

# Overview of BIODEPRO EU Project and Prion Inactivation in the Biodiesel Process

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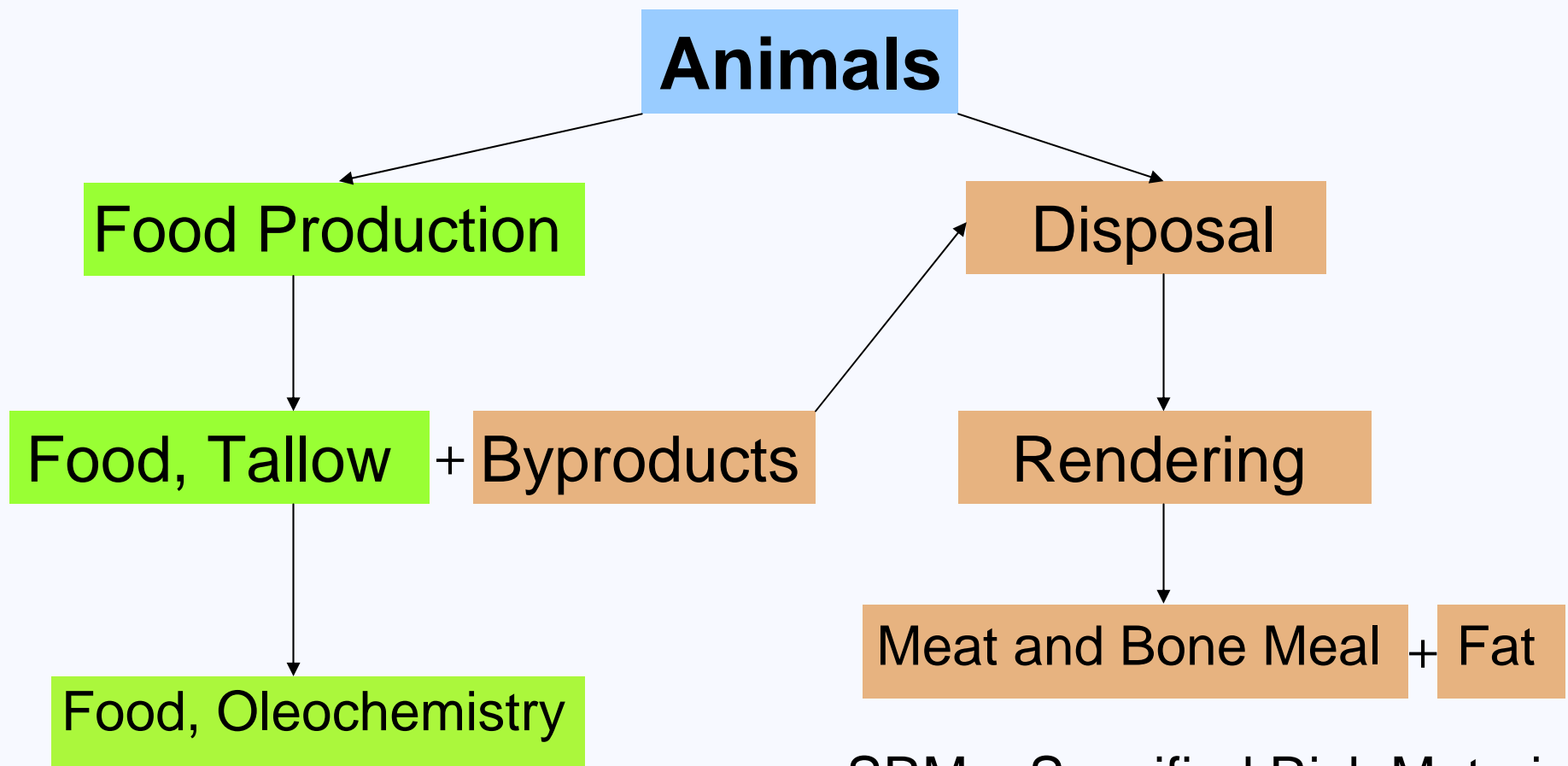
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*Heinrich-Heine University Düsseldorf*  
*Germany*



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# Animal Byproducts as Feedstock



SRM – Specified Risk Material



# Extracted Fat from Meat and Bone Meal

Parameter	Unit	Value
Sulfur	[mg/kg]	316
Nitrogen	[wt-%]	0.3
Phosphorus	[mg/kg]	854
Water	[mg/kg]	185
Free fatty acids	[wt-%]	11
Unsaponifiabiles	[wt-%]	1.97
Total fatty acids	[wt-%]	97



# Biodiesel from MBM Extraction Fat

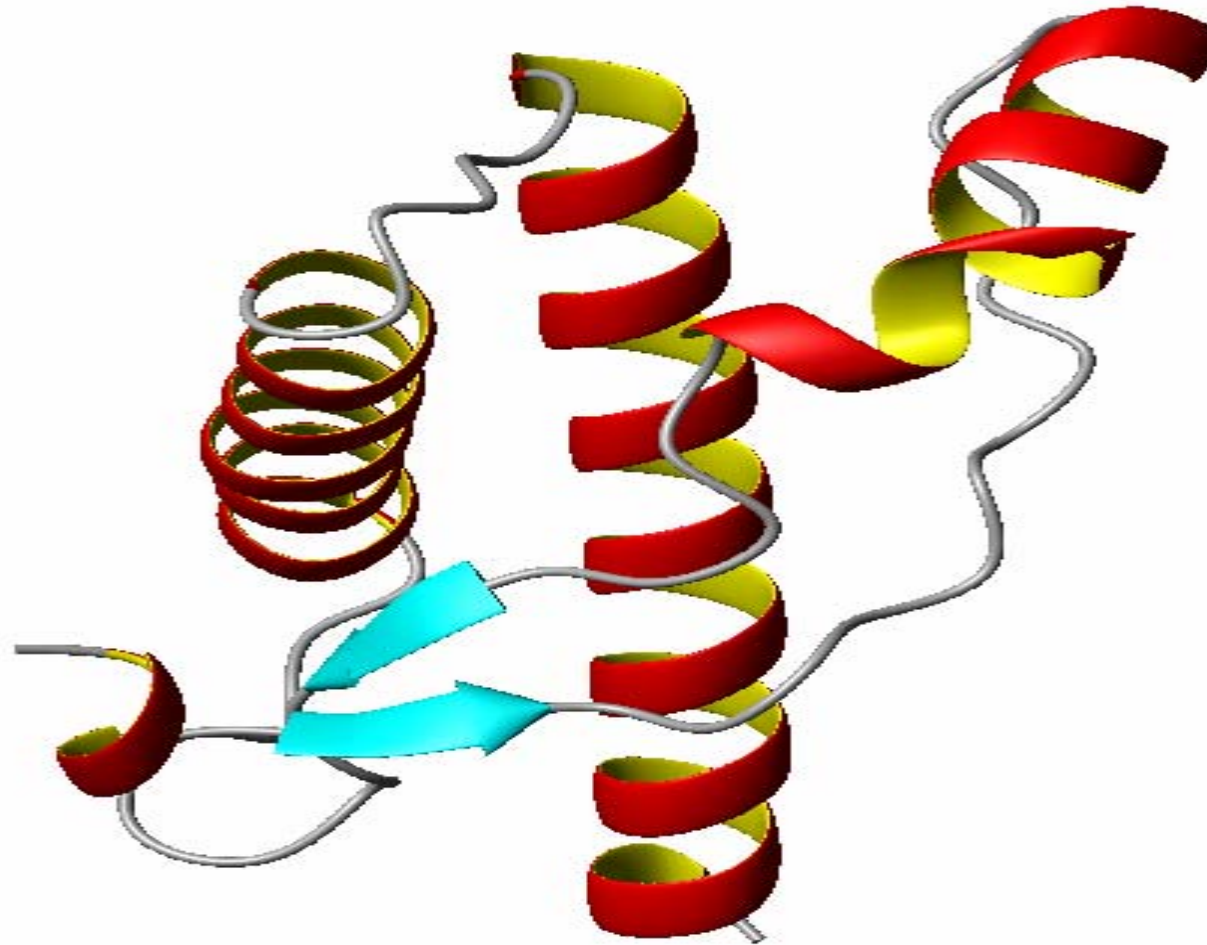
Parameter	Method	Unit	Value	EN 14214
Ester content	EN 14103	[wt-%]	97.4	≥ 96.5
Density at 15°C	ISO 3675	[kg/m <sup>3</sup> ]	875	860-900
Viscosity at 40 °C	ISO 3104	[mm <sup>2</sup> /s]	4.7	3.5-5.0
Flashpoint	DIN EN 22719	[°C]	161	≥ 120
Sulfur content	EN ISO 20846	[mg/kg]	8	≤ 10.0
CFPP	EN 116	[°C]	14	+5/-20
Water content	EN ISO 12937	[mg/kg]	140	≤ 500
Acid value	EN 14104	[mg KOH/g]	0.26	≥ 0.5
Oxidation stability at 110 °C	EN 14112	[h]	5.21	≥ 6.0
Monoglyceride content	EN 14105	[wt-%]	< 0.01	≤ 0.8
Diglyceride content	EN 14105	[wt-%]	< 0.01	≤ 0.2
Triglyceride content	EN 14105	[wt-%]	< 0.001	≤ 0.2
Free glycerol	EN 14105	[wt-%]	0.058	≤ 0.02
Total glycerol	EN 14105	[wt-%]	< 0.5	≤ 0.25
Phosphorous content	EN 14107	[mg/kg]	< 1	≤ 10.0
Group I metals : Na	EN 14108	[mg/kg]	< 0.5	≤ 5.0
Group I metals : K	EN 14109	[mg/kg]	< 0.5	≤ 5.0
Group II metals : (Ca+Mg)	EN 14538	[mg/kg]	< 0.5	≤ 5.0



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# Prion Protein



# Prion Protein Deactivation

## 2 Strategies

### Bioassay:

- + Tests with animals
- + Absolute results
- Time consuming
- High number of animals
- Injection of biodiesel in brain ??

### Analysis of prions:

- + Separation; antibody reaction
- + In vitro, no animals necessary
- low sensitivity of analysis
- no test for infectivity



# BIODIEPRO

## „Demonstration of the Production of Biodiesel from Tallow and Recovered Vegetable Oil (RVO)“

“Energy, Environment and Sustainable Development – Energy Program”

**Contract No.:** NNE5/2001/00832

**Coordinator:** ARGENT Energy Ltd., London

**Partner:** Institute for Chemistry, KF Uni Graz  
Institute for Biotechnology, TU Graz  
Institute for Resource Efficient and Sustainable Systems,  
TU Graz

**General plant construction and plant engineering:**

Biodiesel International (BDI), Graz, Austria

**Duration:** 1.1.2003 - 31.12.2005

**Overall costs:** € 17.438.593.-

**EU-funding:** € 3.150.000.-



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## Aim of WP 3, R&D

- Investigation whether prion proteins are inactivated and/or destroyed by the Biodiesel-production process
- Spike experiments with model proteins (ApoA<sub>1</sub>) as well as infectious prion proteins
- New applications for FAME and by-products (glycerol, solids)

# Work Packages

- WP 1 – Plant Design & Construction
- WP 2 – Commissioning & Testing
- WP 3 – Research & Development (IFC, IBTUG)
- WP 4 – Analysis & Evaluation (RNSTUG)
- WP 5 – Dissemination Activities
- WP 6 – Project Management Activities



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# Safety Evaluation for a Biodiesel Process Using Prion-Contaminated Animal Fat as a Source

*B.Seidel, M.Alm, R.Peters, W.Kördel, A.Schäfer  
Environ.Sci.&Pollut.Res. 13 (2) 2006*

## ***Experimental Setup:***

- Spiking of fat and biodiesel with Scrapie-infected hamster brain
- Performing of biodiesel production process
- Estimation of degradation via SDS-Gel and Western Blot



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# Safety Evaluation for a Biodiesel Process Using Prion-Contaminated Animal Fat as a Source

*B.Seidel, M.Alm, R.Peters, W.Kördel, A.Schäfer  
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## **Results:**

- Each production step: degradation approx.  $10^4$  ID<sub>50</sub>
- Whole Biodiesel production process:  $10^{12} - 10^{16}$  ID<sub>50</sub>
- Whole process including sterilisation:  $10^{20}$  ID<sub>50</sub>



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# Experimental Design - BIODIEPRO

## Model Protein:

Apolipoprotein A<sub>1</sub> was used for preliminary studies and for the statistical calculation of the results

## Prion Spike Experiments:

In respect of the security rules all prion spike experiments were done at a S2+ - Laboratory

# Prion Spike Experiments

**Location:** Institute of Physical Biology,  
Heinrich Heine University Duesseldorf  
Head: Prof. D. Riesner

**Safety Precautions:**

Biochemistry Laboratory S2+

**Used Spike Material:**

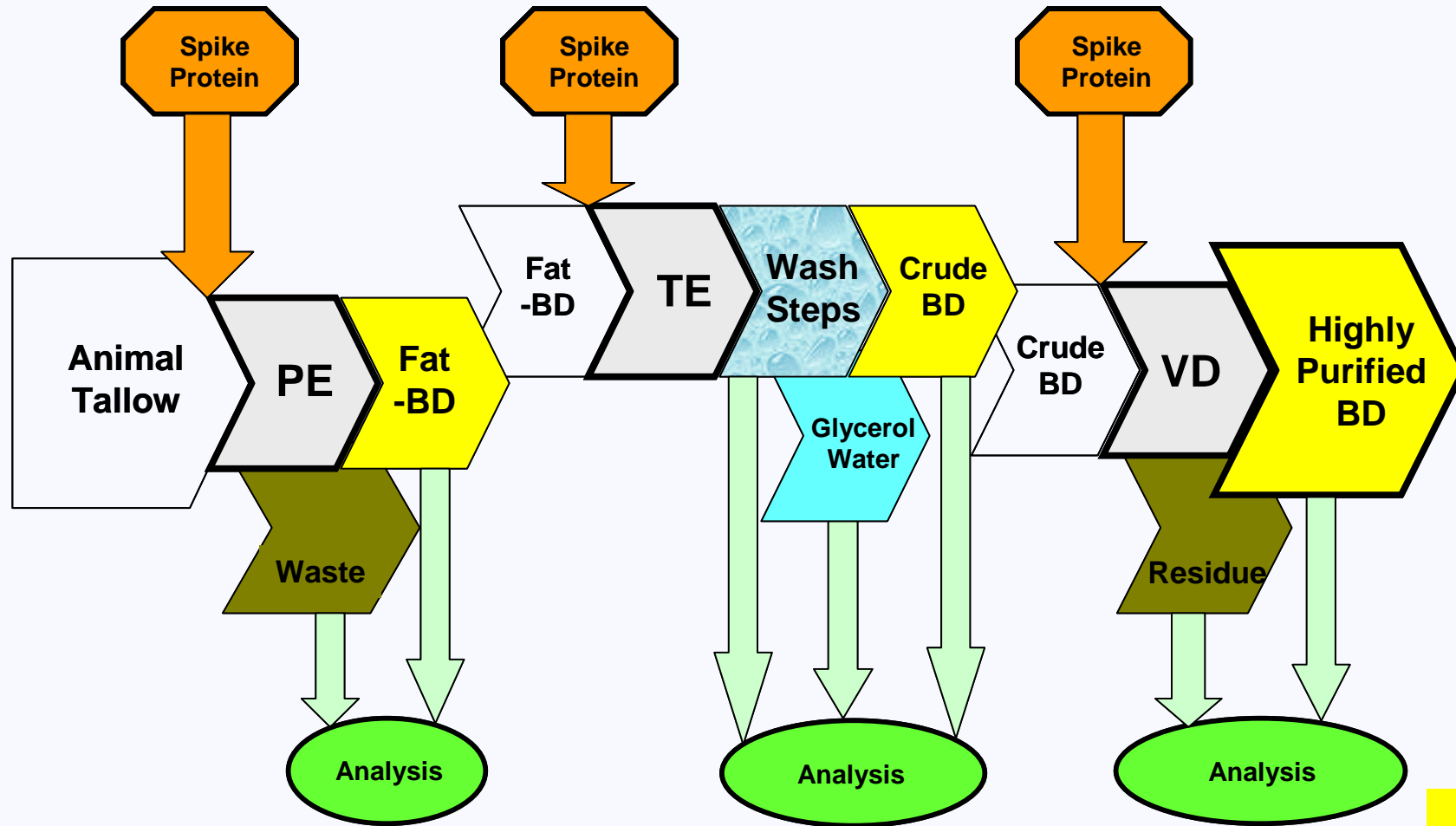
„Prion Rods“ 263K (Prep. out of Syrian Golden  
Hamster Brain)



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# Spike Experiments

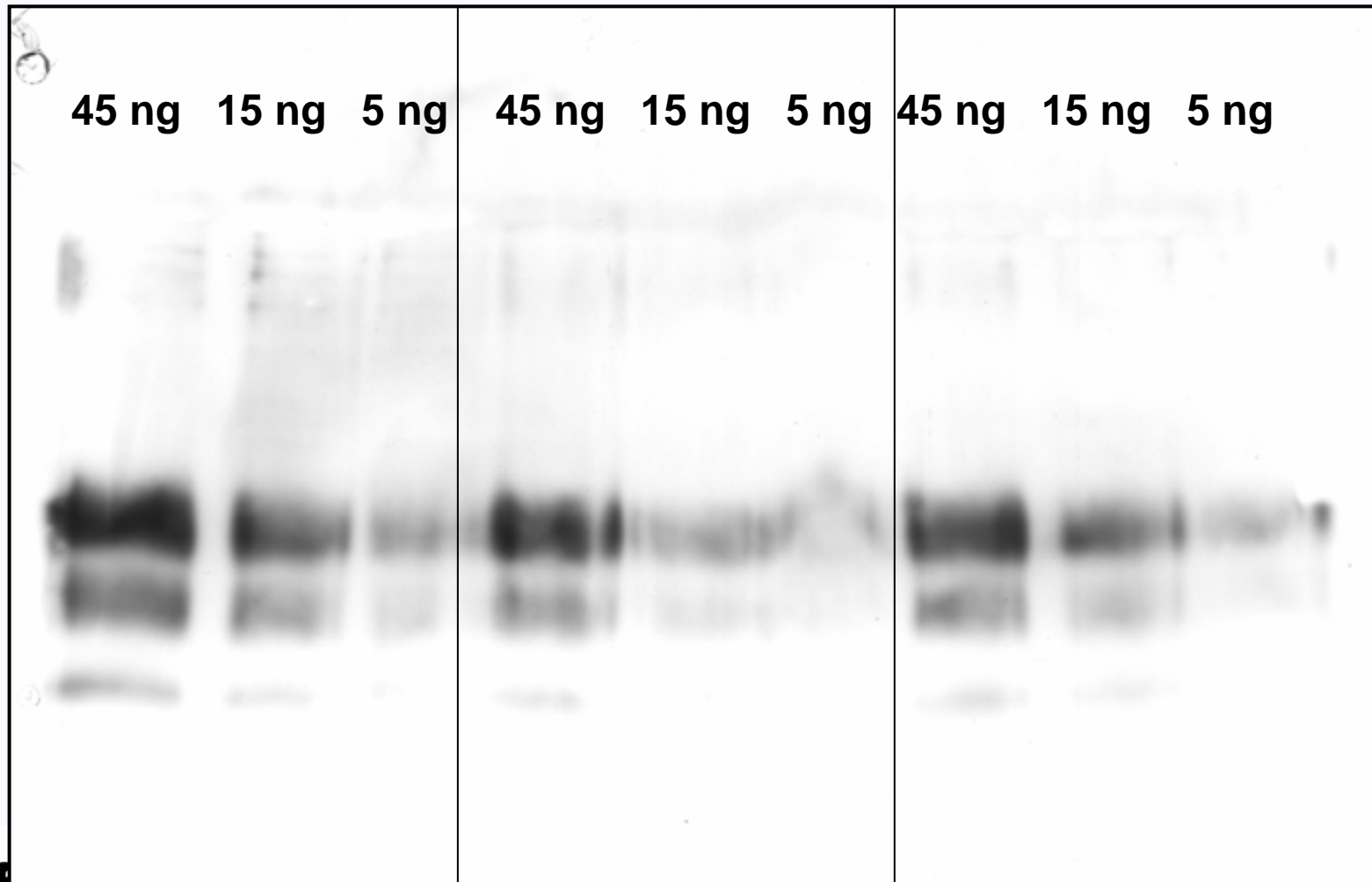


# SDS Gel Electrophoresis & Western Blot Working - Laminar Flow Bench

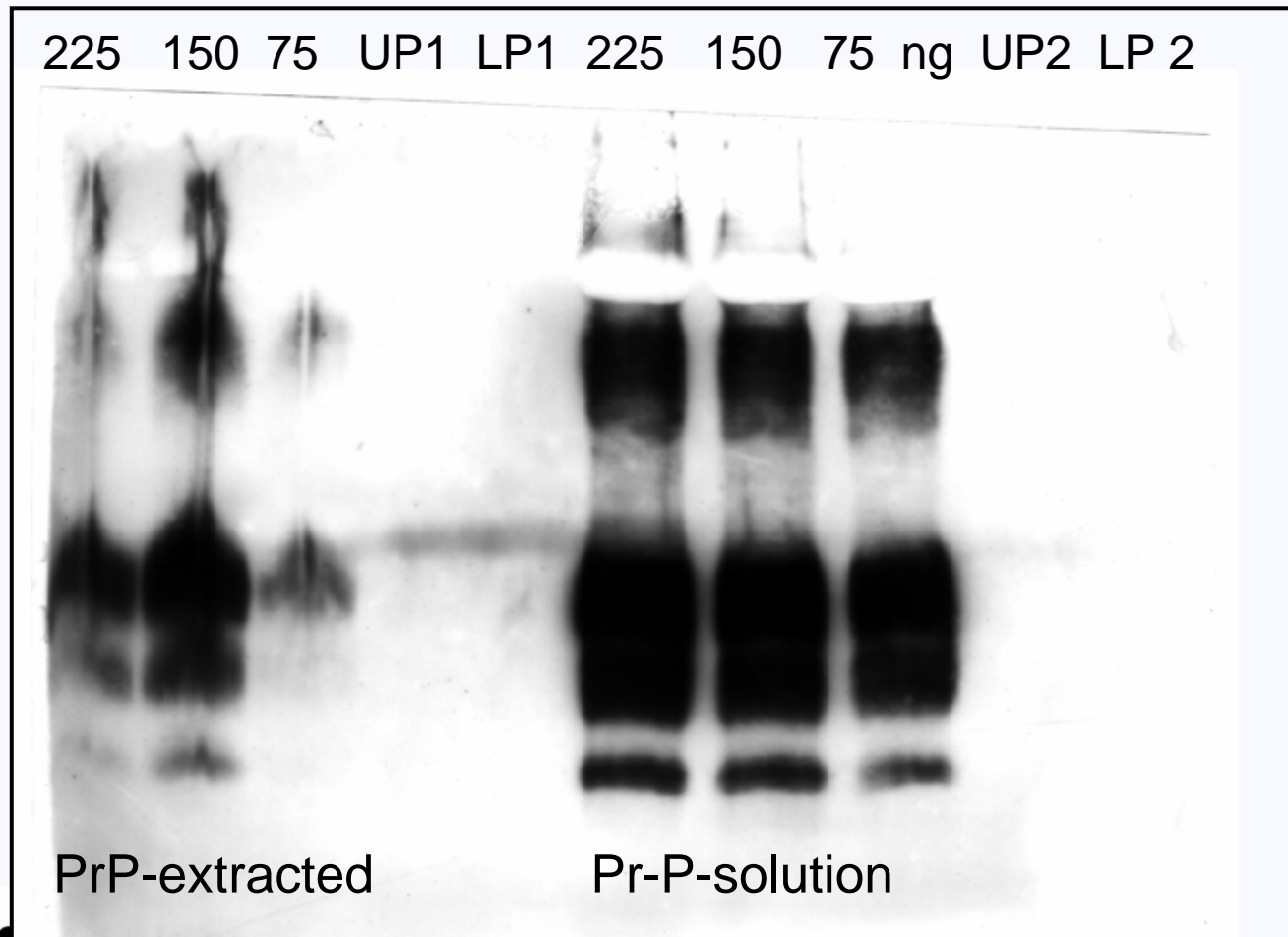


# Recovery Rate of $\text{CHCl}_3/\text{MeOH}$ - Extraction

PrP solution    Extracted from glycerol    Extracted from tallow



# SDS PAGE & Western Blot Pre-Esterification



UP: Upper Phase  
LP: Lower Phase

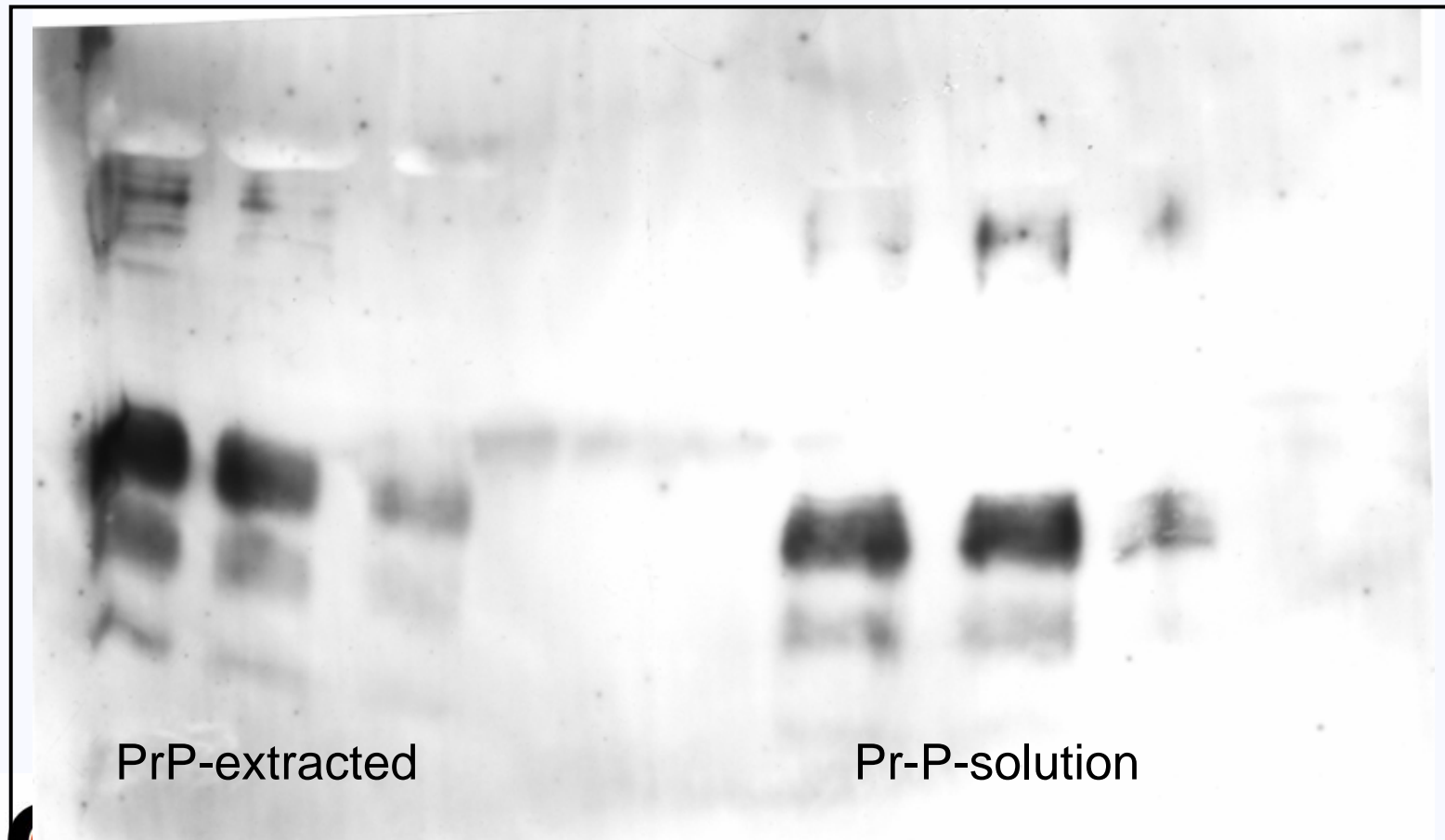


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# SDS PAGE & Western Blot Transesterification 1 & 2

300 150 75 ng UP1 LP1      300 150 75 ng UP2 LP2



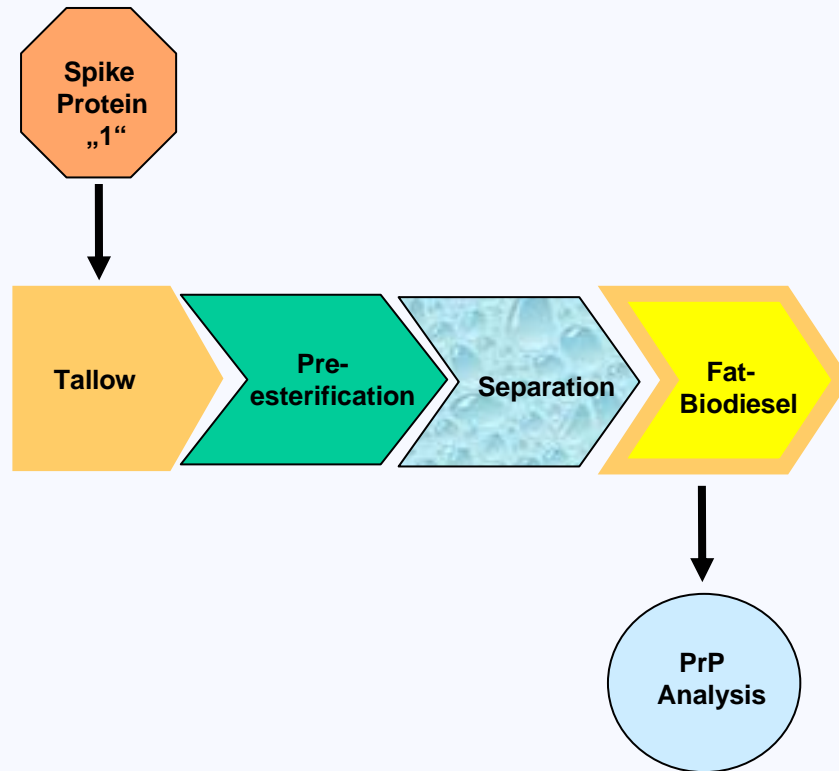
# SDS PAGE & Western Blot Distillation

300 200 100 ng R1 BD1 300 150 75 ng R2 BD2



# Biodiesel-Conversion Step 1

## Preesterification



### Conditions:

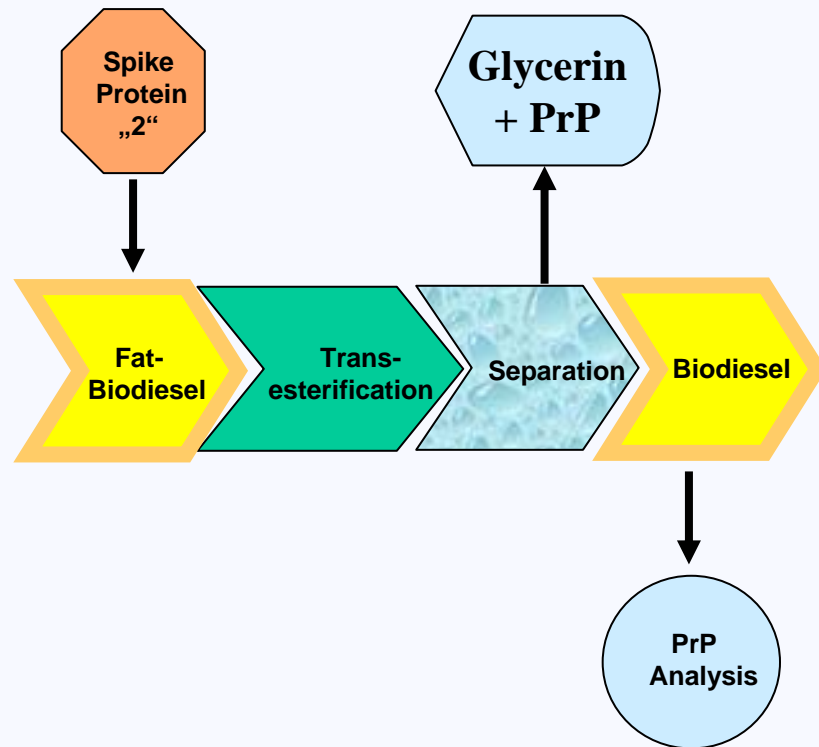
pH~1, 2 hours, 70°C

- Edible Tallow: 50 g
- Spike: 1000 ng PrP
- Det. Limit: 10 ng
- Degradation Factor:  
~ 10<sup>2</sup>

**„No PrP - only  
unidentified protein traces  
detectable“**

# Biodiesel-Conversion Step 2

## Transesterification

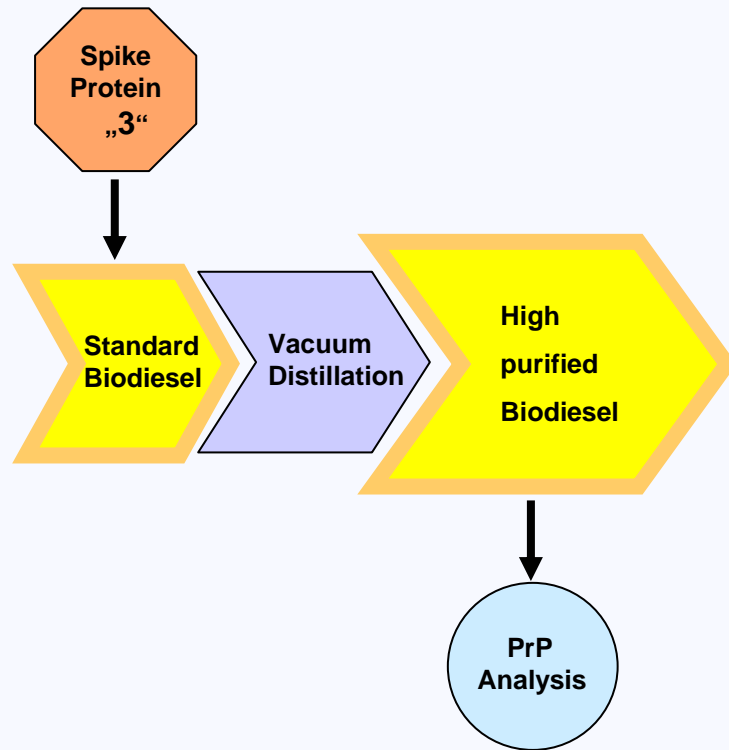


### Conditions:

pH~14, 20 min. 50°C

- Oil/Ester-Mix: 50 g
- Spike: 2500 ng PrP
- Det. Limit: 10 ng
- Degradation Factor:  
~  $2,5 \cdot 10^2$  for each step  
**„No PrP - detectable“**

# Biodiesel-Conversion Step 3 Distillation



- **Conditions:**  
Vacuum (<1 mbar),  
~150°C, 35 min.
- Standard-Biodiesel: 60g  
Spike: **10.000 ng PrP**
- Det. Limit: 10 ng
- Theo. Degr. Factor: 10<sup>3</sup>  
**„No PrP detectable“**

# Degradation Factors

Exp. Step	Apo A1-Spike	PrP-Spike
Pre-Est. – Oil/Ester	$3.4 \cdot 10^4$	$10^2$
Pre-Est. – Water/Acid	-	$10^2$
Trans-Est. – Biodiesel	$3.4 \cdot 10^4$	$2.5 \cdot 10^2$
Trans-Est. – Glycerol	$3.4 \cdot 10^4$	$2.5 \cdot 10^2$
Merged Wash Phases	$3.4 \cdot 10^4$	$10^2$
Distillation – Residue	$3.4 \cdot 10^4$	$10^2$
Distillation – Biodiesel	$3.4 \cdot 10^4$	$10^3$

## Risk Assessment (Worst Case)

Risk Factors	Risk Values
Dose of BSE-infected tissue contaminating animal fat [g]	$1.5 \times 10^{-3}$
Prion infectivity of the contaminating tissue [ $ID_{50}/g$ ]	$2.0 \times 10^5$
Prevalence of BSE infection	$2.6 \times 10^{-4}$
Pre-esterification	$1.0 \times 10^{-2}$
Transesterification	$2.5 \times 10^{-2}$ ; $2.5 \times 10^{-2}$
Distillation	$1.0 \times 10^{-3}$
Rel. efficiency by route of admin.	$1.0 \times 10^{-4}$
Dose of administration [100 g]	$10^2$
<b>Final exposure [<math>ID_{50}</math> units/p.a.]</b>	<b><math>5.0 \times 10^{-12}</math></b>

# Results

Detection limit	10 ng prion rods
Recovery of extraction	65 – 85 %
Esterification step	no prions det.
Transesterification	no prions det. (unident. traces)
Glycerol phase	no prions det. (unident. traces)
Distillation	no prions det.

**Total Degradation**  $> 10^9$

**Risk Assessment**  $5.0 \times 10^{-12}$  [ID50/p.a.]

**Final Risk:**  $2.5 \times 10^{-12}$

**Background Risk:**  $1.0 \times 10^{-6}$



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# European Food Safety Authority (EFSA)

The Scientific Panel on Biological Hazards concludes that the Biodiesel process as described (BDI) is considered as safe for treatment and use of ABP of category 1



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**Biodiesel Plant at Motherwell, Scotland**

**Capacity: 50.000 t/a**

**Raw material: Recycled Cooking Oil, Tallow, SRM-Material**

**Initial Operation: January 2005**

**Construction: Biodiesel International (BDI), Graz, Austria**



