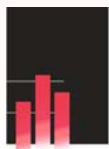


Uncertainty in determination of airborne ENDOTOXIN

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Conclusions

- 1. Determination of endotoxin by a single lab is repeatable**
- 2. However, reproducibility between labs is very poor. Large differences are found between different laboratories**

Why is endotoxin that interesting?

- ❖ **Bioaerosol component with dose-response data of respiratory effects**
 - Cross-sectional surveys
 - Experimental studies
- ❖ **Netherlands Health Council proposed HBR-OEL of 50 EU/m³. Netherlands accepted a OEL of 200 EU/m³ in 2002, but the OEL was withdrawn in 2003.**
 - No support of employers
 - Reproducibility of the endotoxin assay is poor



Method for measurement of airborne endotoxine

- ❖ **European standard EN 14031**
 - **Sampling of inhalable dust**
 - **Extraction of endotoxin from the dust at the filter**
 - **Determination of endotoxine in extract with the kinetic chromogene LAL-assay**
 - ◆ **LAL-assay = color-reaction by activation of a enzym in the lysate of blood of Limulus (= crab)**
 - ◆ **Comparison of the activity to standard endotoxine => activity of sample in EU/m³**

Reason for study of the uncertainty of the method

- 1. Negative experience of composting facilities**
 - Very large difference in airborne concentrations of endotoxine between facilities
 - Large differences from year to year
 - Differences remained after introduction of draft standard NEN-EN 14031 in 2000

- 2. Reports in literature on differences**
 - Interaction by unknown compounds in dust
 - Assay of endotoxine is very sensitive to small differences in lab procedures (sampling, extraction, storage and analysis)

Question of waste composting facilities

Does the the method to measure airborne endotoxines present true values?

In other words, what is the:

- ◆ Reproducibility (bias between laboratories, systematic error)
- ◆ Repeatability within laboratories



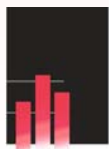
Aim and design of study

Aim: assessment of uncertainty

That means:

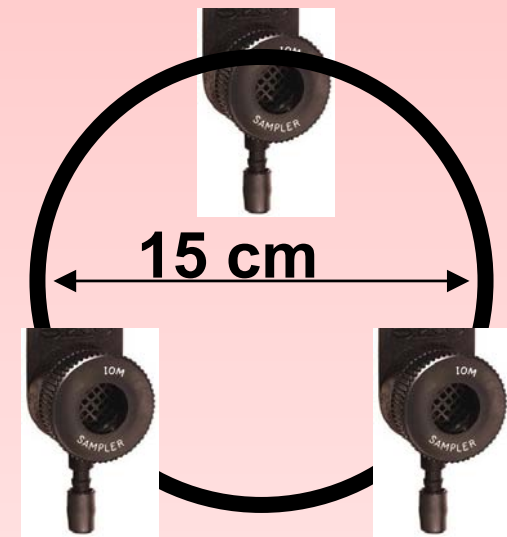
- 1. Test of reproducibility between labs**
 - ❖ **Three equal series of samples**
 - ❖ **Analysed by three different labs**
- 2. Test of repeatability within a lab**
 - ❖ **Duplo samples**
 - ❖ **Analysed by each lab**

Small scale study



Design of study

Test of reproducibility



Three parallel series of inhalable dust samples

- 3 samplers on ring of \varnothing 15 cm
- In 2 composting facilities, on 10 fixed spots
- Dust sampling according to UK-method: MDSH 14/2
 - ◆ IOM-sampler, blanks included

Design of study

Test of repeatability

- ❖ **Repeatability of each of 3 labs**
- ❖ **Each lab received 4 samples in duplo**

Results (1)

Inhalable dust of 3 series

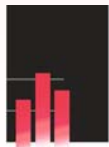
❖ Gravimetric analysis

- All pre- and postsampling weighting was done by one lab according to MDHS dust sampling standard**

Results (2)

Inhalable dust of the 3 series (mg/m³)

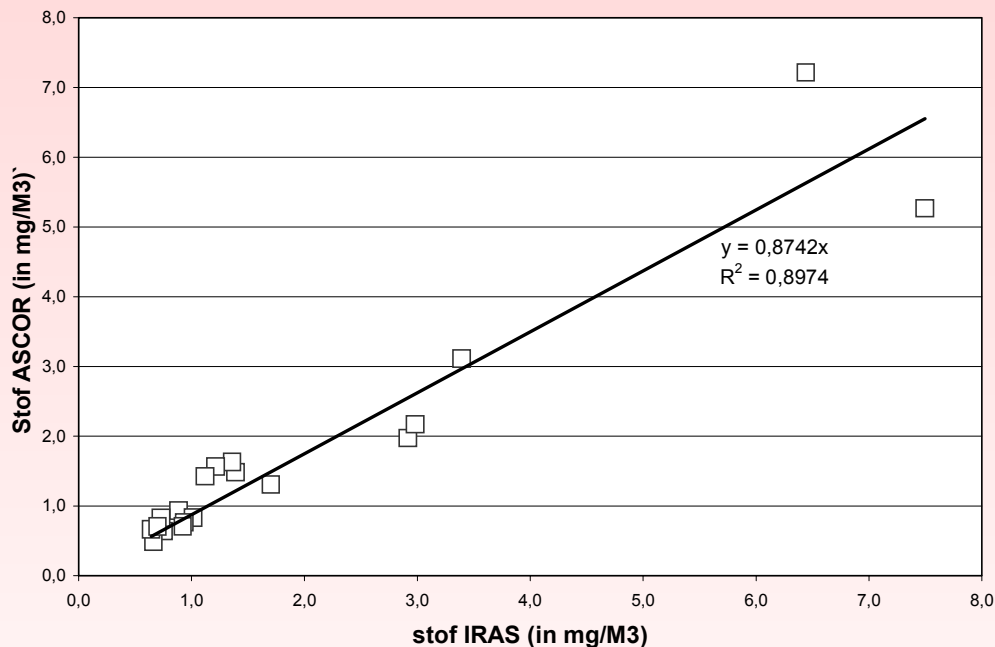
Source		Series Lab A	Series Lab B	Series Lab I
Composting site V	AM (range) (mg/m ³)	2,6 (0,8 - 7,2)	2,7 (0,8 - 7,2)	2,9 (0,9 - 7,5)
	N	10	10	10
Composting site D	AM (range) (mg/m ³)	0,8 (0,5 - 1,4)	0,8 (0,4 - 1,5)	0,9 (0,6 - 1,7)
	N	9	8	9
Blanks	AM (range) (mg/m ³)	0,06 (-0,01 - 0,17)	0,05(-0,02 - 0,12)	-0.02(-0,13 - 0,08)
	N	4	4	4



Results (3)

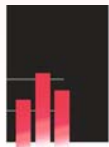
Comparison of inhalable dust of 3 series

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<i>Regression Line</i>	<i>Slope</i>
Lab I-Lab A	0,87
Lab I –Lab B	0,89
Lab A –Lab B	0,95

➔ Dust in the 3 series of samples were equal,
max. difference = $1/0,87 = 1,15$



Results (4)

Endotoxine of 3 series

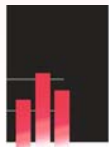


❖ Analysis for endotoxine

- After weighting filters were frozen and 3 series were shipped to 3 different labs for analysis
- 3 different labs: Lab I (NL), Lab A (NL), Lab B (D)
- LAL-assay according to EN 14031
- Each lab did each determination of endotoxine in duplo or triplo. The average was taken.

❖ Problem: one lab did not a direct analysis after extraction, but froze the extracts

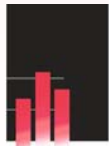
- Solution: a second lab was requested to split the extracts: one fraction for analysis without freezing and other fraction for analysis after freezing.



Results (5)

Endotoxine of 3 series

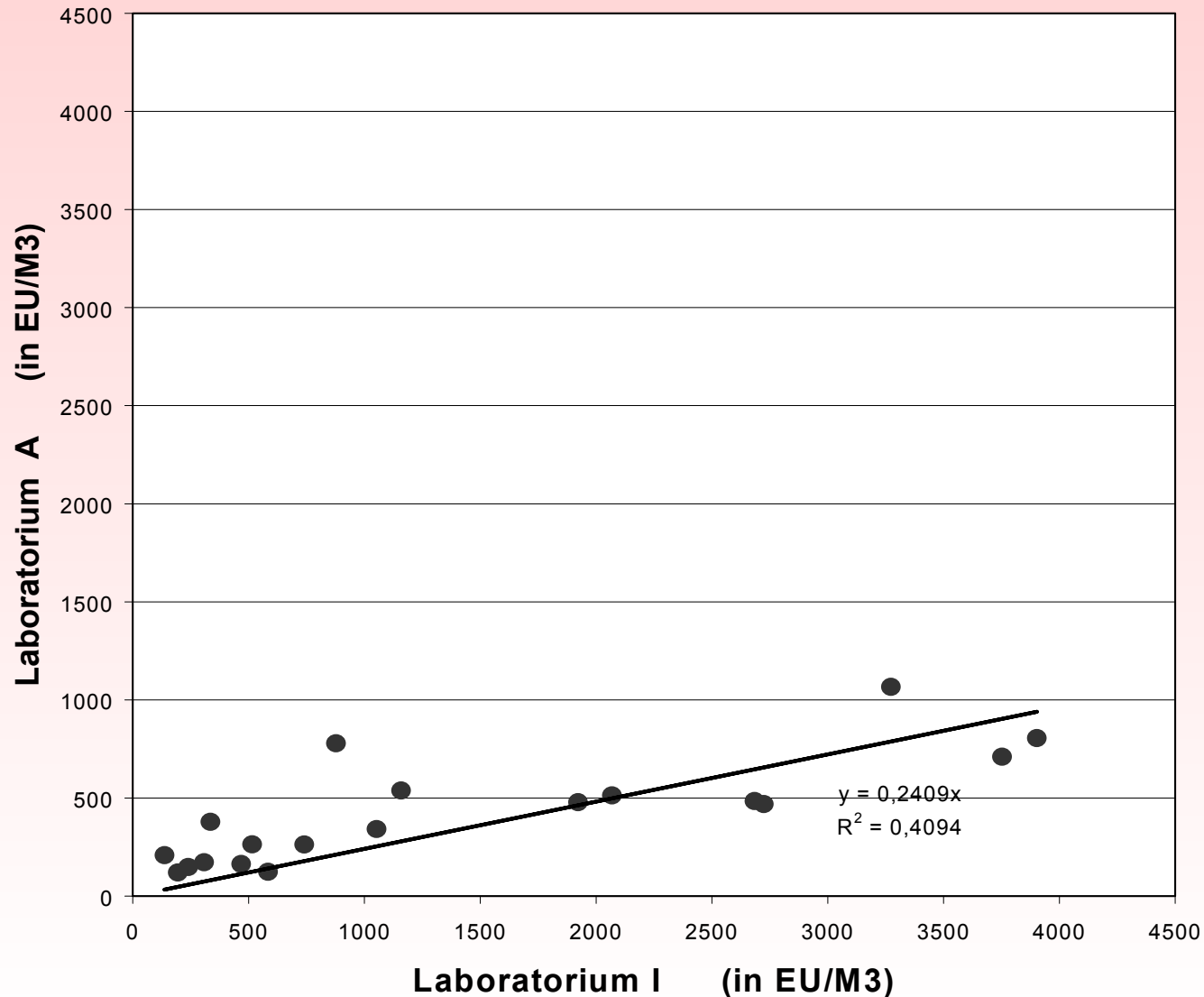
Source		Series Lab A		Series Lab B	Series Lab I
		(- freeze)	(+ freeze)	(- freeze)	(+ freeze)
Composting site V	AM (EU/m ³)	802	498	854	1984
	Range (EU/m ³)	229 - 1597	124 - 1067	208-2880	583 - 3902
	N	10	10	10	10
Composting site D	AM (EU/m ³)	625	338	251	789
	Range (EU/m ³)	209 - 1538	121 - 778	105-625	137 - 3752
	N	9	9	8	9
Blanks	AM (EU/m ³)	<4,9	<1,5	2,0	< 6,25
	Range (EU/m ³)	<0,25 -18,8	<0,25 - 4,0	0,5-4,6	<5 - 11,25
	N	4	4	4	4



Results (6)

Comparison endotoxine Lab A- Lab I

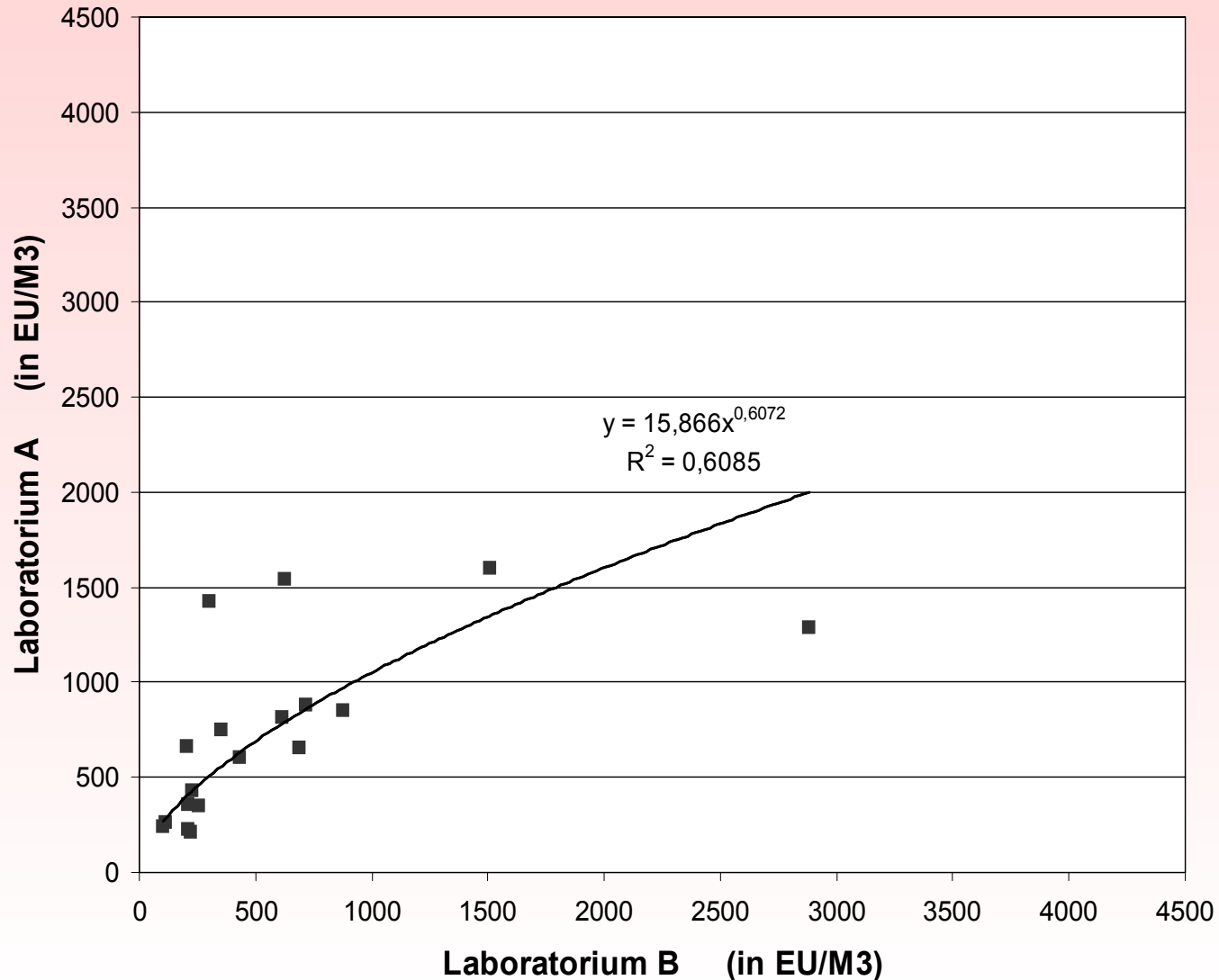
Both labs
froze
extracts !



Results (7)

Comparison endotoxine Lab A - Lab B

**Both labs
did not
freeze
extracts !**



Results (8)

Reproducibility of determination of airborne endotoxin

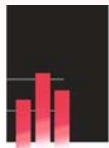
- ❖ **Differences between 3 labs is substantial (up to 4,2-fold)**
- ❖ **Not caused by differences in sampled dust (max. 1,15 - fold) → source is endotoxin-assay**

Results (8)

Repeatability of determination of airborne endotoxin within a lab

	<i>Lab I</i>	<i>Lab A</i>	<i>Lab B</i>
Relative Standard Deviation (RSD)	29%	17%	13%
Number of duplo samples	4	4	4

**Demand of EN-standard 482: RSD < 15%,
Repeatability is little high, but acceptable**



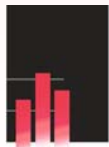
Conclusions

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- 2. However, reproducibility between labs is poor. Large differences are found between laboratories**

Interpretation

Round robin studies by others

- ❖ **Endotoxin in cotton dust** (Chun et al, 2002)
 - Result of 2nd phase of standardisation of procedure
 - Within-lab differences are limited
 - Between-lab differences = maximal 10-fold (13 labs)
- ❖ **Endotoxine in agricultural dust** (Reynolds et al, 2002)
 - Within lab differences are limited (CV < 11%)
 - Between lab differences = maximal 10-fold (6 labs)
- ❖ **Endotoxine in poultry farming dust** (Lindsel 2002)
 - Comparison of 10 German labs
 - Within-lab variation limited (CV < 8%)
 - Difference between labs max 3-fold





Two questions remain:

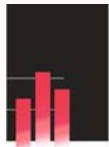
- 1. Which lab has the golden standard?**
- 2. What rank had the lab that published the critical dose-response study of health effects by endotoxin? Low or high?**

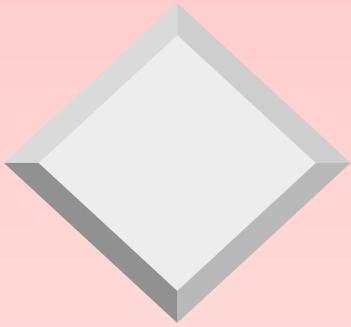
What next?

- 1. Analytical method of endotoxin needs further development and standardisation**
 - ❖ Extraction, freezing, analysis, interferences etc.
- 2. Validation by external quality control is necessary**
 - ❖ Interlaboratory testing program
- 3. Re-evaluation of dose-response of endotoxin**

Last but not least:

Accreditation of labs for the analysis of endotoxin based on proficiency testing is urgently needed !





Thank you,

Merci,

Danke,

Dank u

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See for handout:

www.industox.nl/handouts.htm