedly regarded as irritative, and presumably caused by non-enzymatic ingredients (5, 52).

Ducksbury and Dave reported cases and a questionnaire cross-sectional study among home-helps (24). The prevalence of detergent associated dermatitis was 5%. Patch tests with enzyme containing detergents were negative.

Bolam et al concluded after a study among house-wives that enzymatically active washing powders were not more irritant than conventional powders (14).

Göthe et al studied 50 workers exposed to enzymes during detergent production (38). Forty-seven percent reported work-related skin symptoms and there was a dose-response relationship. Reactions were regarded as irritative. One case reported generalized urticaria upon enzyme contact.

Zachariae et al studied 79 workers with skin symptoms and exposure in enzyme

production, and 12 unexposed controls (81). Patch test with subtilisin were negative. High enzyme concentration was considered to cause irritant dermatitis.

Schirmer et al described one baker with dermatitis (66). He had positive skin prick tests to α-amylase and various bread improvers. The immediate test reaction to α-amylase persisted for 48 hours. A patch test with a-amylase was also positive as well as RAST to α-amylase, malt and bread improvers .

Four cases of contact urticaria developing after 0.5-4 years of exposure to the enzymes cellulase and xylanase was reported by Kanerva and Tarvainen (44). All cases later developed rhinitis and asthma and two of them an allergic contact dermatitis. Skin prick tests were positive to cellulase and xylanase in all cases. A patch test was positive to cellulase in one case and, to xylanase in the other. In a cross-sectional study Brisman and Belin reported on 20 amylase exposed workers (16). They found significantly more skin symptoms compared to controls.

Morren et al (56) reported 32 consecutive bakers patch tested with α-amylase. Seven had an immediate reaction, and two had a positive delayed skin test. Four of the seven were skin prick tested to α-amylase and they were all positive.

10.2.2. Respiratory tract

Detergent enzymes in occupational settings. Studies with registration of respiratory symptoms and sensitization together with exposure are listed in Table 5.

The first account of adverse effects from handling detergent enzymes was reported in 1969 by Michael L H Flindt. When working as medical officer in a detergent powder factory in 1967, he observed an epidemic disease among the workers. Twenty-eight cases with symptoms from the respiratory tract were reported. Positive skin prick tests with detergent enzyme extract indicated an immediate allergy as the explanation of the symptoms. Spirometry and chest X-ray were normal. Precipitating antibodies were found in some of the affected individuals but also in unexposed controls (27).

In the same issue of the Lancet, J Pepys and co-workers reported immunological findings in three selected patients referred from Flindt. Apart from reproducing the skin prick tests, they found immediate and late asthmatic reactions to inhalation tests with enzyme powder. Again, precipitins were found in the examined subjects, but also in sera from controls (63).

The two articles were commented in an editorial, calling for preventive measures against organic dusts causing allergic lung disease (4). Several cross-sectional studies validated the findings of these case reports.

Greenberg et al found that 40 % out of 121 examined workers had positive skin prick test positive to enzymes after about two years of exposure (34). Sensitized persons more often complained of dyspnea and had lower FEV_1/FVC ratio compared with non-sensitized.

There was a follow-up of these 121 cross-sectionally examined workers (34). During the six months that had passed, enzyme had been handled only in six days, the last of which was eleven weeks previously. One worker who was non-sensitized on the first occasion, had a positive skin prick test to enzymes at the

second. Of the 19 who had reduced ventilatory capacity, eight had improved to normal levels.

Ninety-seven of the original 121 workers were followed up by Watt et al in 1973 (75). During six rounds of investigations at 6-monthly intervals, ventilatory capacity improved and no further excess of symptoms developed among prick test positive workers.

Newhouse et al examined 271 out of 277 exposed workers of which 21 % were sensitized according to skin prick test (59). There were significantly more respiratory symptoms, as well as an obstructive ventilatory impairment, among the sensitized workers compared to non-sensitized. None of 27 shipping and wear-house men were sensitized.

Newhouse also made a follow-up in 103 workers after six months (59). Fewer workers now had acute respiratory symptoms and in nine workers negative prick test had converted to positive. The means of FEV_1 of both sensitized and non-sensitized workers were unchanged.

Weill et al studied workers in two detergent manufacturing plants handling enzymes (76). Fifty workers in a factory employing dust control (plant A) and 60 workers from a factory without such control (plant B) were selected into the study. Workers were skintested after six months of employment in factory A and after varying employment time in plant B. There were no differences in ventilatory capacity between the exposure groups. None of the workers in plant A had any respiratory symptoms while 22 % of the workers in plant B had symptoms indicating lower respiratory tract disease such as wheezing, chest tightness, breathlessness or nocturnal cough.

Göthe et al studied 50 enzyme exposed workers and 14 controls (38). Respiratory symptoms occurred in about 50 %, positive skin prick tests to enzyme in 21 % and positive RAST in 5 %. There were significantly more positive prick tests in symptomatic workers compared with non-symptomatic.

Shapiro and Eisenberg studied 93 workers of a plant where enzymes were used in the production of detergent powders (67). Thirty-four had strongly positive intradermal tests, 13 of whom complained of asthma or rhinits associated with exposure. Only one non-sensitized had similar symptoms. Non-exposed and new personal had no symptoms and were not sensitized.

McMurrain reported of 207 cases with rhinitis, pharyngitis, cough, bronchitis and asthma among approximately 3500 workers with enzyme exposure in the United States since 1966 (52). Intradermal tests with enzyme material in 1727 employees evoked a positive reaction in 588 cases. Up to 50 % of workers handling concentrated enzyme products were sensitized.

Mitchell and Gandevia (55) found that 50 % of 98 workers periodically exposed to high levels of detergent enzyme developed symptoms suggestive of asthma. Sensitization (skin prick and intradermal tests) was not significantly more frequent among symptomatic workers. Tests of ventilatory (FEV_1, FVC, FEV_1/FVC) and diffusion capacity showed no evidence of permanent lung damage compared with predicted values.

Re-investigation of all available employees or ex-employees from the study of Mitchell and Gandevia (n=67) was undertaken three years after the original survey (57). No enzyme was handled during this time. Thirteen heavily exposed persons showed a significant loss of pulmonary elastic recoil compared with 42 lightly or moderately exposed. There were no differances in other parameters of lung function or respiratory symptoms.

Liss and co-workers studied the effect of encapsulation in order to prevent sensitizing of enzyme exposed workers (47). In spite of the preventive measures, specific IgE antibodies to enzyme were detected in three out of twelve currently exposed workers. IgG-antibodies were detected in 4/12 exposed workers and in one out of two previously exposed workers, but in none of the non-exposed workers.

Witmeur et al studied 335 workers in two Danish enzyme producing factories. RAST showed 3.3 % to be sensitized to subtilisin. There was no indication of deterioration of respiratory functions in relation to sensitization or enzyme exposure (79).

Juniper et al reported a seven year longitudinal study of 1 642 workers in the factory where Flint's original observation was made (43). All workers were examined at the beginning of the enzyme exposure and thereafter at six-monthly intervals 1968-1975. Sensitization ranged between 73 % (high exposed atopics, original employees) and less than 1 % (low exposed, non-atopics starting after September 1969). Average exposure time before detection of positive skin test ranged 12-20 months. There was no evidence of chronic ventilatory impairment or significant pathology on chest X-rays.

An eleven year observation report was presented by Flood and co-workers (30). Apart from the previously mentioned factory, the study included two other enzyme detergent factories in the UK. Frequency of sensitization varied between factories, exposure levels (classification method not reported) and atopic status. Four and a half percent of the workers "had experienced respiratory hypersensitivity for enzymes". They were all skin prick test positive to the detergent enzymes and had respiratory symptoms, but not necessarily frank asthma. These workers are excluded from Table 5.

Zachariae et al followed at total of 667 enzyme producing workers during ten years (80). They were exposed to subtilisins and another detergent enzyme. Thirty-one were found by RAST to be sensitized. No incidence rate was calculated. Twenty-six of the 31 workers were followed, none was RAST positive at follow-up, but the follow-up time is not reported. Ten workers remained in their jobs with safety precautions.

Domestic exposure to proteolytic detergent enzymes. In 1970 Belin et al demonstrated three cases of enzyme allergy in housewives (11). They had symptoms while handling detergent enzymes, positive skin prick tests and presence of circulating IgE-antibodies. No IgG-antibodies were found. Falleroni and Schwartz reported another, similar case (26). Shapiro and Eisenberg tested 35 consecutive allergic housewives without occupational exposure to enzymes (67).

Table 5. Respiratory symptoms and sensitization in studies with exposure classification or air-measurements. (Exposure to subtilisins in all studies except the one by Briman 1991)

Exposure	N	Respiratory symptoms (%)	Sensitization (%)	Ref.
Factory A				
Low	15	0	0	76
Intermediate	15	0	53	
High	15	0	45	
Factory B				
Low	20	?	16	76
Intermediate	20	? total 22 %	35	
High	20	?	52	
Contact				
Direct	33	55	83	37
Indirect	17	41	59	
None	14	0	43	
None-atopics				
High	430	39		43
Intermittent high	372	4.3		
Medium	95	9.4		
Low	137	3.6		
Atopics				
High	78	73		43
Intermittent high	60	20		
Medium	30	17		
Low	45	8.9		
Filling, blending	13	46	25	47
Controls	9	44	0	
Mixing, packing	20	45	30	16
Controls	9	22	11	
None-atopics				
Factory A				
Maximum	80	0	18	30
Medium	111	0	23	
Minimum	317	0	6	
Factory B				
Maximum	118	0	24	30
Medium	65	0	14	
Minimum	186	0	12	
Factory C				
Maximum	503	0	29	30
Medium	405	0	3	
Minimum	451	0	3	

Table 5 cont.

Exposure	N	Respiratory symptoms (%)	Sensitization (%)	Ref.
Atopics				
Factory A				
Maximum	14	0	57	30
Medium	40	0	73	
Minimum	78	0	22	
Factory B				
Maximum	12	0	42	30
Medium	27	0	37	
Minimum	51	0	16	
Factory C				
Maximum	70	0	63	30
Medium	71	0	11	
Minimum	114	0	8	

Four had strongly positive intradermal tests, including one with asthma from detergent powders.

Bernstein found positive skin tests to enzymes in 25 % of 353 consecutive allergic patients in the US (13). There has been concern over the methods used in this study. The enzyme extracts used for skin testing may have been too concentrated causing non-specific reactions which may have been regarded as positive (12, 62). Positive passive transfer results were obtained as well as positive nasal and bronchial challenge tests in fourteen patients with respiratory symptoms related to prior exposure to enzyme-containing products.

Zetterström and Wide tested 1132 patient sera referred to a laboratory for routine RAST testing against various allergens (82). Twentyone (1.9 %) were positive to subtilisin. Of 391 blood donors two had positive RAST to *subtilisin*. All 122 consecutive patients referred to an allergy out-patient clinic were prick tested, four of them were positive to subtilisin. Eight selected patients with positive RAST as well as positive skin test to enzyme referred symptoms to exposure to enzyme detergents and three to possible residues of subtilisin present in the laundry after washing.

Pepys et al studied 2500 consective patients of which only two had weak prick test reactions to enzyme preparations (64). RAST tests were performed on sera from enzyme exposed workers (with negative pricktests) and the 2500 consecutive patients. The latter were interviewed about the use of detergents and divided into unexposed, light or heavy consumer groups. There were significant differences in RAST counts between unexposed and light exposed, between light-and heavy exposed as well as between heavy exposed patients and exposed workers.

Other proteolytic enzymes. The first account of *papain* hypersensitivity dates 1928 (25). Eyermann reported a pharmacist who, upon contact with powdered papain, experienced nasal symptoms which were reproduced by an oral challenge. A

scratch test with papain was positive. Beecher reported a case, also a pharmacist, with rhinitis and asthma when handling papain (10). Another pharmacist with severe asthma, was reported to be unconscious on two occasions after exposure to papain. Milne and Brand found two cases of food technologists with occupational asthma (54). Scratch tests with papain were positive. Flindt described one case of occupational asthma and positive skin prick test (28). He briefly accounts another case of occupational asthma to papain with fatal outcome.

Tarlo reported two cases of occupational rhinitis and asthma (72). Skin prick tests and RAST were positive to papain. Eighteen co-workers of one index case were also studied as well as 330 consecutive patients in an allergy clinic. 231 consecutive blood samples sent to a clinical hospital laboratory were also analysed for circulating antibodies to papain. There were no positive findings in the co-workers. In the group of allergy clinic patients, seven (2.1 %) had positive skin prick tests, none of these had a history of papain - induced symptoms. RAST was positive in 2.1 % of blood specimens obtained at random.

Flindt performed a cross-sectional study in a factory handling several different enzymes (29). Of eight individuals tested, seven gave positive skin prick test-reactions to papain. Five of those had work-related symptoms from the respiratory tract.

Baur investigated eleven papain exposed workers of which seven reported work-related respiratory symptoms (7). All seven had positive skin prick reactions, while non-symptomatic workers were negative. Sera from six workers with a history of asthma had strong positive RAST reactions, one worker with rhinitis and two asthmatics with high total IgE-levels showed weak positive RAST results.

Novey made a cross-sectional study of 23 workers at a pharmaceutical plant exposed to papain (60). Twelve had work-related respiratory symptoms suggestive of asthma. Seventeen had pulmonary function tests performed, 21 sera were obtained. Eight workers with pulmonary symptoms had IgE antibodies to papain and nine had papain precipitins. Of the eleven tested non-symptomatic, two had IgE-and three precipitating antibodies to papain. One unexposed control out of 52 was RAST positive. Lung volumes and expiratory flowrates were generally lower in the IgE-positive workers compared with the negative.

McLaren reported a graduate student with hay-fever who was working in a laboratory with *chymotrypsin* (51). Intradermal and passive transfer tests were positive to chymotrypsin.

Two similar cases were reported by Howe et al (41). The first case was a non-atopic research chemist with rhino-conjunctivitis during enzyme exposure. An intradermal test was positive as well as a transfer test with chymotrypsin. The other case was an atopic student but without work-related symptoms. Intradermal test with *trypsin* was positive.

Zweiman et al reported one case of asthma in a trypsin- and chymotrypsin exposed individual (83). Intradermal and bronchial provocation tests with trypsin were positive.

Colten and co-workers reported a cross-sectional study of fourteen workers

exposed to hog trypsin (22). Four workers with work-related respiratory symptoms, four non-symptomatic and other controls were investigated. Intradermal, passive transfer, bronchial challenge and in vitro tests (antigen-mediated histamine release) tests were all positive in the four workers but otherwise negative.

Hartmann et al reported two cases of asthma (39). In one case RAST to trypsin was positive, in the other an intradermal test.

Maisel (50) reported the first case of asthma after *pepsin* exposure in a pharmacist. An intradermal test was positive.

Cartier et al reported one case of work-related asthma in a pepsin exposed atopic worker (21). Skin prick and specific inhalation tests were positive, and monitoring of PEF showed pathological variability.

The first case-report describing asthma due to allergy against bromelain, a purified *protease* of pineapple, was from Galleguillos (31). Baur and Fruhmann (6) reported a case from a pharmaceutical company of work-related asthma and rhinitis after exposure to bromelain. RAST and prick test were positive as well as both an inhalation test and an oral challenge. Six patients sensitized to papain were also investigated and five of them showed positive RAST and skin tests to bromelain, two of them also immediate asthmatic reactions after bronchial challenge. In 60 non-exposed asthmatics, two had positive skin tests and eight had positive RAST to bromelain, but in no case there was a clear evidence for sensitization of any clinical importance.

Wiessmann and Baur studied fourteen workers in a pharmaceutical company exposed to porcine pancreatic dust (78). All had work-related respiratory symptoms. In twelve out of thirteen tested skin prick tests with pancreatic extract were positive. Bronchial challenge tests were positive in eight cases. Chest X-rays revealed an infiltration in two cases, mild fibrosis in another two and emphysema in one. Changes in respiratory mechanics were described as "a combination of restrictive and obstructive distrubance". The authors propose three possible explanations to their findings:

1 allergic type I reaction
2 allergic type III reaction causing alveolitis and fibrosis
3 toxic - enzymatic effects of proteases causing emphysema.

Pauwels reported three cases of allergic asthma in pharmacists exposed to a proteolytic enzyme from the fungus Aspergillus niger (61).

Non-proteolytic enzymes. The first report on adverse effects from the non-proteolytic enzyme *α-amylase* was also reported by Flindt (29). He reported eight workers with respiratory symptoms in an enzyme-handling factory. Five of them were skin prick test positive to α-amylase.

Adverse effects from α-amylase as a baking additive was reported by Baur et al (8). Sera from 118 bakers were studied. Ninety-one were screened at random and

27 because of work-relatad respiratory complaints. Thirty-four percent of the symptomatic had positive RAST to a-amylase, but none of the symptom free. Skin prick tests and bronchial provocation tests in selected individuals were also positive.

The same author tested 140 sera from symptomatic bakers with RAST to a number of enzymes (9). Twenty-four percent were positive to α-amylase, 8 % to *hemicellulase* or *cellulase*, 5 % to *amyloglycosidase* and 1 % to *papain* or *subtilisin*. Carmona et al published a case report of one baker with rhinitis and asthma who had positive skin prick test, RAST and bronchial provocation with α-amylase (20).

Quirce reported five cases of α-amylase sensitized bakers (65). Different immunologic tests and bronchial provocations were positive. Four were also sensitized to cellulase.

Brisman and Belin reported four cases (all with rhinitis and three with asthma) from an enzyme handling factory (16). A subsequent cross-sectional study in the same factory, and reported in the same paper, showed significantly more work related nasal and skin problems in 20 exposed workers compared with controls. Six exposed had positive skin prick tests. Nasal challenge tests validated three cases of α-amylase rhinitis. Specific IgG antibodies to α-amylase were detected in two non-symptomatic workers.

Losada reported a cross-sectional study of 83 pharmaceutical workers exposed to powdered α-amylase (49). Fifty-nine % of the workers reported rhinitis and 30 % asthma. Their mean working time was 9.5 years. Twenty-six (31 %) were skin prick test positive to α-amylase, among these were six non-symptomatic.

Tarvainen et al reported four cases which were RAST-positive to both cellulase and xylanase (73, 74). There was cross reactivity between the two enzymes.

Losada described two cases from the packing department of a pharmaceutical firm, both with asthma (48). They had immediate hypersensitivity to cellulase.

10.3. Genotoxic effects

There are no data related to these questions found in the literature.

10.4. Carcinogenic effects

There are no data related to these questions found in the literature.

10.5.Reproductive and developmental toxicity

There are no data related to these questions found in the literature.

11. Dose-Effect and Dose-Response Relationships

11.1. Single/short-term exposure

There are no data related to this question found in the literature.

11.2. Long-term exposures

There are only a few studies which enables you to investigate the relation between exposure, effect and response. Exposure data are scarce and the measured response is usually sensitization to enzymes. Exposure estimates obtained in different studies are usually not comparable since different enzyme standards were used at analysis. Studies which report respiratory symptoms and sensitization in relation to exposure are found in Table 5.

11.2.1. Respiratory tract

In the study of Weill et al (76) there were no lower respiratory symptoms among workers exposed for six months in plant A. None in the low-exposed group was sensitized, about 50 % of the moderate- and high-exposed were sensitized. Workers in plant B were exposed up to three years, and the levels in the high-exposed groups exceeded those in plant A. Twenty-two percent of these workers had lower respiratory symptoms, but the exposure classification is not mentioned. The frequency of sensitization was about the same in the two plants.

Göthe et al studied workers in two different factories, with mean exposures of 5.4 GU/m^3 (Factory A) and 1 GU/m^3 (Factory B) (37, 38). However, previous exposure in Factory B had probably been higher due to a different production process. Symptoms of respiratory allergy in sensitized workers occurred in 13 % in Factory A and in 21 % in Factory B. Lung function and chest X-ray did not differ between the two factories.

Juniper et al (43) studied sensitization rates and lung function during five years in workers of an enzyme detergent producing factory. When the workers were categorised into exposure groups (classification method not reported) a greater proportion of "high exposed" workers were skin prick test positive to enzymes compared with lower exposed groups.

Liss and co-workers studied workers exposed to encapsulated enzyme (47). Exposure levels were below the TLV of 0.06 µg/m^3. Five out of 14 examined workers were sensitized. Six out of 14 exposed workers reported work-related respiratory symptoms, the same proportion was found in non-exposed. There was no abnormality in the spirometry of the exposed workers.

Brisman and Belin studied 20 α-amylase exposed workers during packing and mixing operations (16). Amylase levels of 30 µg/m^3 were measured during packing, but there were no detectable air borne enzyme during mixing. Eleven of the workers reported work-related symptoms, 3 of them rhinitis. There was no difference in spirometry between exposed and non-exposed. Of the workers,

30 % were sensitized to α-amylase.

11.2.2. Skin

Exposure defined as time between first enzyme-exposure and onset of contact urticaria was reported by Kanerva and Tarvainen (44). The time was 1.5-4 years for cellulase and xylanase.

The prevalence of skin symptoms at enzyme contact was reported to 26 % and 22 % in the two factories studied by Göthe (38).

12. Previous Evaluation by (Inter)National Bodies

Two previous evaluations are found in the literature. One is the above mentioned document by ACGIH (2), the other is from Australia (58).

13. Evaluation of Human Health Risks

13.1. Groups at extra risk

There is a number of studies focusing on whether atopics have a greater risk to become sensitized during enzyme exposure compared with non-atopics. Atopy is usually defined as having a history of atopy (childhood eczema, asthma, hay fever) and/or a positive prick test to one or more of common allergens. Studies in which sensitization rate is given in relation to atopic status are listed in Table 6. Thus, it is evident that both atopics and non-atopics can be sensitized to enzymes but atopy increases the risk for sensitization.

Table 6. Prevalence of positive skin tests to enzyme according to atopic status

Number of tested workers	Percent with positive test		Ref.
	Atopics	Non-atopics	
121	64	33	34
640	43	13	30
459	26	18	30
1614	24	13	30
56	28	11	37
1642	37	15	43
155	77	45	55
103	82	37	59
65	83	23	64

13.2. Assessment of health risks

There is a substantial body of studies showing that exposure to enzymes can cause sensitization with IgE antibodies. These antibodies can be demonstrated by skin prick test, RAST or similar in-vitro techniques (40, 12). Sensitization has been showed to occur against several industrially used enzymes, proteolytic and non-proteolytic and is regarded as the critical effect (62).

The clinical relevance of sensitization is not clear. Usually there are more workers showing signs of sensitization than of respiratory symptoms. In some cases sensitization may lead to overt illness as rhinitis or asthma. The proportion of clinically diseased persons among sensitized is not well known, but may be only a minority. Merget et al (53) examined 42 enzyme exposed workers with work-related respiratory symptoms. All were sensitized in skin prick tests, but only 13 (31 %), had a positive bronchial challenge test with the sensitizing enzyme.

Precipitins or specific IgG antibodies are inconsistently found in the sera of exposed persons but also in controls. The clinical relevance of these findings is unclear.

Emphysema occurs in laboratory animals after papain exposure, most probably due to a direct enzymatic action. In man, early findings of alveolitis, chest X-ray pathology and impaired lung function were essentially confined to pioneer works from around 1970, and have not, with some exception been repeated. This may be due to decreased exposure levels when health hazards became known. The findings may have been caused by direct proteolytic activity rather than sensitization.

The most prominent exception is the study on pancreatic dust exposed workers (78).However, it is not obvious that the findings were work-related and the exposure was complex and not confined to enzyme alone.

There is evidence that subtilisin exposure exceeding the TLV proposed by ACGIH (or its equivalent in GU) for six months causes sensitization in a majority of exposed workers but in most cases no work-related respiratory symptoms. Higher exposure and longer exposure-time causes work-related respiratory symptoms as well (76).

Exposure around the TLV proposed by ACGIH causes sensitization and work-related respiratory symptoms (37, 38). Even exposure at 13-50 % of the proposed TLV causes sensitization in 3.6-73 % of exposed workers (43, 47). The risk of work-related respiratory symptoms elicited by this exposure can not be estimated from the literature.

α-Amylase levels of 30 $\mu g/m^3$ (with a commercial enzyme preparation as standard) causes sensitization and respiratory symptoms (16). Lower levels (0.2-7.3 $\mu g/m^3$) probably also causes sensitization and respiratory symptoms in bakers (8, 42, 19).

In conclusion, the present body of knowledge does not permit the setting of a NOAEL (No Observable Adverse Effect Level) for any industrial enzyme. Sensitization has been observed at levels of about 0.012 µg subtilisin/m^3 and

0.2-7.3 µg α-amylase/m^3, with commercial enzyme prepartions as standards.

Skin irritation have been shown for proteolytic enzymes but patch tests in the sixties and seventies were negative. Recent case reports show positive patch tests to non-proteolytic enzymes (i.e. cellulase, amylase, xylanase). It is worth noticing that these persons also have positive prick tests to the same enzymes. This may indicate that the observed patch test reactions in fact are type I IgE-mediated allergic reactions and not necessarily the expected type IV reactions, at least in some cases. This may also indicate that these enzymes are causative agents in developing contact urticaria and that this urticaria may proceed into an allergic contact dermtitis.

14. Research Needs

The development of specific and sensitive air-measurement techniques for several industrial enzymes would be of considerable value. In order to assure a high sensitivity these assays should probably be immunochemical. More air measurements are needed, especially in occupational settings with none or only a few reported exposure studies.

Attention has to be paid to the problem of standards. If the enzyme content of a standard is not well known, comparisons to well-standardized enzyme preparations are necessary. The using of common standards should be encouraged as well as interlaboratory controls between laboratories employing enzyme analyses.

Only well defined symptoms should be reported in clinical studies. When performing skin tests it is important to use test extracts with an appropriate concentration in order not to cause false positive tests. Tests must also be performed in non-exposed controls.

In order to establish a NOAEL or LOAEL (Lowest Observable Adverse Effect Level) for an enzyme it is necessary to undertake clinical and immunological investigations together with exposure measurements in low exposed groups.

Are enzymes more prone to cause sensitization than other proteins because of their enzymatic capacity? The question is worth exploring since several common allergens e.g. mite, ragweed, Alternaria and cat most probably are enzymes (4, 45, 69,70). The role of a specific action of the enzymatic capacity, that is important for sensitization, could be studied in experimental systems.

15. Summary

Brisman J. 111. Industrial Enzymes. Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals. *Arbete och Hälsa* 1994;28:1-26.

The literature on adverse reactions caused by exposure to industrial enzymes is reviewed. The critical effect is sensitization and the forming of IgE-antibodies. This sensitization may cause clinical respiratory allergic disease such as rhinitis and asthma, or skin disease such as contact dermatitis or contact urticaria. Exposure levels can be measured by air-sampling and enzyme activity or immunological analysis. Experiences from enzyme production plants show that workplace hygienic measures are effective in reducing air-levels and sensitization rates. However, the pesent ACGIH TLV for subtilisins (the most common proteolytic detergent enzyme) of 0.06 µg/m^3 does not totally prevent the occurrence of sensitization.

Keywords: air-measurements, allergy, analysis, asthma, dermatitis, enzymes, exposure, rhinitis.

16. Summary in Swedish

Brisman J. 111. Industriella Enzymer. Nordiska Expertgruppen för Kriteriedokumentation av Kemiska Hälsorisker. *Arbete och Hälsa* 1994;28:1-26.

Genomgång av litteraturen om hälsoeffekter vid exponering för enzym. Den kritiska effekten bedöms vara sensibilisering med bildande av IgE-antikroppar. Sensibiliseringen kan orsaka klinisk allergisk sjukdom som rinit, astma, kontakt dermatit och kontakt urtikaria. Exponeringsnivåer kan mätas genom luftprovtagning och enzymaktivitets- eller immunokemisk analys. Erfarenheter från enzymproducerande fabriker visar att hygieniska åtgärder effektivt minskar luftnivåer och sensibiliseringsförekomst. Det av ACGIH föreskrivna gränsvärdet för subtilisiner (det vanligase proteolytiska tvättmedelsenzymet) på 0.06 µg/m^3 förhindrar dock inte helt förekomsten av sensibilisering.

Nyckelord: allergi, analys, astma, eksem, enzymer, exponering, luftmätningar, rinit.

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Appendix 1.

Permitted or recommended maximum levels of subtilisins in air.

Country	ppm	mg/m^3	Comments	Year	Ref.
Denmark	-	-		1988	1
Finland	-	-		1993	2
Iceland	-	1 GU/m^3 3 GU/m^3	 CEIL	1989	3
Netherlands		0.00006	CEIL	1994	4
Norway	-	0.00006	CEIL	1989	5
Sweden	-	1 GU/m^3 3 GU/m^3	S CEIL	1993	6
USA (ACGIH)	-	0.00006	CEIL	1991-92	7
(NIOSH)	-	0.00006	60 min	1990-91	8

CEIL: ceiling limit value
GU: glycine unite. One GU corresponds to an activity which releases as many amino groups as are present in 1 mg glycine (under standard conditions)
S: the substance is sensitizing

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CRITERIA DOCUMENTS FROM THE NORDIC EXPERT GROUP

The Criteria Documents are in a Scandinavian language, with summary in English. Those marked with * are in English. Those marked with D are published in collaboration with the Dutch Expert Committee for Occupational Standards (DECOS). Those marked with N are published in collaboration with NIOSH, USA.

Acetaldehyde	Arbete och Hälsa 1986:25
Acetone	Arbete och Hälsa 1986:39
Acetonitrile	Arbete och Hälsa 1989:22, 1989:37*
Acrolein	Arbete och Hälsa 1991:45
Acrylates	Arbete och Hälsa 1983:21
Acrylonitrile	Arbete och Hälsa 1985:4
Allyl alcohol	Arbete och Hälsa 1986:8
Aluminium	Arbete och Hälsa 1992:45, 1993:1*
Ammonia	Arbete och Hälsa 1986:31
Arsenic, inorganic	Arbete och Hälsa 1981:22, 1991:9, 1991:50*
Arsine	Arbete och Hälsa 1986:41
Asbestos	Arbete och Hälsa 1982:29
Benomyl	Arbete och Hälsa 1984:28
Benzene	Arbete och Hälsa 1981:11
Boric acid, Borax	Arbete och Hälsa 1980:13
1-Butanol	Arbete och Hälsa 1980:20
Cadmium	Arbete och Hälsa 1981:29, 1992:26, 1993:1*
7/8 Carbon chain aliphatic monoketones	Arbete och Hälsa 1990:2*D
Carbon monoxide	Arbete och Hälsa 1980:8
Chlorine, Chlorine dioxide	Arbete och Hälsa 1980:6
Chloromequat chloride	Arbete och Hälsa 1984:36
4-Chloro-2-methylphenoxy acetic acid	Arbete och Hälsa 1981:14
Chlorophenols	Arbete och Hälsa 1984:46
Chromium	Arbete och Hälsa 1979:33
Cobalt	Arbete och Hälsa 1982:16
Copper	Arbete och Hälsa 1980:21
Creosote	Arbete och Hälsa 1988:13, 1988:33*
Cyclohexanone, Cyclopentanone	Arbete och Hälsa 1985:42
n-Decane	Arbete och Hälsa 1987:25, 1987:40*
Deodorized kerosene	Arbete och Hälsa 1985:24
Diacetone alcohol	Arbete och Hälsa 1989:4, 1989:37*
Diesel exhaust	Arbete och Hälsa 1993:34, 1993:35*
2-Diethylaminoethanol	Arbete och Hälsa 1994:25*N
Diethylamine, Diethylenetriamine, Dimethylamine & Ethylenediamine	Arbete och Hälsa 1994:23*
Diisocyanates	Arbete och Hälsa 1979:34, 1985:19
Dimethyldithiocarbamates	Arbete och Hälsa 1990:26, 1991:2*
Dimethylethylamine	Arbete och Hälsa 1991:26, 1991:50*
Dimethylformamide	Arbete och Hälsa 1982:28
Dimethylsulfoxide	Arbete och Hälsa 1991:37, 1991:50*
Dioxane	Arbete och Hälsa 1982:6
Epichlorohydrin	Arbete och Hälsa 1981:10
Ethyl acetate	Arbete och Hälsa 1990:35*D
Ethylbenzene	Arbete och Hälsa 1986:19
Ethylene bisdithiocarbamates	Arbete och Hälsa 1993:24, 1993:35*
Ethylenediamine	Arbete och Hälsa 1994:23*
Ethylene glycol	Arbete och Hälsa 1980:14