



Content

Prevention of Enzyme Allergy in the Manufacturing of Detergent Enzymes

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Cancun, March 2012

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- Novozymes and a little bit of history
- Enzymes and exposure
- Occurrence of enzyme allergy at Novozymes
- Prevention of enzyme allergy
- Recent observations
- Moving forward

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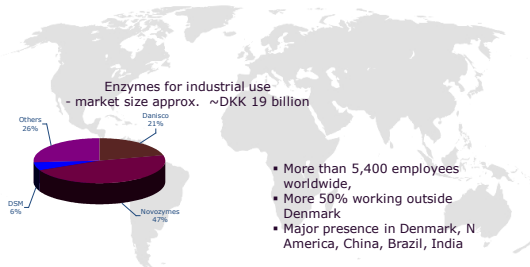
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NOVOZYMES

Novozymes in brief



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Source: Novozymes 2010 estimates

Novozymes operates within nine different industries



History of Novozymes - and enzyme allergy

History overall

- 1925 **Novo** founded: Extracting insulin from the animal pancreas
- 1941 Novo's launches Trypsin, extracted from the pancreas, used for cleaning hides prior to tanning
- 1952 Termozym® the first microbial enzyme, followed by Aquazym® in 1954. Both are used in the textile industry to remove starch from fabrics
- 1963 Alcalase®, the first detergent enzyme produced by fermentation
- 1987 Lipolase®, the first fat-splitting enzyme for detergents manufactured with genetically engineered microorganisms
- 2000 **Novozymes** founded as a result of the split of Novo into a pharmaceutical company and a bioindustrial.
- Additional enzymes and applications and other biological solutions

And the medical angle

- 1969 The Lancet publication by Flindt on allergy / asthma related to exposure to enzymatic detergents.
- Drop in sales and a reduction in Novo's workforce from 2,100 to 1,700
- The US health authorities: Detergent enzymes are safe to use. In 1972 up again
- 1972 Novo published own observations on sensitization and asthma (Acta Allergologica)
- Since then a huge amount of case stories and epidemiological reports showing that most enzymes from bacteria and fungi are allergenic
- Formulation of products into not dusty granulates a major step forward in safety
- Occupational allergy to enzymes established as a classical occupational disease
- - and still challenge in various industries and trades
- Additional Novozymes publications in 1997 and 2007

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A picture of Lipolasis



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Stages in manufacturing of enzymes

- 1) R&D and pilot
- 2) Production itself
 - Fermentation
 - Recovery
 - Formulation
 - Tapping
 - Quality control

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Laboratories

Steps

- 1) R&D
- 2) Production itself
 - Fermentation
 - Recovery
 - Formulation
 - Tapping
- Quality control

Processes and procedures

- Screening of microorganisms for useful metabolites (enzymes) and genes
- Transferring genes into well known non pathogenic microorganisms (bacteria and fungi)
- Developing new formulations and applications for enzymes
- A number of methods to measure enzymes and other components of the product

Risk factors for enzyme allergy

- Spills and aerosols generated through classical laboratory procedures
- Cleaning
- Tiny exposures still relevant for the vulnerable person

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Top of fermentation tank 80 m3



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Fermentation

Steps

- 1) R&D and pilot
- 2) Production itself
 - Fermentation
 - Recovery
 - Formulation
 - Tapping
- Quality control

Processes and procedures

- Inoculation of tanks filled with substrate
- Monitoring the fermentation process (pH etc.)
- Harvesting the output (ferment)

Risk factors for enzyme allergy

- Taking and handling of samples
- Cleaning

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Recovery

Steps

- 1) R&D and pilot
- 2) Production itself
 - Fermentation
 - Recovery
 - Formulation
 - Tapping
- Quality control

Processes and procedures

- Separating and concentrating the enzyme from the ferment (filtering, centrifugation, evaporation etc.)

Risk factors for enzyme allergy

- Exposure to aerosols and spills due to leaking equipment or accidents
- Sampling
- Cleaning

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Granulates



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Formulation

Steps

- 1) R&D and pilot
- 2) Production itself
 - Fermentation
 - Recovery
- Formulation
 - Tapping
 - Quality control

Processes and procedures

- Drying, granulating and encapsulating the enzymes

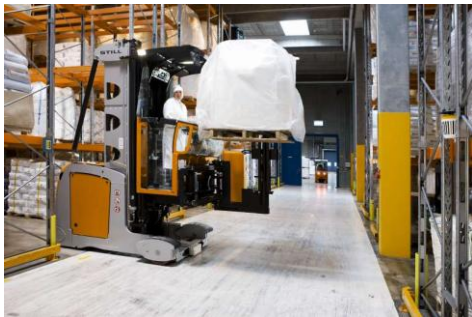
Risk factors for enzyme allergy

- Exposure to powder including spills in case of leaking equipment or accidents
- Sampling
- Cleaning

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Handling of finished enzymes



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Tapping

Steps

- 1) R&D and pilot
- 2) Production itself
 - Fermentation
 - Recovery
- Formulation
- Tapping
 - Quality control

Processes and procedures

- Transferring enzymes between containers (tanks, bags etc.)
- Standardizing the product

Risk factors for enzyme allergy

- Exposure to powder or aerosols including spills in case of leaking equipment or accidents and procedures violating the granulate
- Sampling
- Cleaning

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OCCURRENCE OF ENZYME ALLERGY AND SENSITIZATION AT NOVOZYMES

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Enzyme allergy versus sensitization



Sensitization

- Antibodies (IgE) to enzymes relevant for the exposure detected directly through serum test (CAP) or indirectly through skin test
- Sensitization is *not* a disease
 - but evidence of exposure
 - and a raised **flag** of possible elevated allergy risk
- Rate or incidence (e.g. yearly) of sensitization measured
OS = [First time sensitized persons](#)
Tested persons never sensitized before

Allergy

- Respiratory or mucous symptoms timely related to exposures to enzymes and associated with a sensitization
- Grades in terms of dissemination:
 - Rhinitis (+/- conjunctivitis)
 - Asthma
- ... in terms of severity:
 - Light / transient / exposure associated
 - Severe / persistent
- *OBS: In accordance with a Danish tradition we have a relatively inclusive definition of occupational diseases to protect individuals' right*

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Research together with Danish Universities and clinics of occupational medicine

Occupational and Environmental Medicine 1997;54:671-675 671

Allergy risk in an enzyme producing plant: a retrospective follow up study

Claus R Johnsen, Torben B Sorensen, Anders Ingemann Larsen, Anne Bertelsen Secher, Erling Andreassen, Gertrud S Kosfeld, Lise Fredlund Nielsen, Finn Gyntelberg

ORIGINAL ARTICLE

Incidence of respiratory sensitisation and allergy to enzymes among employees in an enzyme producing plant and the relation to exposure and host factors

A I Larsen, C R Johnsen, J Frickmann, S Mikkelsen

Occup Environ Med 2007;66:1-7. doi: 10.1136/sem.2005.02304

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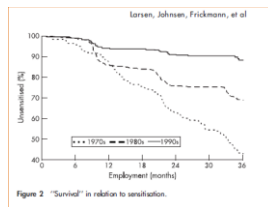
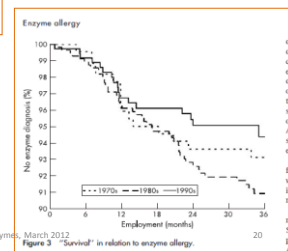


Figure 2 "Survival" in relation to sensitization.

In the nineties 10 % sensitized after three years and 5 % allergic

"Surviving" with enzymes.... Observed sensitizations and allergies by decade



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Figure 3 "Survival" in relation to enzyme allergy.

Summary of results

Enzyme allergy still an issue!

Determinant	Sensitization	Allergy	Comment
Decade of employment	Reduced risk across decades	No association	Decade a proxy for exposure? Changed criteria for disease over time?
Predefined exposure indices	No association	No association	Poor exposure measures (misclassification)
Smoking	Increases risk	Increases risk	Plausible
Preemployment atopy	Increases risk	No association	Consistent with other reports

RESULTS: During the first three years of a person's employment, the enzyme sensitization and allergy incidence rates were 0.13 and 0.03 per person-year at risk, respectively. In the fitted models, exposure class did not correlate with the outcome variables. The risk of sensitization decreased along the three decades, whereas the risk of allergy remained unchanged. The risk of sensitization and allergy was doubled among smokers. Pre-employment atopy was only associated with sensitization risk.

CONCLUSION: Sensitization to enzymes decreased during the study period, possibly reflecting improvements in the working environment. A similar decrease could not be demonstrated for allergy to enzymes. Neither of the two outcomes correlated with exposure estimates, possibly because of the low precision of the estimates.

ZEAL – THE TURN AROUND IN 2004-5

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Overall approach in ZEAL

We understand and acknowledge

- That every case of enzyme allergy conflicts with our social responsibility
- That an uncontrolled epidemic of allergy is a threat for the business

Our vision in 2005

- By being ZEAL (= determined)
- we go for ZEAL ZERO ENZYME ALLERGIES

Our strategy

- Most important: To internalize the vision en every corner of the company; to "talk" and "walk the talk"
- More technical elements – explained in Novozymes "standards", e.g.
 - Exposure control: Concerning product and process design, work procedures, personal employee protection.
 - Procedures > Engineering.
 - Monitoring of dust and other indicators for exposure.
 - Monitoring of sensitizations and doing root cause analyses
 - Surveillance of allergies and clinical handling of cases.

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Elements of ZEAL medicals

At pre-employment

- **Baseline allergy status:** History, pulmonary function, blood sample for the bank
- **Advice not to work with enzymes in case of asthma**
- **Information on allergy and prevention**

Ad hoc

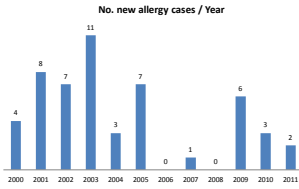
- Due to outcome of screening (sensitization or may be symptoms) or initiated by employee between terms
- Diagnostic tests depending of the problem
- Advise related to prevention, work situation or treatment

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NZ Denmark: Allergies

(conjunctivitis, rhinitis, asthma)



"Average"
• 2000-11: 52/12 ~ 4-5 per year

• 2000-2005: 40/6 ~ 6-7 per year

• 2006-2011: 12/6 ~ 2 per year

* Allergy eradicated in labs?
* And soon in Pilot og warehouse / tap?
* Also decreasing trend in production?

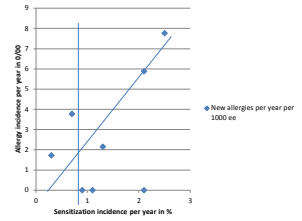
Area	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	I total
Production		1		10	1	2			1		6	2	25
Pilot	2	1	2			1						1	7
Warehouse and tapping		3	3		1	1							8
Qlab	1	2	1	1	1	3							9
R&D lab	1	1	1										3
Total	4	8	7	11	3	7	0	1	0	6	3	2	52

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DK 2007-11: Allergies vs. sensitizations

Incidence of allergy vs. sensitization over 5 year

"Function"	Sensitization rate: %		Allergies No.
	2011	2007-11	
R&D lab	0.8	0.9	0
Qlab	2.2	1.1	0
Ware house / tapping	2.2	2.1	0
Pilot	0	0.3	1
Fermentation	0	0.7	1
Recovery	0.7	2.1	5
Granulation	1.4	2.5	4
Maintenance	2.5	1.3	1



Sensitization Incidence in % per year	Allergy risk in 0/00 per year
<1	0.7
>1	3.2

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"Must do's" for preventing enzyme allergy



- Proactively: Constantly aim to control exposure (peaks, visible dust, spills, measurements < 60 ng enzyme protein/m³)
- Retrospectively correct any deviations from norms – e.g.: A spill is always a spill
- Keep level of new sensitizations low, probably around 1 %
- Rapid and offensive caretaking in case of allergy symptoms
- A company culture where management and employees are committed to walk the talk

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A lot of challenges, such as



- Prioritizing resources for prevention when resources are scarce
- Dust measurements
 - The true TLV and dose-response curve?
 - Valid methods for sampling and analyses
 - The sampling program – keeping it cost-effectively
- Employee attitudes and behavior
 - Keeping the spirit – new ways to keep on track: Cleaning, using masks etc.
 - Willingness to take the yearly test

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Medical challenges



- Better understanding of sensitization in relation to subsequent allergy development
- Understand the dose-response relations
- Having credible and trustworthy alternatives for symptomatic individuals ensuring that they do not ignore symptoms

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Thanks for your attention

And thanks to colleagues at Novozymes

- Stine Fangel
- Birger Stjernholm Madsen
- June Christensen
- Anne Mette Lykke

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