



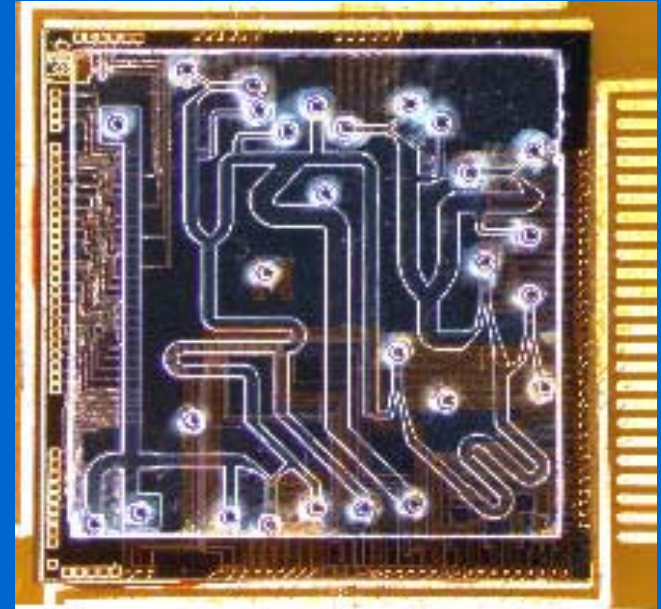
Oude technieken de deur uit?



Terug naar de natuur



Het Laboratorium van morgen



ZomerBloemen bv

Klassieke visie analytische chemie

- wat?
- hoeveel?
- klassieke analytisch chemische vragen
- stof gerelateerd (normen-waarden)
- analyse door stofeigenschap
- spectroscopie (UV-Vis, IR, NMR)
- massa spectrometrie
- chromatografie

Klassieke analyse drinkwater

Aantal pompstations met normoverschrijding

Bestrijdingsmiddel

	1995	1996	1997	1998	1999	2000	2001	2002	2003
Bromacil	0	3	2	1	1	0	0	0	0
Bentazon	3	1	2	4	3	1	1	1	1
Dichloorpropaan	3	3	1	1	0	0	0	0	0
Atrazin	0	0	0	0	0	0	0	0	0
Dikegulac *)	0	0	0	1	1	1	0	0	0
DNOC	0	0	0	0	0	0	0	2	0
AMPA *)	0	1	0	0	0	0	0	0	0
Dinoterp	1	0	0	0	0	0	0	0	0
Fosfamidon	1	0	0	0	0	0	0	0	0
Azinfos-metyl	1	0	0	0	0	0	0	0	0
Diuron	0	1	0	0	2	0	0	0	0
Simazin							0	1	0
MCPP	0	0	1	2	1	1	1	1	1
BAM *)						2	3	5	4

Bron: Waterleidingbedrijven (2005)

MNP/MNC/sept05

*) deze metabolieten zijn beoordeeld als humaan toxicologisch niet relevant, behoeven daarom niet te voldoen aan de norm

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Onze visie analytische chemie

Stof, Effect, Proces

- is het gevaarlijk, veilig
- effect gerelateerd
- analyse door effect eigenschap
- proefkonijn, vogeltje in de mijn
- effect op heel celsysteem
- effect op enzymatische reactie

Onze visie analytische chemie



Is it safe??

Onze visie analytische chemie

- Procescontrole / sturing (PAT)
- Screening "oude" chemicalien

Process Analytical Technology

Process Analytical Technology is:

a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes with the goal of ensuring final product quality.

It is important to note that the term *analytical* in PAT is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner.

Registration Evaluation Authorisation Chemicals

The Commission proposed a new EU regulatory framework for the Registration, Evaluation and Authorisation of Chemicals (REACH) on 29 October 2003. The aim is to improve the protection of human health and the environment through the better and earlier identification of the properties of chemical substances. At the same time, innovative capability and competitiveness of the EU chemicals industry should be enhanced. The benefits of the REACH system will come gradually, as more and more substances are phased into REACH.

Het betreft hier meer dan 100000 "oude" chemicalien waarvan de eigenschappen in kaart moeten worden gebracht.

context

- Enzym inhibitoren (geneesmiddel, bestrijdingsmiddel, zenuwgas, etc)
- Screenen van oude chemicaliën (REACH)
- Reporter genen in vitro (CALUX, etc)
- Reporter genen in vivo (Tumoren, effect op immuunsysteem)

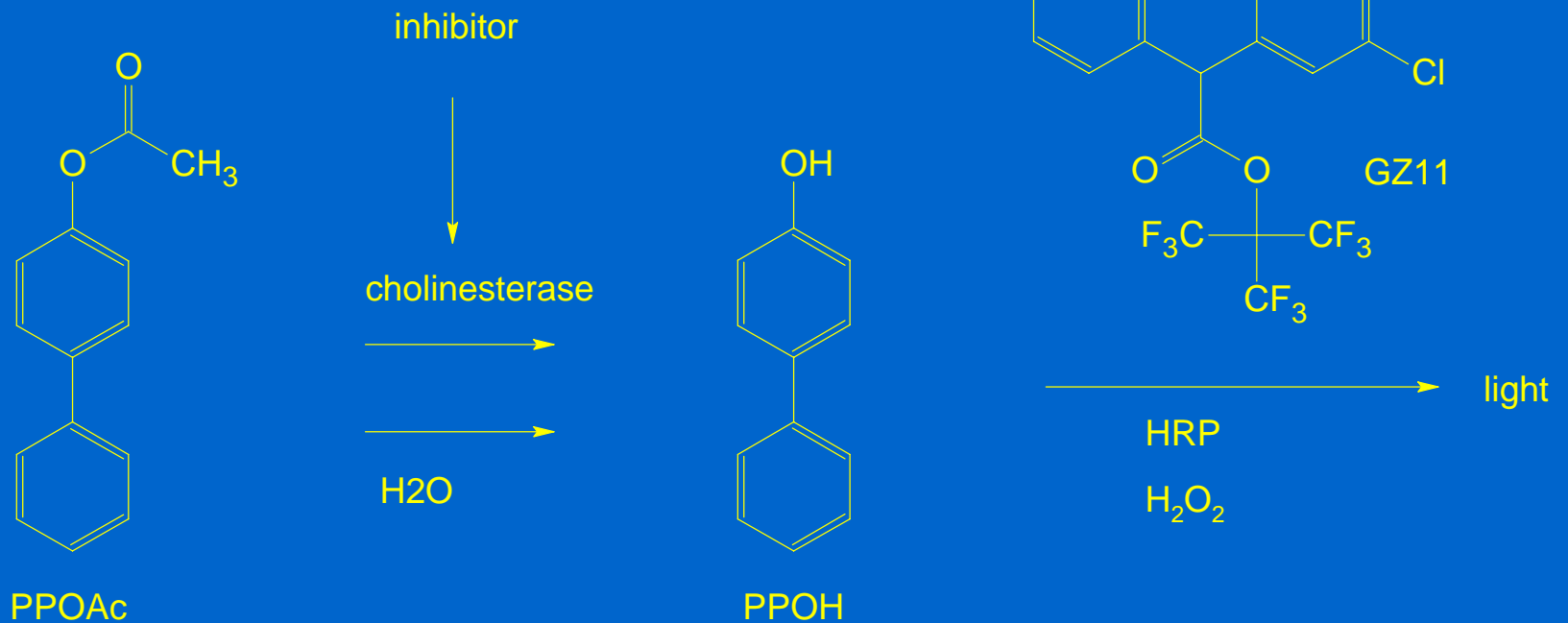
Analyse op basis van effect

- Specificiteit door enzym, antilichaam, receptor
- Analytisch signaal door substraat/inhibitor, antigeen (gelabeld), agonist/antagonist
- Analytisch signaal: kleur, fluorescentie, chemiluminescentie

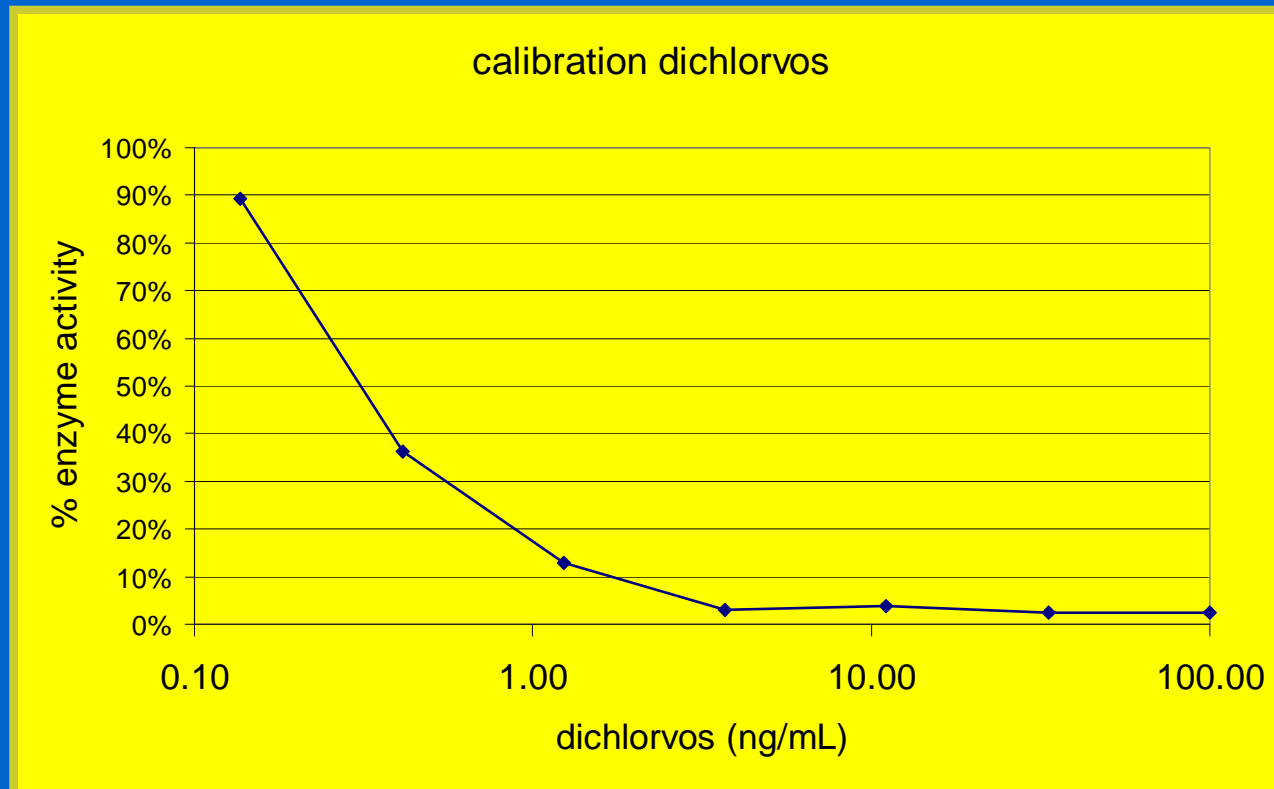
Voordeel: specifiek, gevoelig, high througput (parallel)

Nadeel: niet in natuurlijke omgeving, gevoelig voor storingen (zoutgehalte, temperatuur, etc)

Bepaling enzym inhibitoren

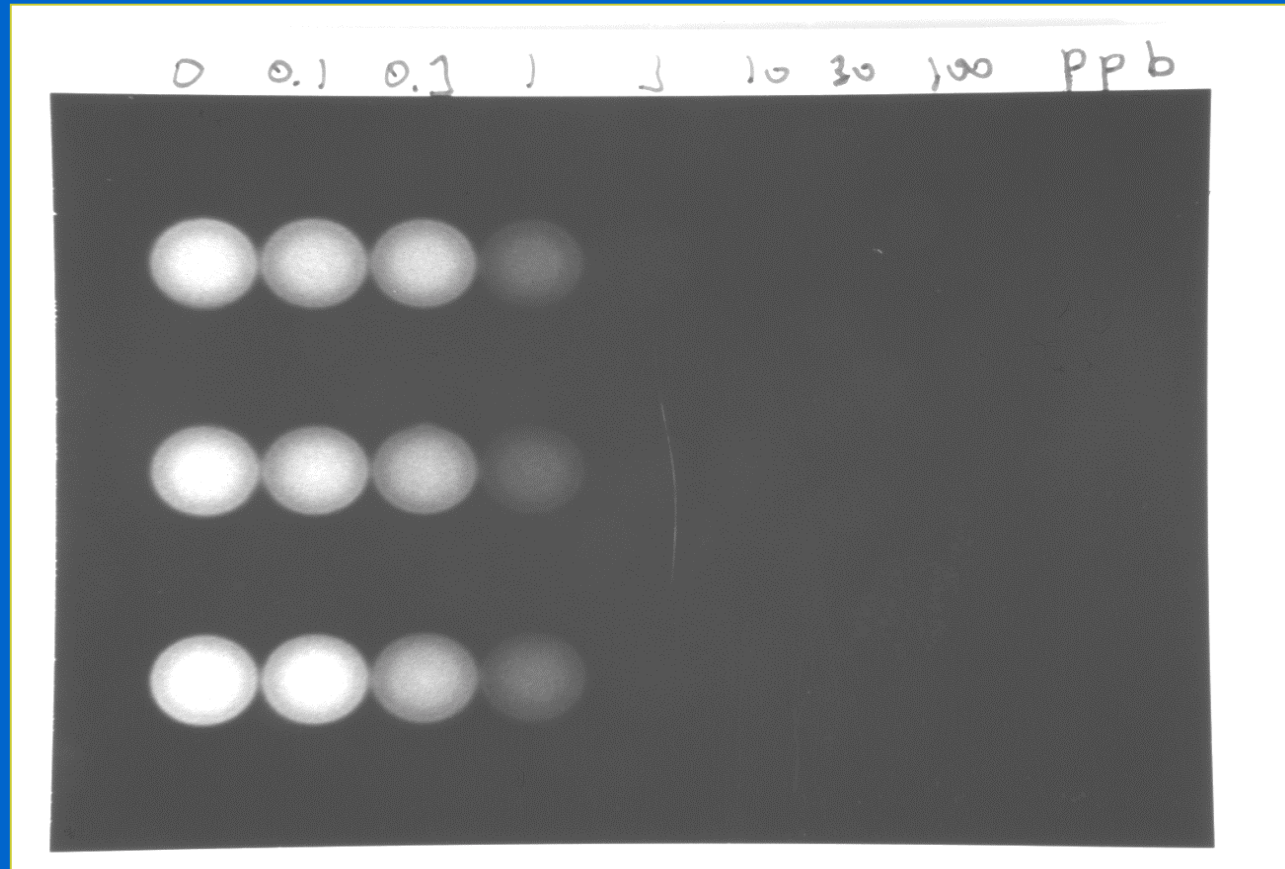


Toepassing: bepaling dichloorvos



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Dichloorvos met polaroid
analysetijd 30 minuten

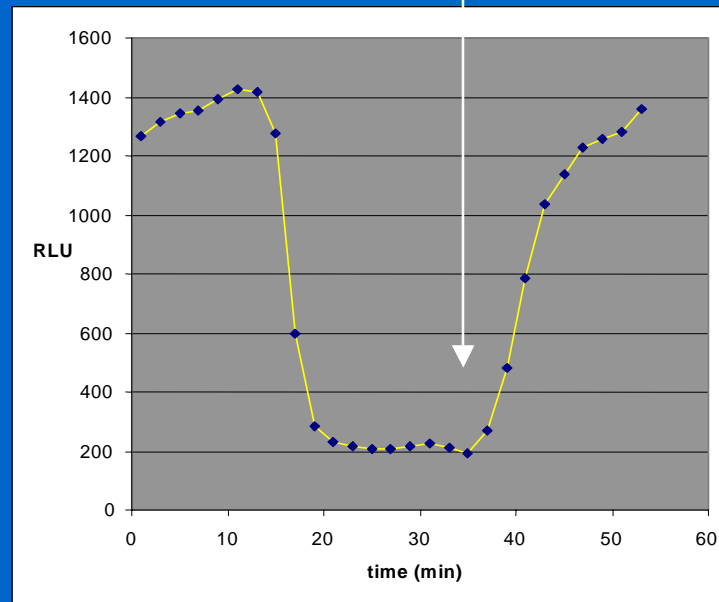


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Resultaten flow systeem (off-line meting)

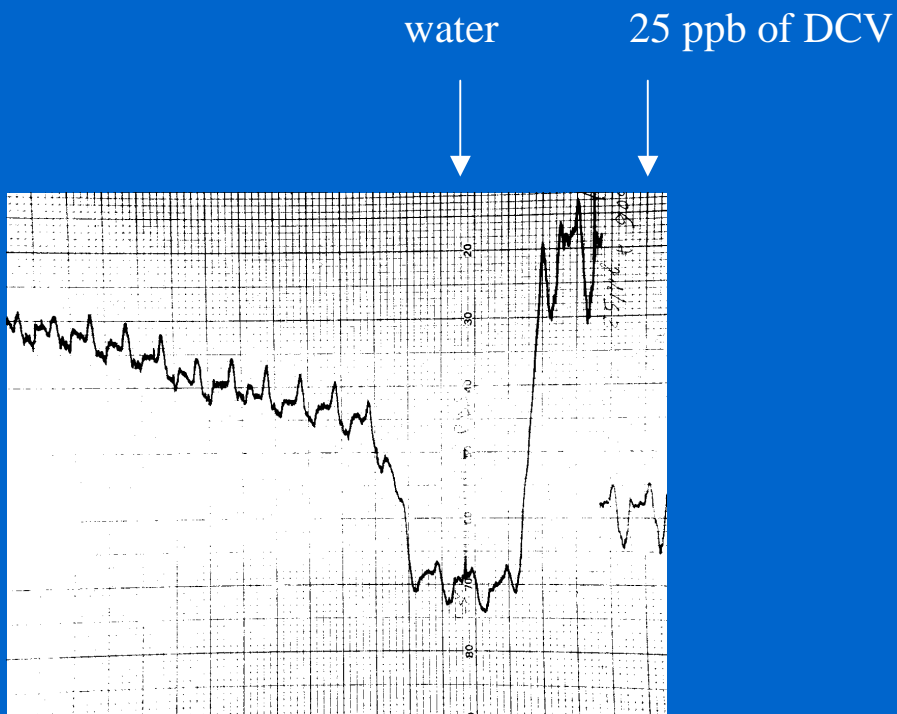
10 ppb dichloorvos (DCV)

water



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Resultaten flow systeem (on-line meting)

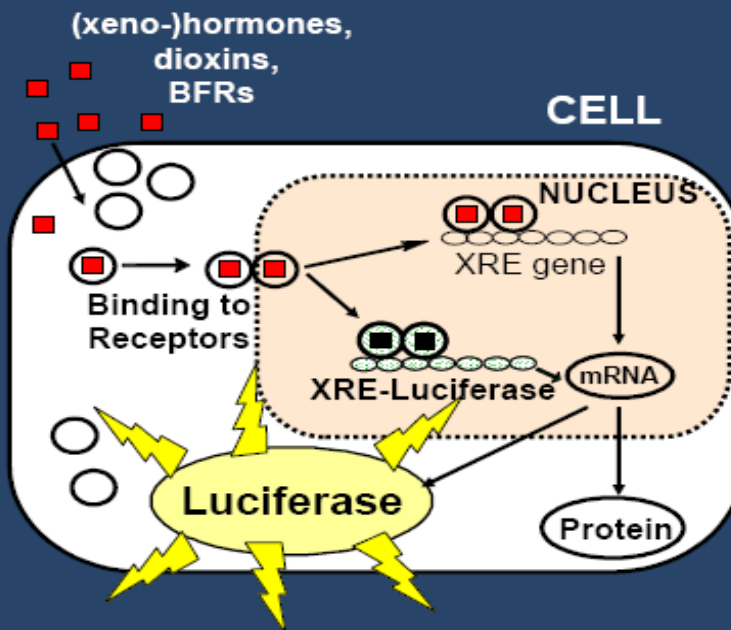


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Reporter genen in vitro

- CALUX, detectie en kwantificering dioxinen, PCB's, etc (op basis van binding aan Ah-receptor)
- Endocrien disruptors (op basis van binding aan estrogeenreceptor)

Principle of CALUX® reporter gene assays



Reporter gene assays in stably transfected cell lines

Four different receptors:

1. Estrogen (ER-CALUX)
Legler et al., 1999
2. Dioxin (DR-CALUX)
Aarts et al., 1996
3. Androgen (AR-CALUX)
Sonneveld et al., 2005
4. Progesteron (PR-CALUX)
Sonneveld et al., in prep

Selectie van reporter gen assays voor milieucontaminanten

Recombinant cell	Reporter gene	Analyte
<i>Escherichia coli</i>	<i>luc</i>	mercury
<i>Escherichia coli</i>	<i>luc</i>	arsenite
<i>Staphylococcus aureus</i>	<i>luc</i>	cadmium, lead
<i>Staphylococcus aureus</i>	<i>luc</i>	antimonite, arsenite
<i>Escherichia coli</i>	<i>lux</i>	mercury
<i>Escherichia coli</i>	<i>luc</i>	benzene
<i>Pseudomonas fluorescens</i>	<i>lux</i>	naphthalene
<i>Pseudomonas putida</i>	<i>lux</i>	benzene, toluene, ethylbenzene, xylene
<i>Escherichia coli</i>	<i>lux</i>	alkanes
<i>Escherichia coli</i>	<i>lux</i>	tetracyclines
Human hepatoma, breast tumor, <i>luc</i>		polyhalogenated aromatic hydrocarbons
Mouse hepatoma cells	<i>luc</i>	carbaryl
Human breast cancer cells	<i>luc</i>	estrogen-like molecules

Reporter genes in vivo

Expressie van bacterieel luciferase
Voor de detectie van tumorcellen.
In tumorcellen kunnen bacterien zich
wel vermenigvuldigen.

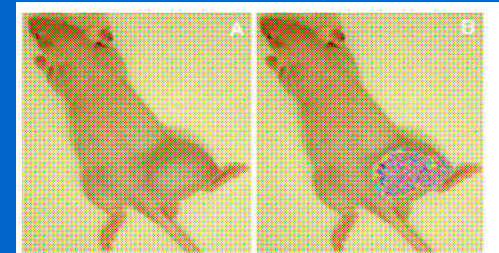


Fig. 1 Visualization of s.c. C6 glioma tumor with light-emitting *Vibrio cholerae*. Nu/nu⁺ mouse bearing an 11-day old s.c. implanted C6 glioma tumor on the right lateral thigh was injected i.v. with 1×10^8 cells of *Vibrio cholerae* carrying *luxCDABE* operon sequence. Three days after injection of bacteria, the mouse was anesthetized and a photon emission image taken under a Hamamatsu ARGUS100 low light imager. The resulting photon acquisition image was superimposed onto a photographic image (panel A) of the mouse to show the location of luminescence emission specifically localized to the tumor region (panel B)

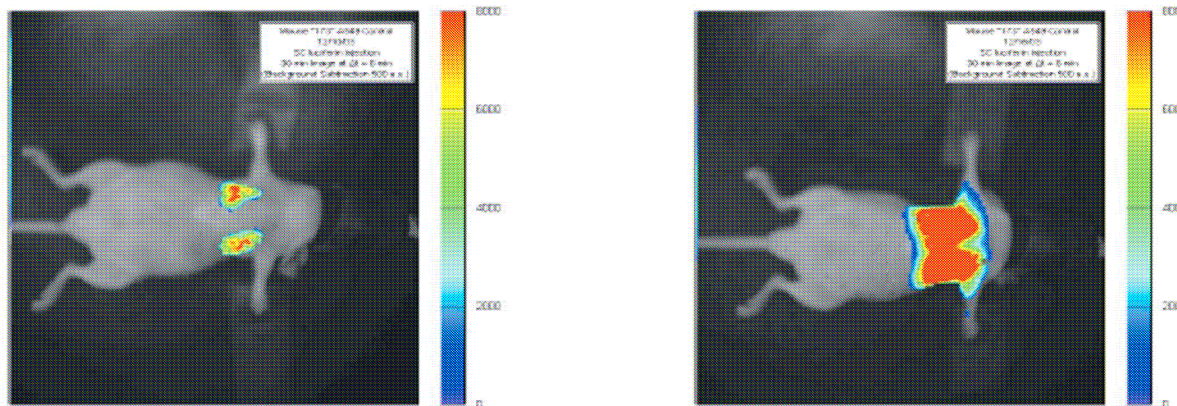


Figure 3. Lung metastases formed 3 weeks after tail vein injection of A549-Luc cells (1×10^6). The image on the left side is 3 weeks after injection, the right one is 4 weeks after injection.

Wat nu?

- Oude technieken de deur uit? Nog even niet
- Nieuwe technieken de deur in? Ja!
 - Analytisch chemicus ook thematische kennis
 - Naast fysisch-chemische ook biochemische meetmethoden