Bioresponse-linked instrumental analysis: Bridging the gap between Cause and Effect?

Risk Analysis – Risk Assessment: Detection of bio-effect environmental compounds by bioactivity based analysis in HPTLC.

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Berlin 15.10.2006

Bioactivity Based Analysis

- **Motivation concerning bioactivity based analysis**
- **The principle of bioactivity based analysis in HPTLC**
- **Progress and Results**
  Detection of estrogenic compounds
  Verification of chemicals
- **Outlook**

Motivation concerning bioactivity based analysis

Studie: Lebensmittel-Kontrolle im Saarland mangelhaft

Greenpeace prangert Schwachstellen der Überwachung an – Ministerium weist Kritik zurück

Im Saarland werden Lebensmittel zu selten auf giftige Rückstände untersucht. Das ist das Ergebnis einer Greenpeace-Studie. Das Saar-Gesundheitsministerium weist die Vorwürfe scharf zurück.

VON SZ-REDAKTEURIN SABINE SCHORR

Saarbrücken. Die Lebensmittelkontrollen im Saarland reichen nach Ansicht der Umweltorganisa- [illegible] Greenpeace bei weitem nicht [illegible] die Verbraucher vor pesti- [illegible] totem Obst und Gemüse zu [illegible]. In einer jetzt vorgestell- [illegible] die bewertete Lars Neumies- [illegible] erte für Schädlingsbekämp- [illegible] ittel, im vorigen Jahr die Le- [illegible] telüberwachung aller Bun- [illegible]er. Die Ergebnisse waren insgesamt enttäuschend. Nur fünf Länder erhielten als Gesamtnote „ausreichend", alle anderen – darunter auch das Saarland – schnitten mit „mangelhaft" ab.

Das Saar-Gesundheitsministerium lässt diese Bewertung nicht gelten. Das seien „alte Hüte", sagte gestern der Sprecher von Gesundheitsminister Josef Hecken (CDU), Stephan Kolling. Bei der Greenpeace-Studie sei die Gesamtzahl der Kontrollbesuche im Saarland zu Grunde gelegt worden. Entscheidend sei aber „die Zahl der Besuche bezogen auf die Zahl der Betriebe im Land". Greenpeace widerspricht: „Genau diese Relation wird mit dem Bewertungskriterium Kontrolldichte berücksichtigt", sagt Chemie-Experte Manfred Krautter zur SZ. Das Saarland habe die Note „mangelhaft" erhalten. Zudem sei unter der Rubrik „Probenahmedichte" benotet worden, „wie viele Lebensmittel-Proben pro 100 000 Einwohner ein Kontrolleur untersucht". Ergebnis für das Saarland: „mangelhaft".

Die so genannte Aufdeckungsquote im Land wird in der Studie sogar mit „ungenügend" bewertet. Die Quote basiert auf den entdeckten Überschreitungen von erlaubten Giftmengen bei frischem Obst und Gemüse. Baden-Württemberg erhielt hier mit „gut" die beste Note. Positiv hob Greenpeace hervor, dass das Saarland „relativ viel" in seine Laborausstattung investiert habe. Allerdings heißt es in der Studie: „2004 wurden im Saarland nur 177 Proben auf Pestizid-Rückstände untersucht. Es ist mehr als fraglich, ob diese geringe Zahl hohe Investitionen rechtfertigt." Scharfe Kritik übte der Autor der Studie an der mangelnden Auskunftsbereitschaft des Hecken-Ministeriums. Neumeister: „Die Auskunftsfreude war im Saarland saumäßig." Öffentlichkeitsarbeit dürfe keine lästige Pflicht sein.

Greenpeace warnt vor einem „schleichenden Lebensmittel-Skandal". Als Reaktion auf die alarmierenden Ergebnisse der Studie fordert die Umweltorganisation bundesweit intensivere Kontrollen, mehr Personal und wirksame Strafen für Lebensmittelhersteller und -händler, die Obst und Gemüse mit zu hoher Giftbelastung verkaufen. Krautter: „Wer gegen das Lebensmittelgesetz verstößt, muss öffentlich benannt werden. Die Behörden decken durch ihr Nichtstun die schwarzen Schafe."

Saarbrücker Zeitung, vom 12.4.2006

Berlin 15.10.2006

Motivation concerning bioactivity based analysis

Drugs in the environment, Example: Ivermectin

Comparison of excrements of cattle, food of insects and birds after one week

UBA 29.9.2004 Dr. Boers: Ausgewählte Therapeutika in der Tierzucht – Applikation und Umweltrelevanz, aus Arzneimittel in der Umwelt – Zu Risiken und Nebenwirkungen fragen Sie das Umweltbundesamt, Texte 29/05 ISSN 0722-186X

Berlin 15.10.2006

Motivation concerning bioactivity based analysis

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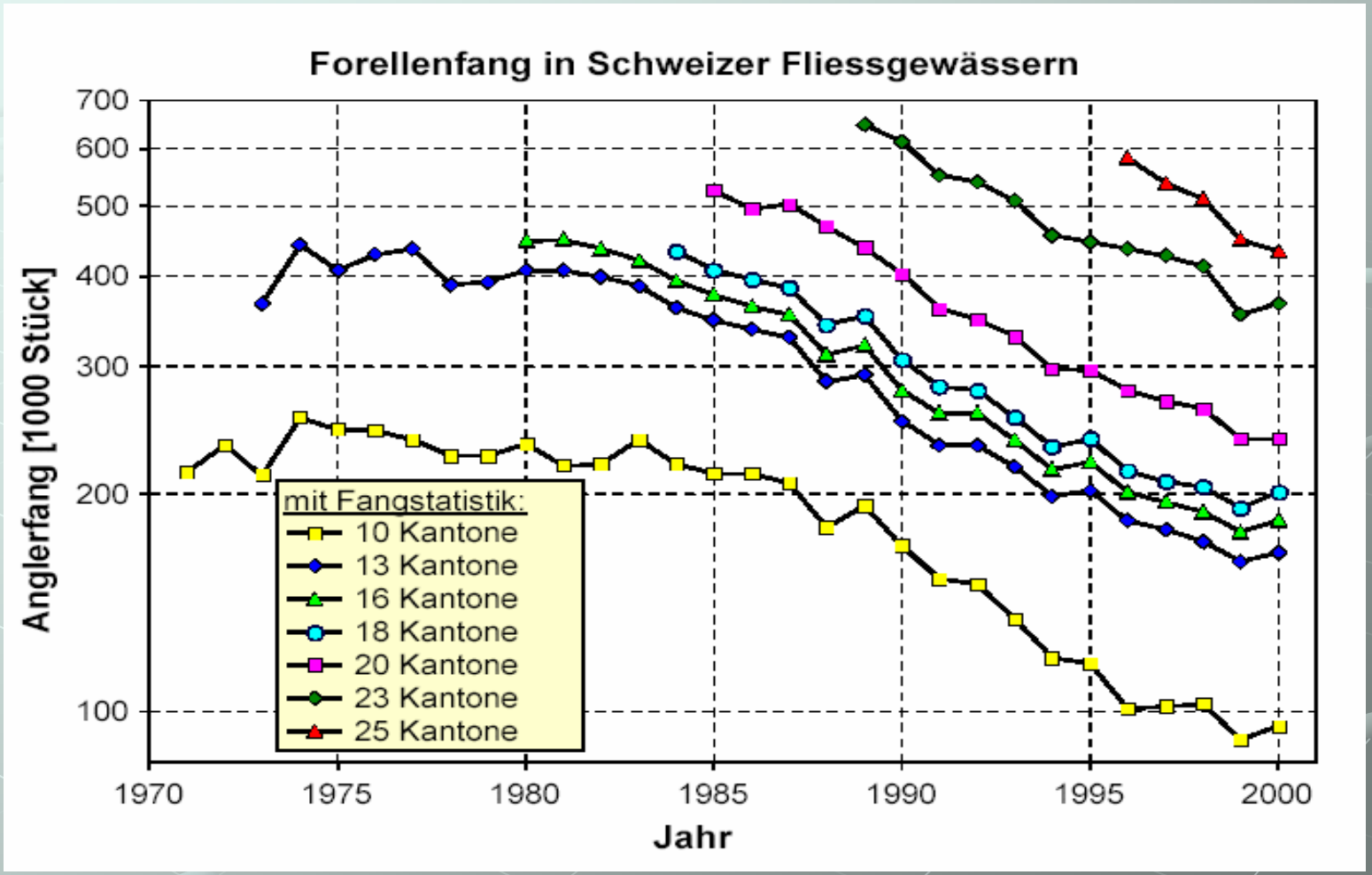
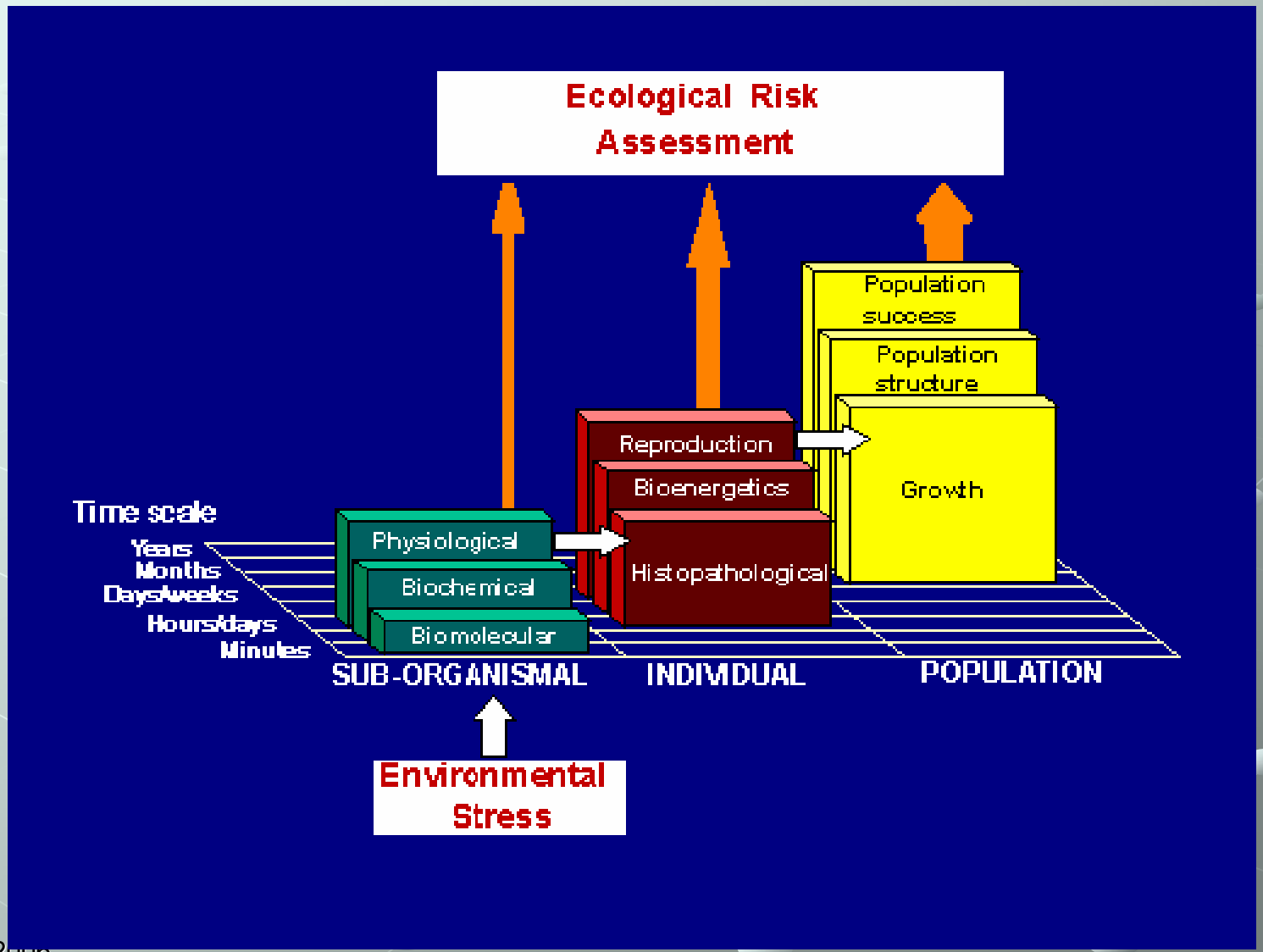


Trouts, fished by anglers from Switzerland

Motivation concerning bioactivity based analysis



Initial Situation

Environmental Illness Society of Canada
La société canadienne pour les sensibilités environnementales

1. In 1989, the world production of pesticides amounted to 5 billion pounds or about 1 pound for every person in the world annually.
2. This production included 1600 chemicals.
3. Pesticide use in the United States alone amounts to 2.2 billion pounds a year, roughly 8.8 pounds per capita.
4. Annually, American farmers use over 560 million pounds of herbicides and fungicides (not counting other pesticides).
5. Every year about 600 different species of pesticides are spread out into the environment. Every year about 500 – 1000 new pesticides are produced

Bioresponse-linked instrumental analysis: Bridging the gap between Cause and Effect?

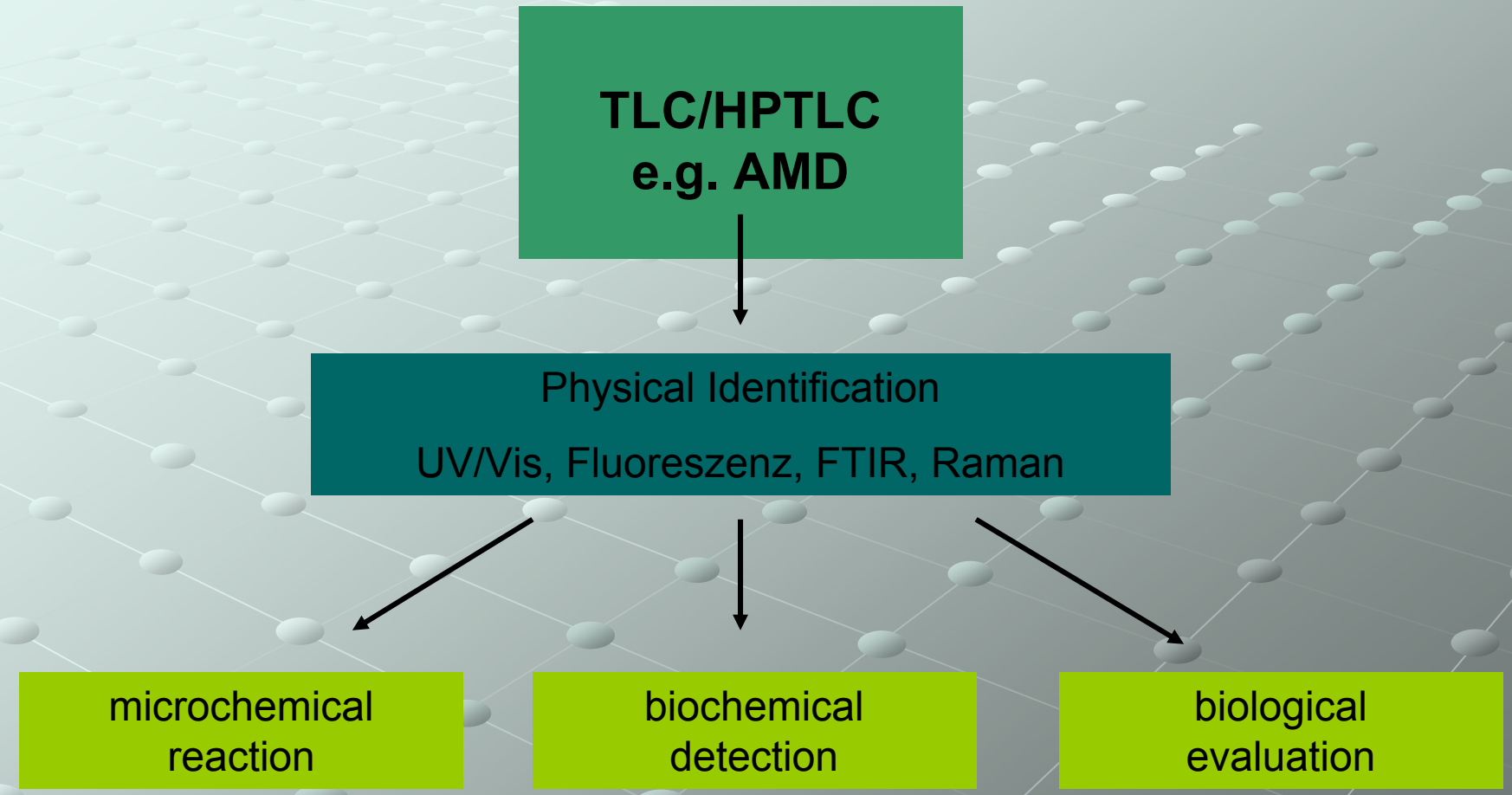
Biotests

1. The Bioactivity of water soluble toxins on test organisms in unprocessed water samples are detected.
2. Discussion of synergistic effects
3. It is not possible to identify only one single substance

Trace Analysis

1. The toxins in a water sample are selectively enriched
2. Analytical separation
3. Toxins are identified using selected reference substances
4. Identified toxins can be quantified

Principle of Bioactivity Based Analysis in HPTLC/AMD



The principle of the AMD procedure

(AMD = Automated Multiple Development)

GRADIENT ELUTION IN PLANAR CHROMATOGRAPHY

1. *The chromatogram is developed repeatedly in the same direction.*
2. *Each partial run goes over a longer solvent migration distance than the one before.*
3. *Between partial runs, the solvent is completely removed from the developing chamber and the layer is dried under vacuum.*
4. *Each partial run uses a solvent of lower elution strength than the one used before. In this way, a stepwise elution gradient is formed.*
5. *The combination of focusing effect and gradient elution results in extremely narrow bands. Their typical peak width is about 1mm.*

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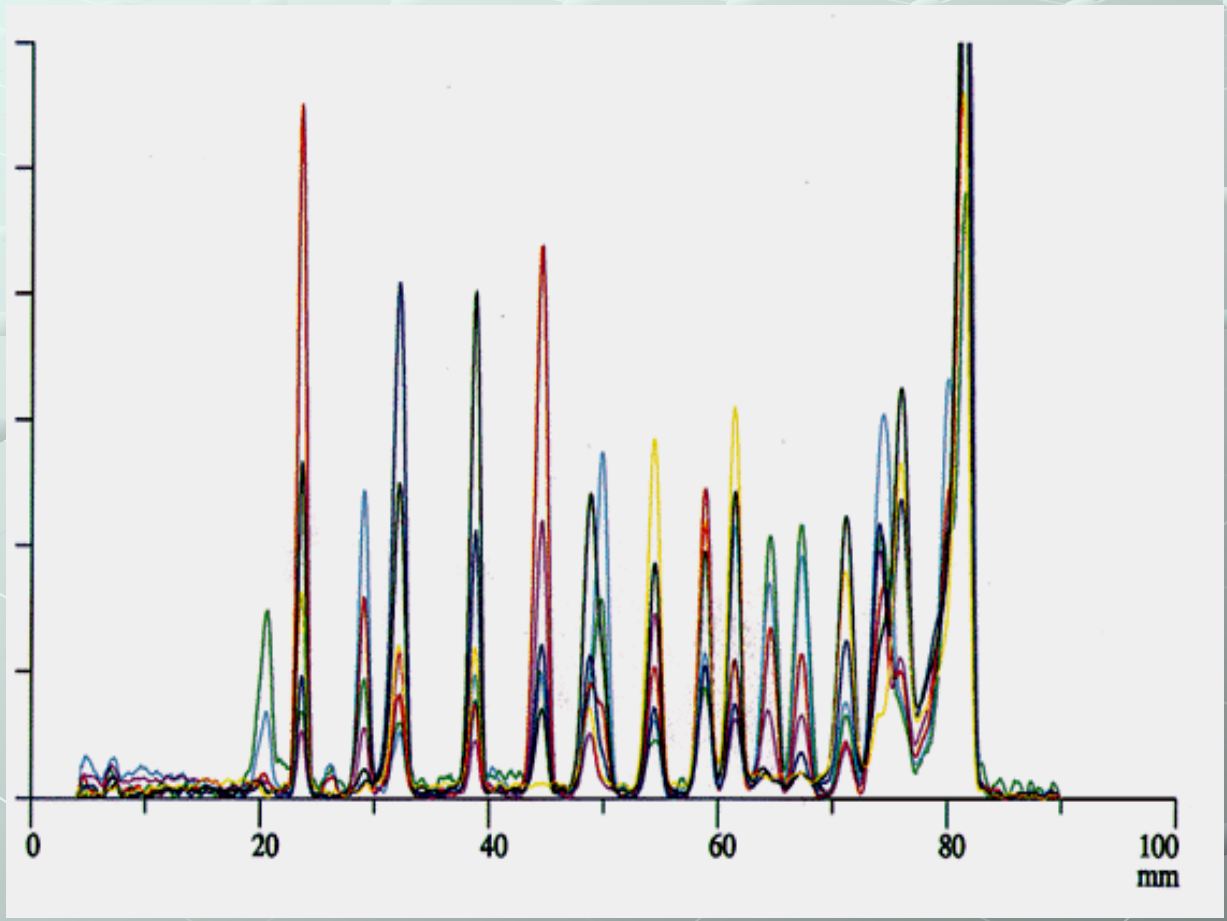
GRADIENT ELUTION IN PLANAR CHROMATOGRAPHY



1. Pesticides in drinking water (German Standard Method DIN 38407 Part 11)
2. Complex samples from environmental analysis
3. Phospholipids
4. Carbohydrates
5. Anabolics in the meat and urine of cattle

The principle of the AMD procedure

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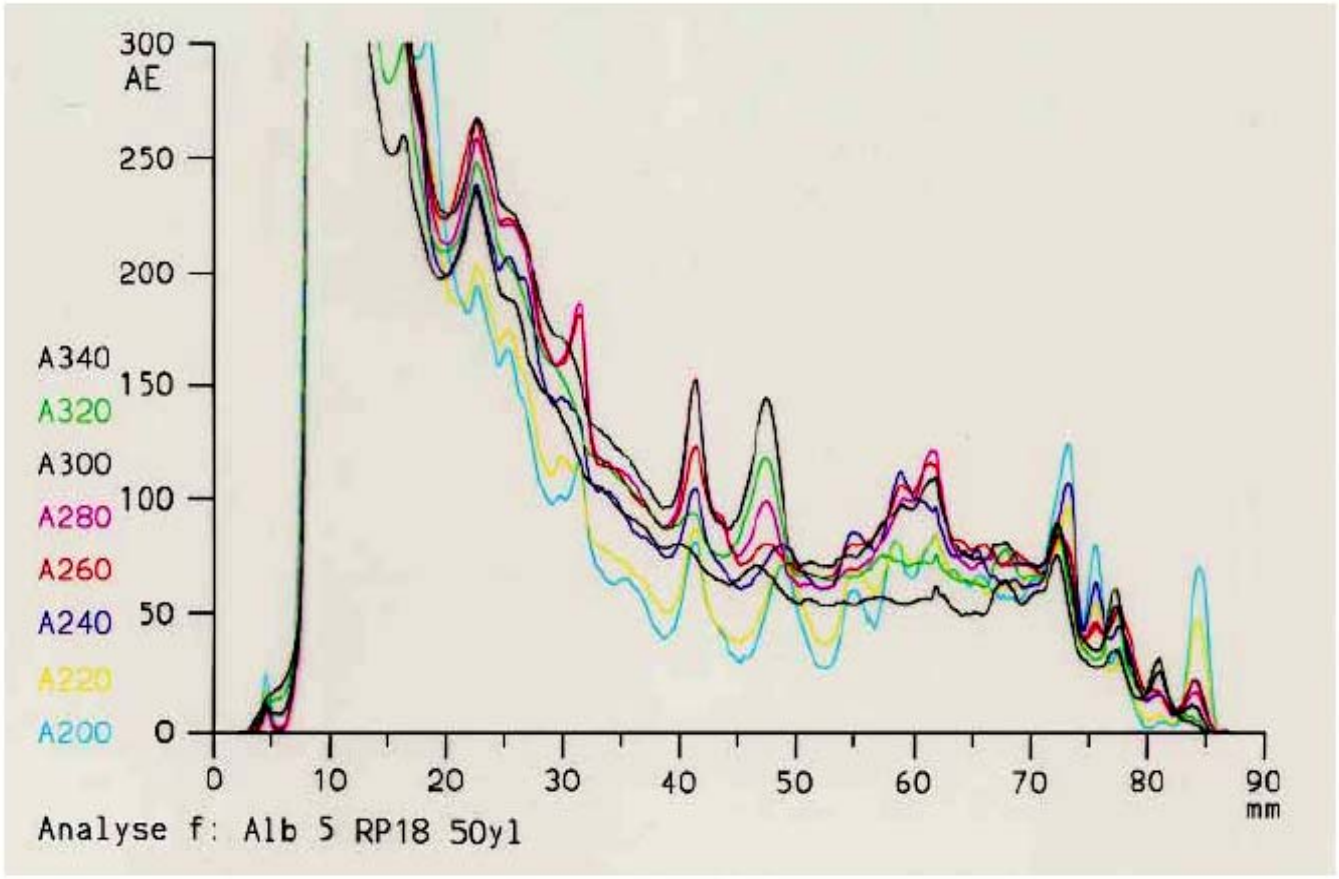

5: sewage treatment plant effluent

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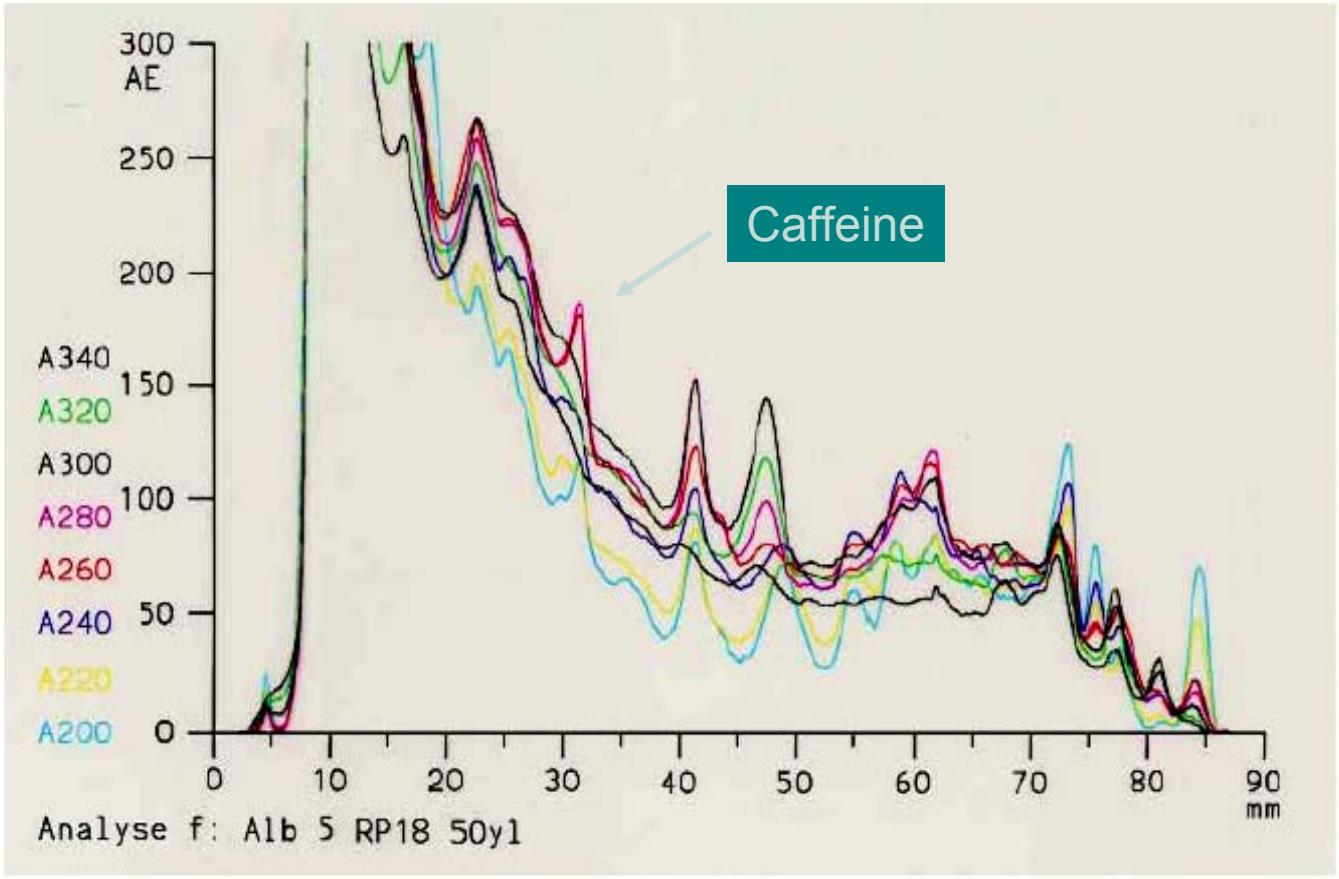
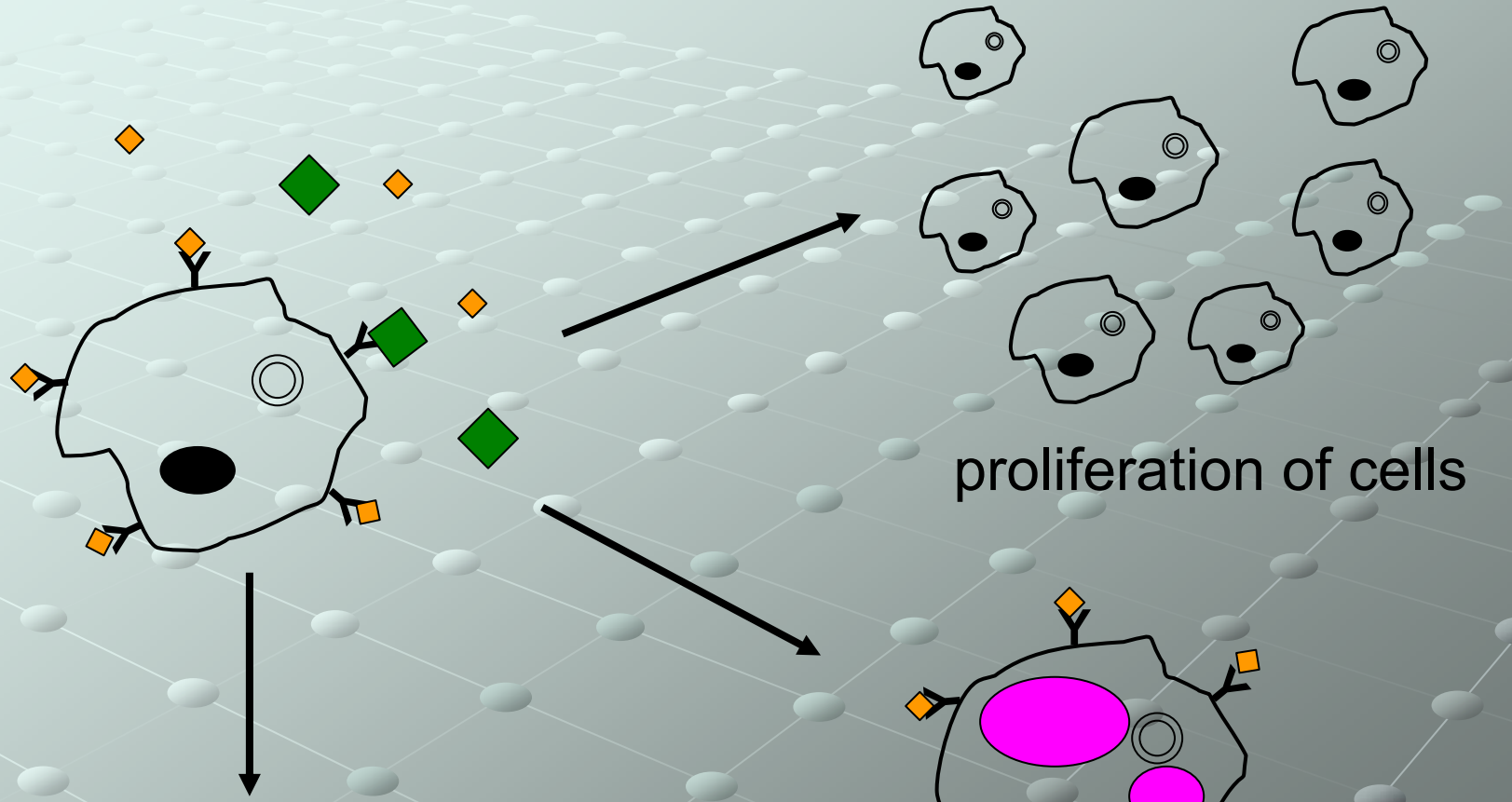
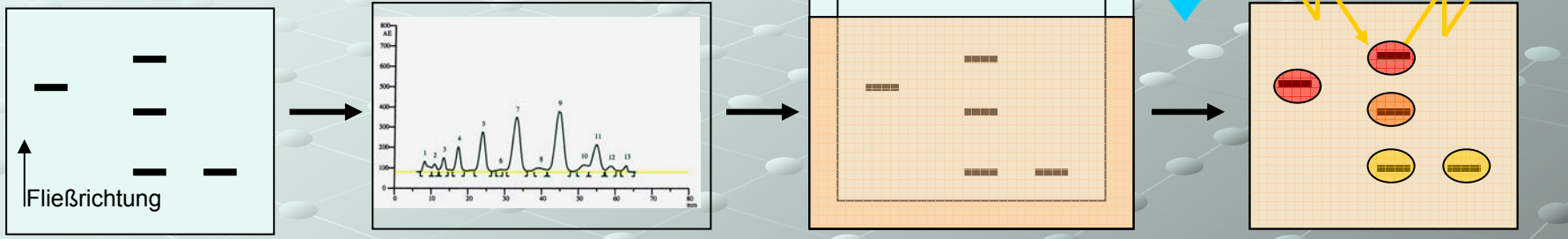


5: sewage treatment plant effluent

Principle of biological indicators and responses



Principle of Bioactivity Based Analysis in HPTLC/AMD

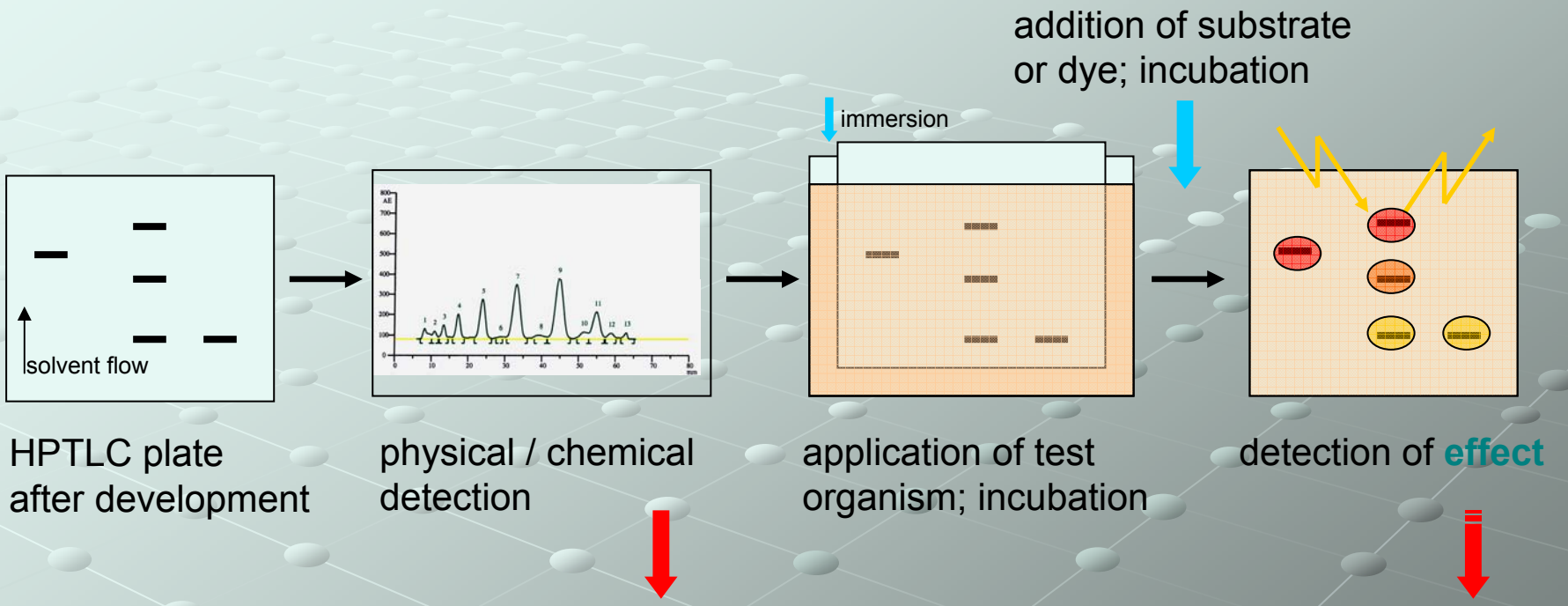


Application of a test-Isolution; incubation

detection of effects

identification, quantification

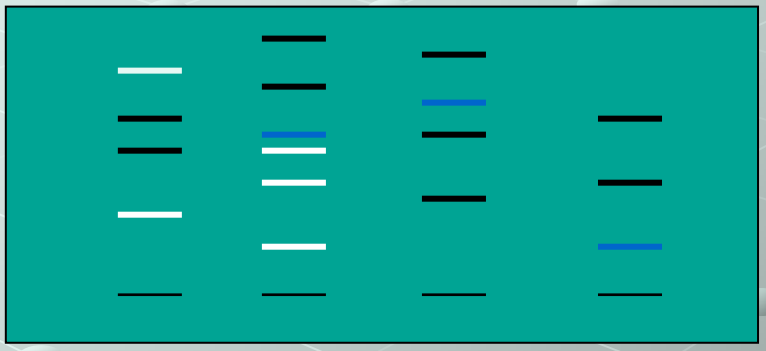
Principle of Bioactivity Based Analysis in HPTLC/AMD



- Screening methods for detection of bioactive compounds in complex mixtures
- Pharmaceutical research[1], environmental analysis[2, 3]

[1] Hostettmann et al. Phytochem. Anal. 1991, 2, 199-203.
[2] Weins, C.; Jork, H. J. Chromatogr. A 1996, 750, 403-407. [3] Eberz, G. et al. Chromatographia 1996, 43, 5-9.

Examples for toxicological tests in situ



Bioluminescent inhibition

Gentoxicity
UMU-test

Fungicides
Penicillium exp.

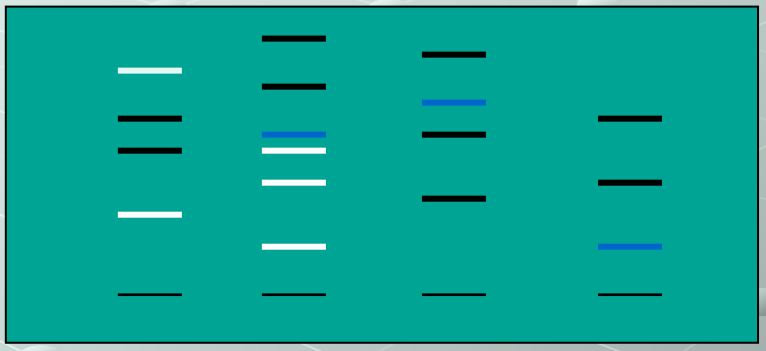
Hormones
Yeast Test (Sumpter)

Antibiotics
Bacillus subtilis

Herbicides
Algae, Chloroplasts

Insecticides
Enzyme Inhibition

Examples for toxicological tests in situ



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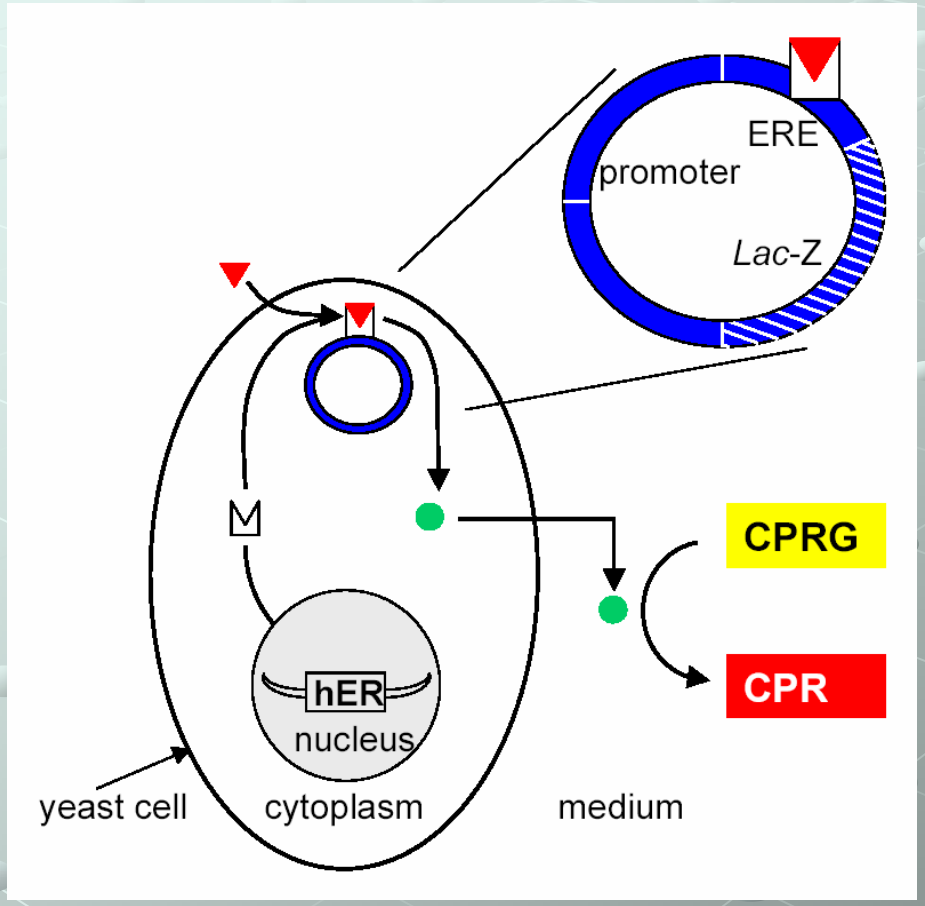
Detection of Hormones - HPTLC-YES test

Yeast Estrogen Screen (YES) According to Routledge and Sumpter[1]



estrogen

estrogen receptor

activated receptor

β -galactosidase

hER:	human estrogen receptor gene
ERE:	estrogen responsive element
lac-Z:	reporter gene
CPRG:	chlorophenol red-β-D-galactopyranoside

sensitivity:
c (E2) = 10^{-11} mol/L (2.7 ng/L)

[1]Routledge, E. J.,Sumpter, J. P. (1996),Environ.Toxicol. Chem. 15, 241-248

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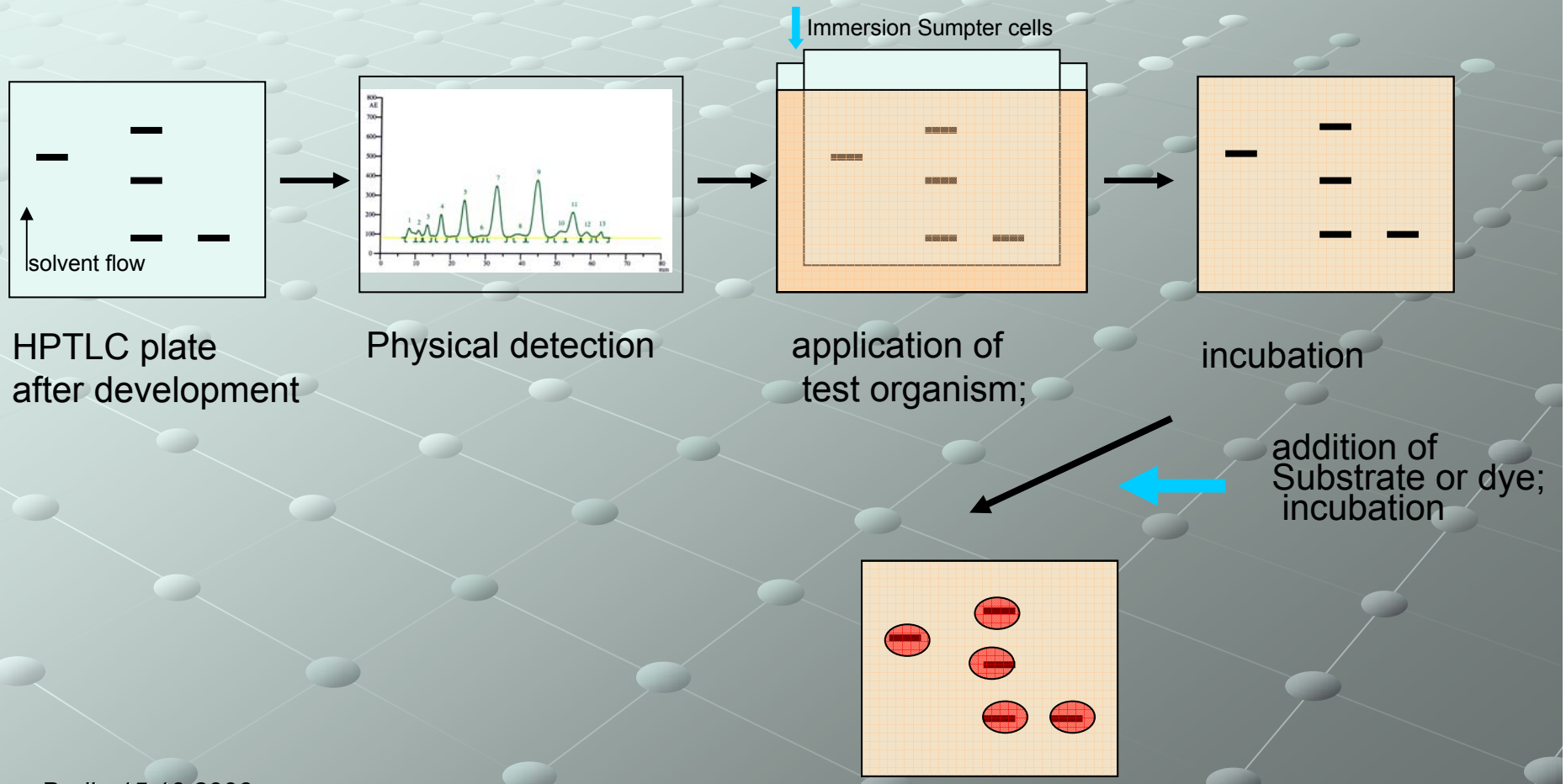

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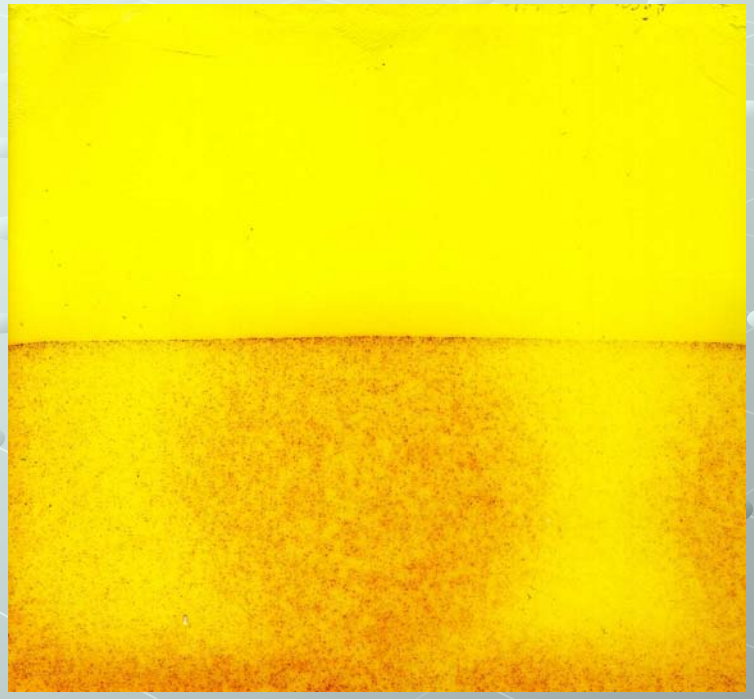
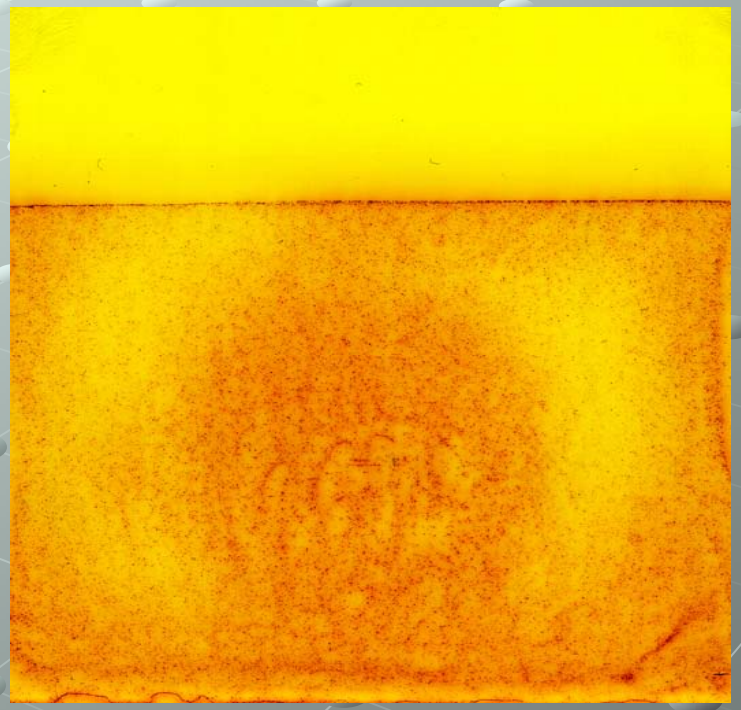
Time of incubation 48 h



10 ng 17-ß Estradiol



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Detection of Hormones - HPTLC-YES test

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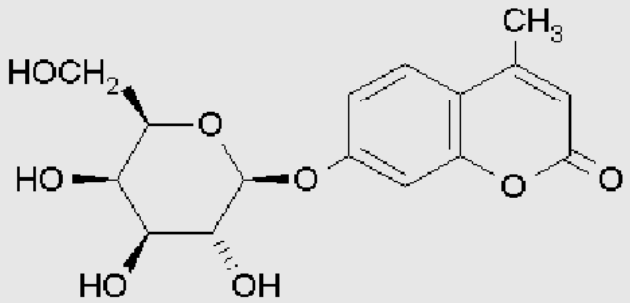
Detection of β-Galactosidase

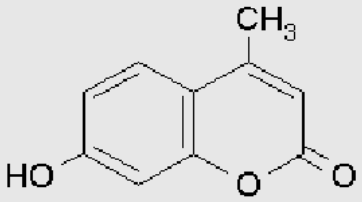
MUG

4-Methylumbelliferyl
β-D-galactopyranoside



β-galactosidase

+ H_2O



4-MU

4-Methylumbelliferone

non-fluorescent

fluorescent at pH > 8
λ_{ex} ~ 360 nm, λ_{em} ~ 460 nm

Detection of Hormones - HPTLC-YES test

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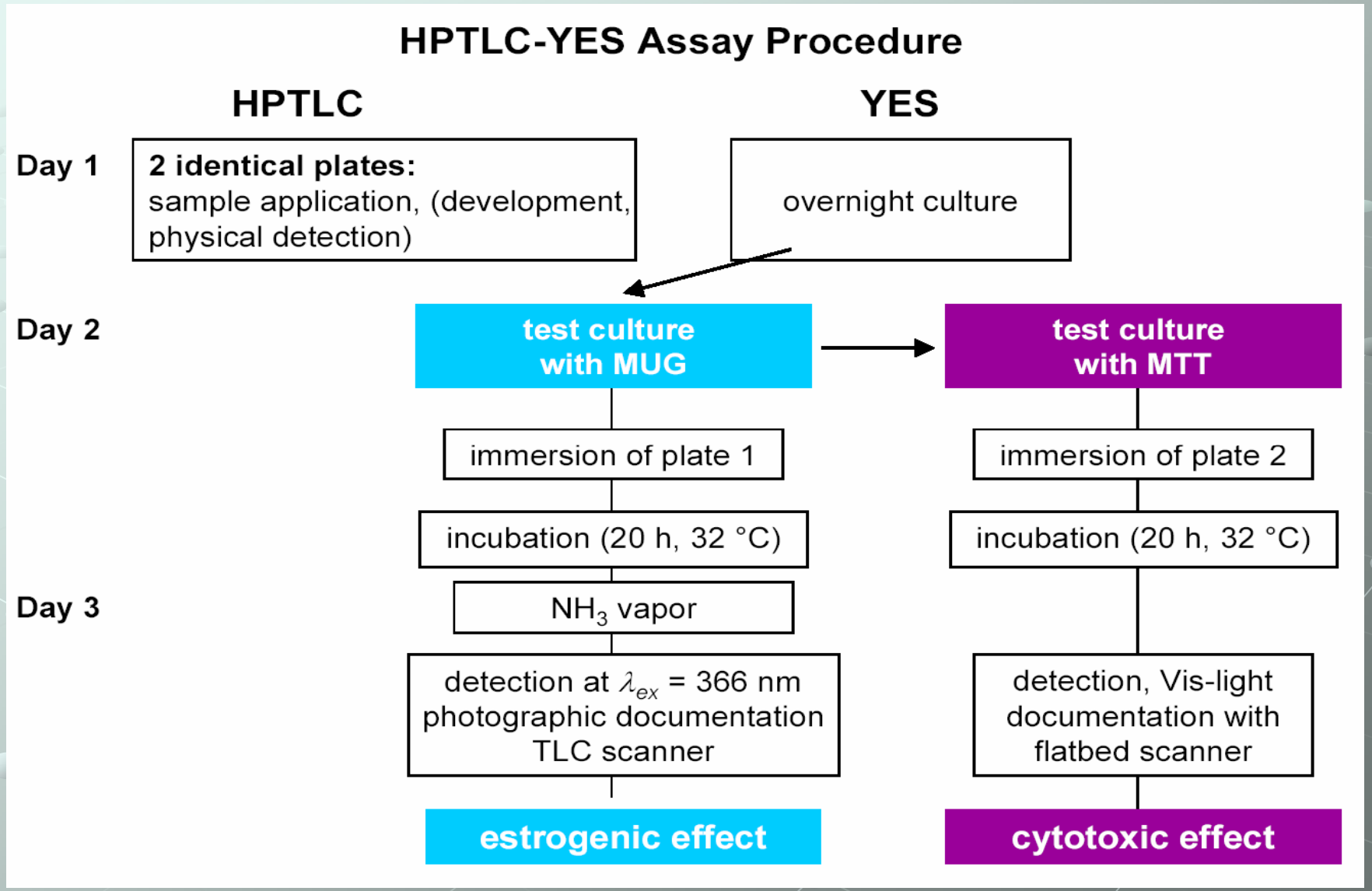

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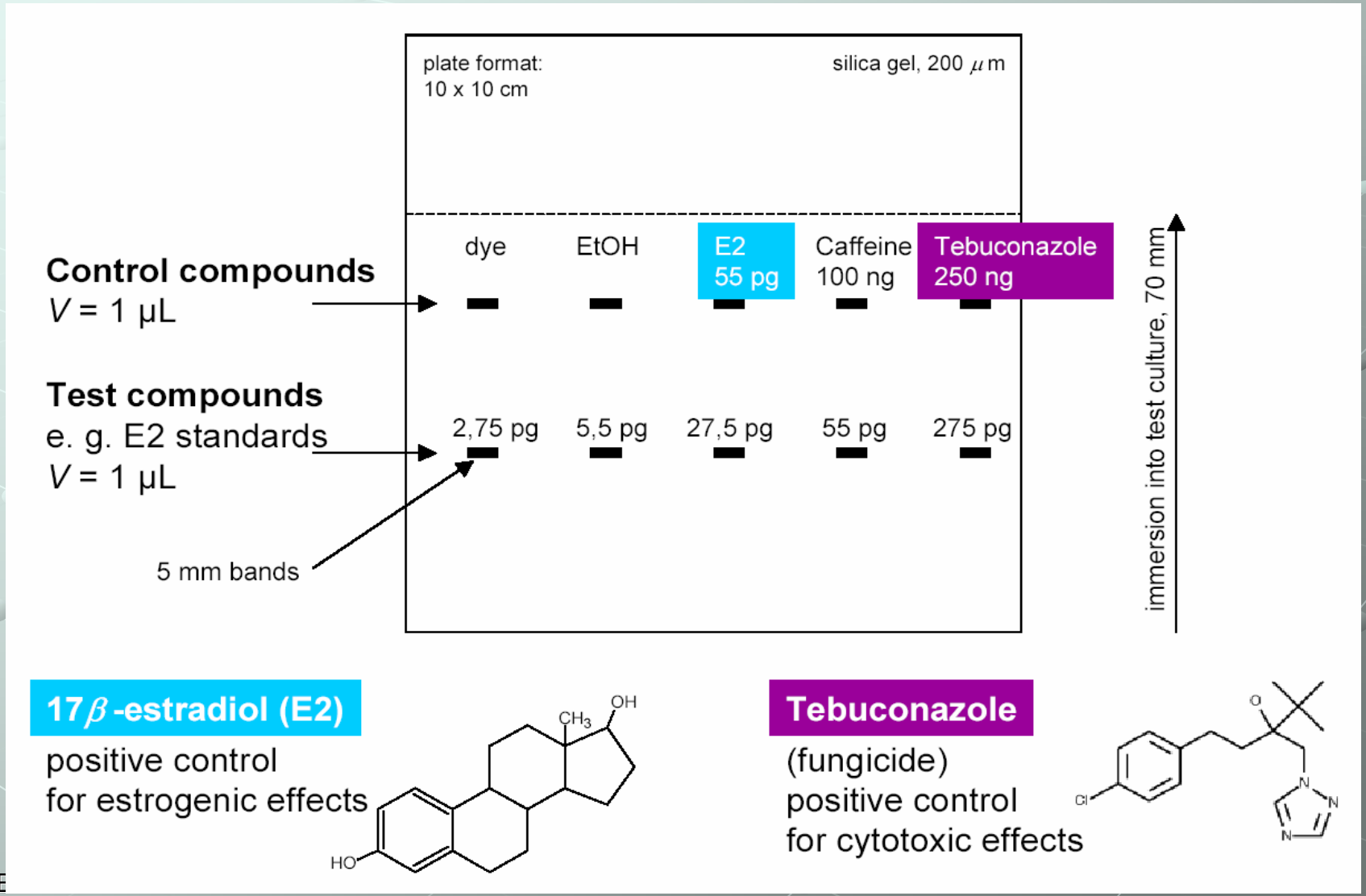
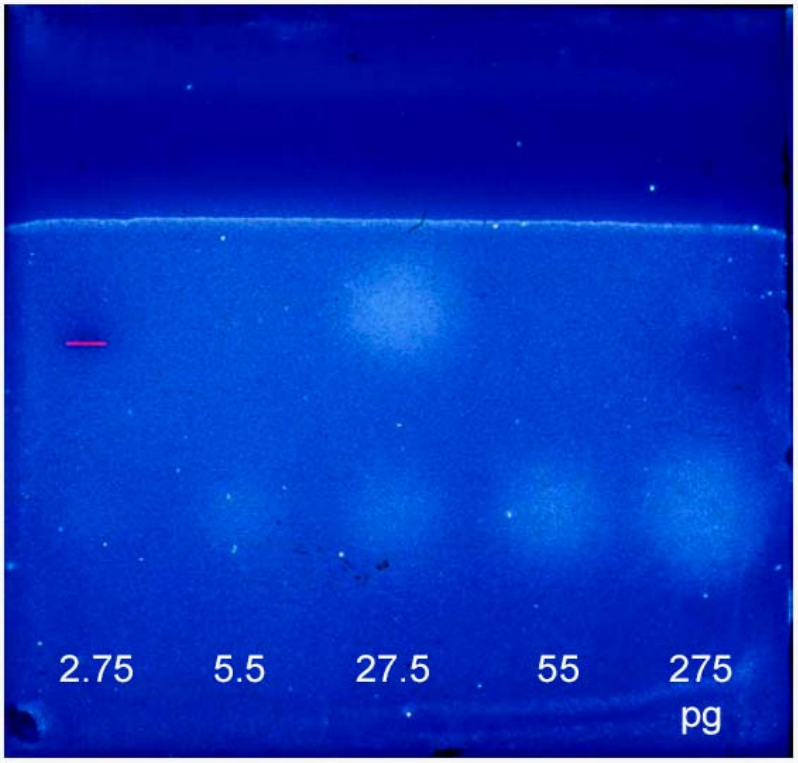


Detection of Hormones - HPTLC-YES test

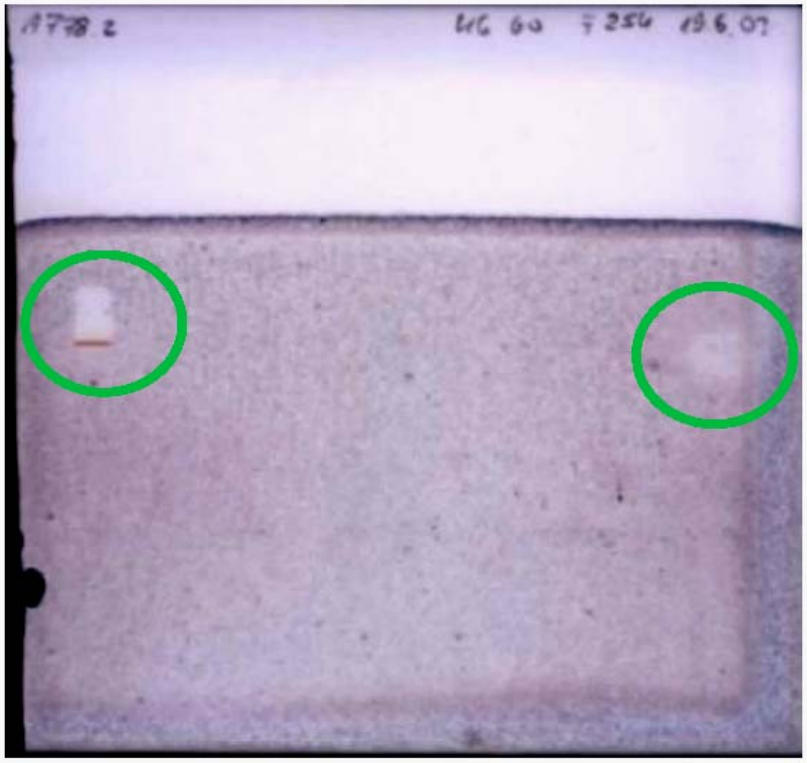
Yeast Estrogen Screen (YES) According to Routledge and Sumpter[1]

Estrogenic effects



Photographic documentation under UV-light (λ_{ex} = 366 nm, Hg-lamp)

Cytotoxic effects

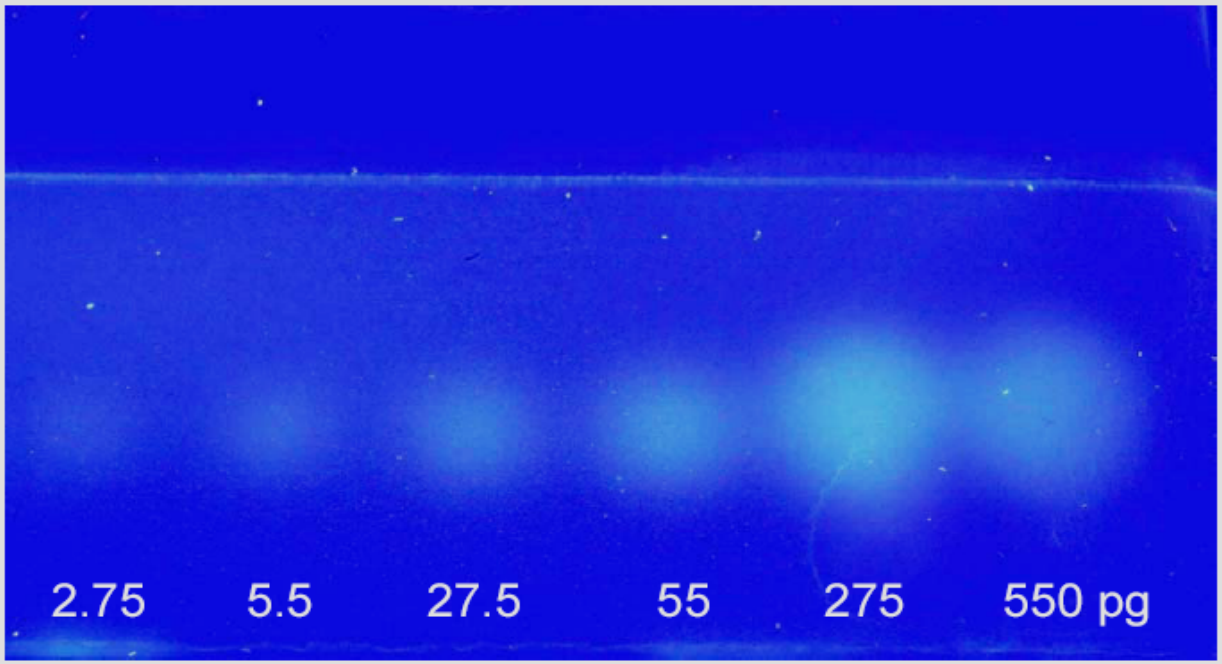


Documentation with flatbed scanner, Vis-light

Detection of Hormones - HPTLC-YES test

Yeast Estrogen Screen (YES) According to Routledge and Sumpter[1]

Detection limit of the HPTLC-YES test with β-Estradiol



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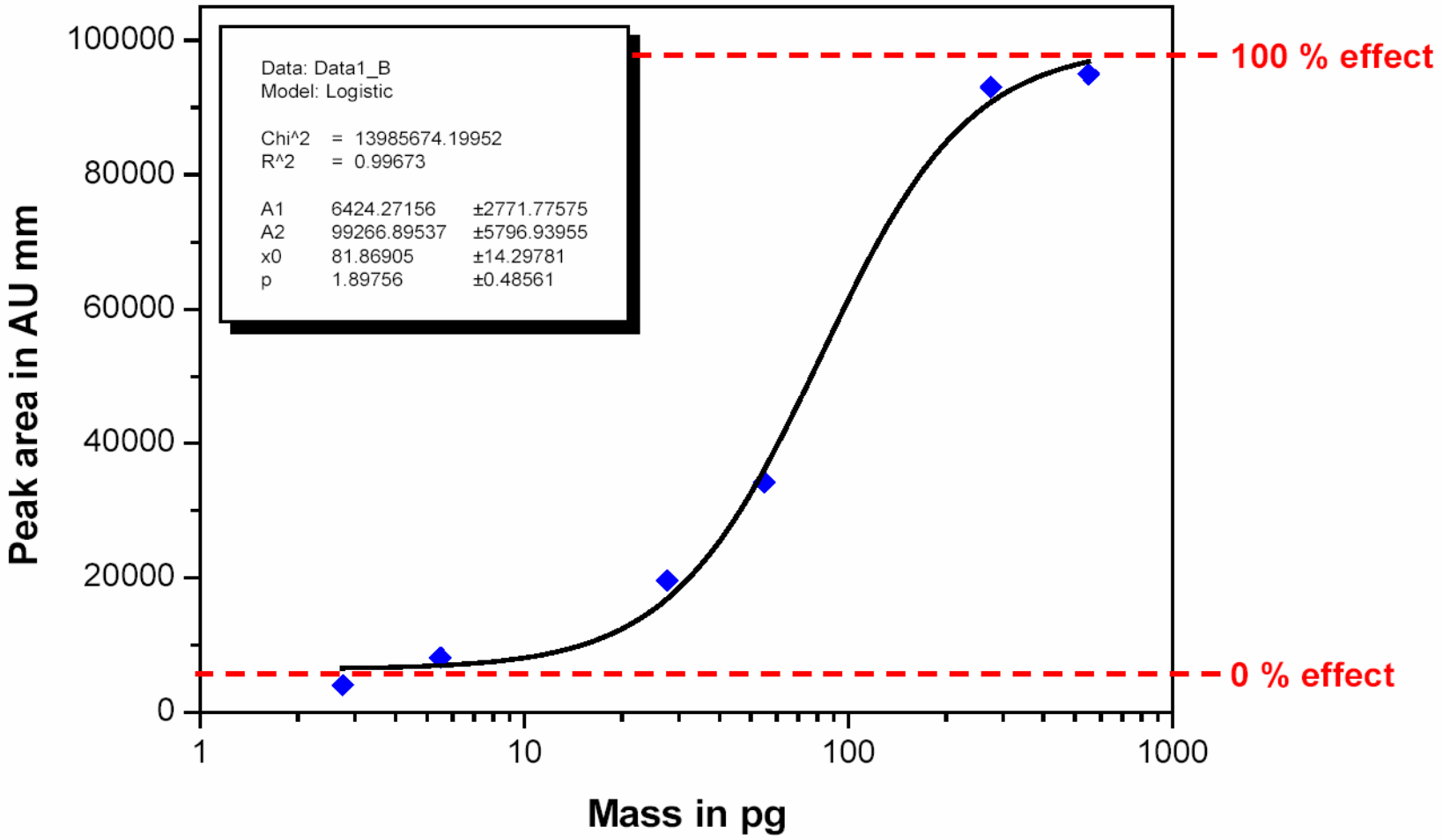
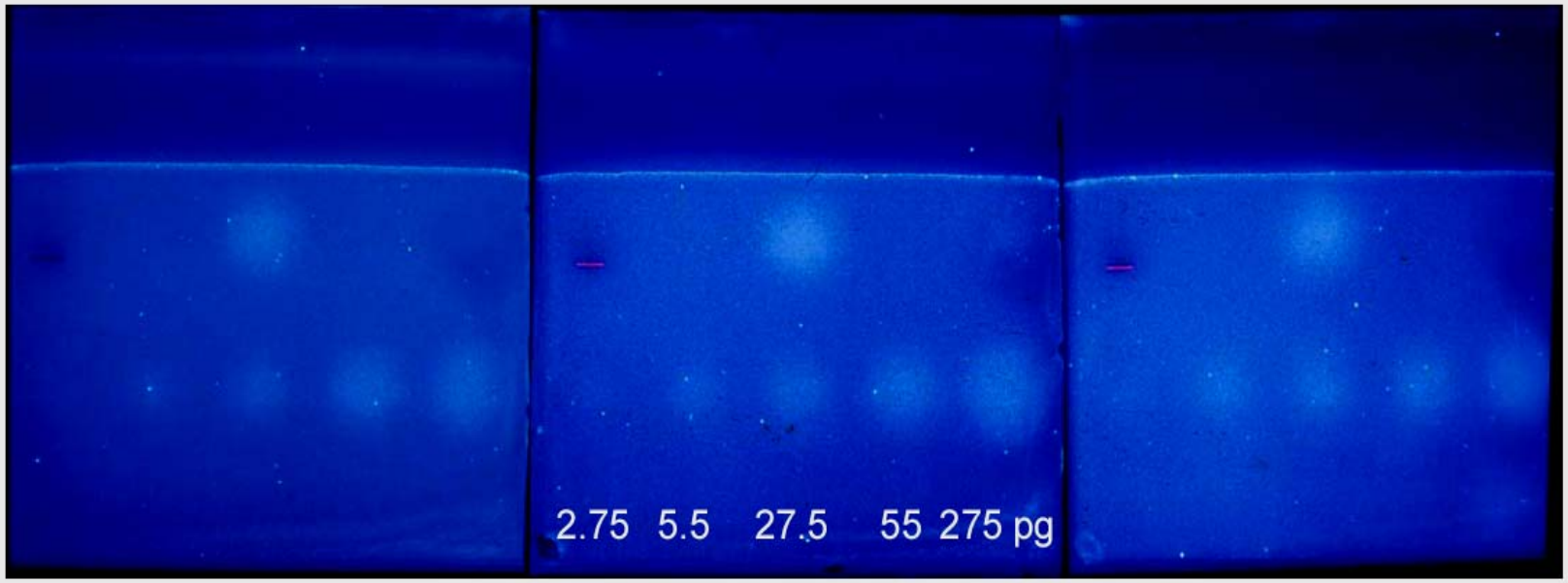


Detection of Hormones - HPTLC-YES test

Yeast Estrogen Screen (YES) According to Routledge and Sumpter[1]

Reproducibility of the HPTLC-YES with 17 β-estradiol



HPTLC plates with standard position of control and test compounds,
3 replicates immersed into same test culture;
Photographic documentation under UV-light (λ_{ex} = 366 nm, Hg-lamp)

Detection of Hormones - HPTLC-YES test

Yeast Estrogen Screen (YES) According to Routledge and Sumpter[1]

Procedure in general

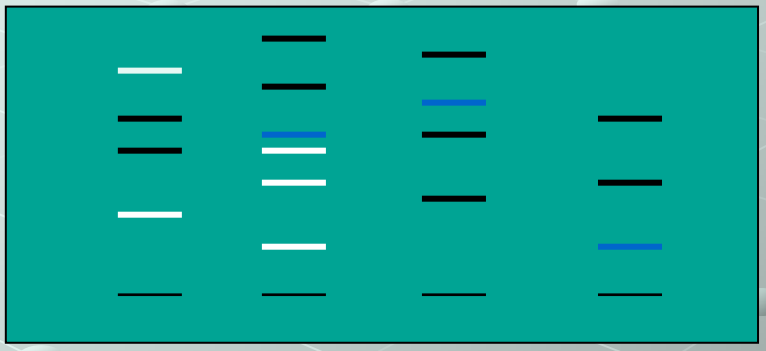
- **Step 1**: Immobilization of the test organism on to the HPTLC plate and create optimized condition of cultivation on the layer (HPTLC plate)

  induction of the target enzyme within the testorganism

- **Step 2**: Optimized condition for the enzymatic turnover of selectiv substrates (pH, buffer,..)

- **Step 3.** Optimized condition for detection the signal of the accumulated product of the enzymatic turnover

Examples for toxicological tests in situ



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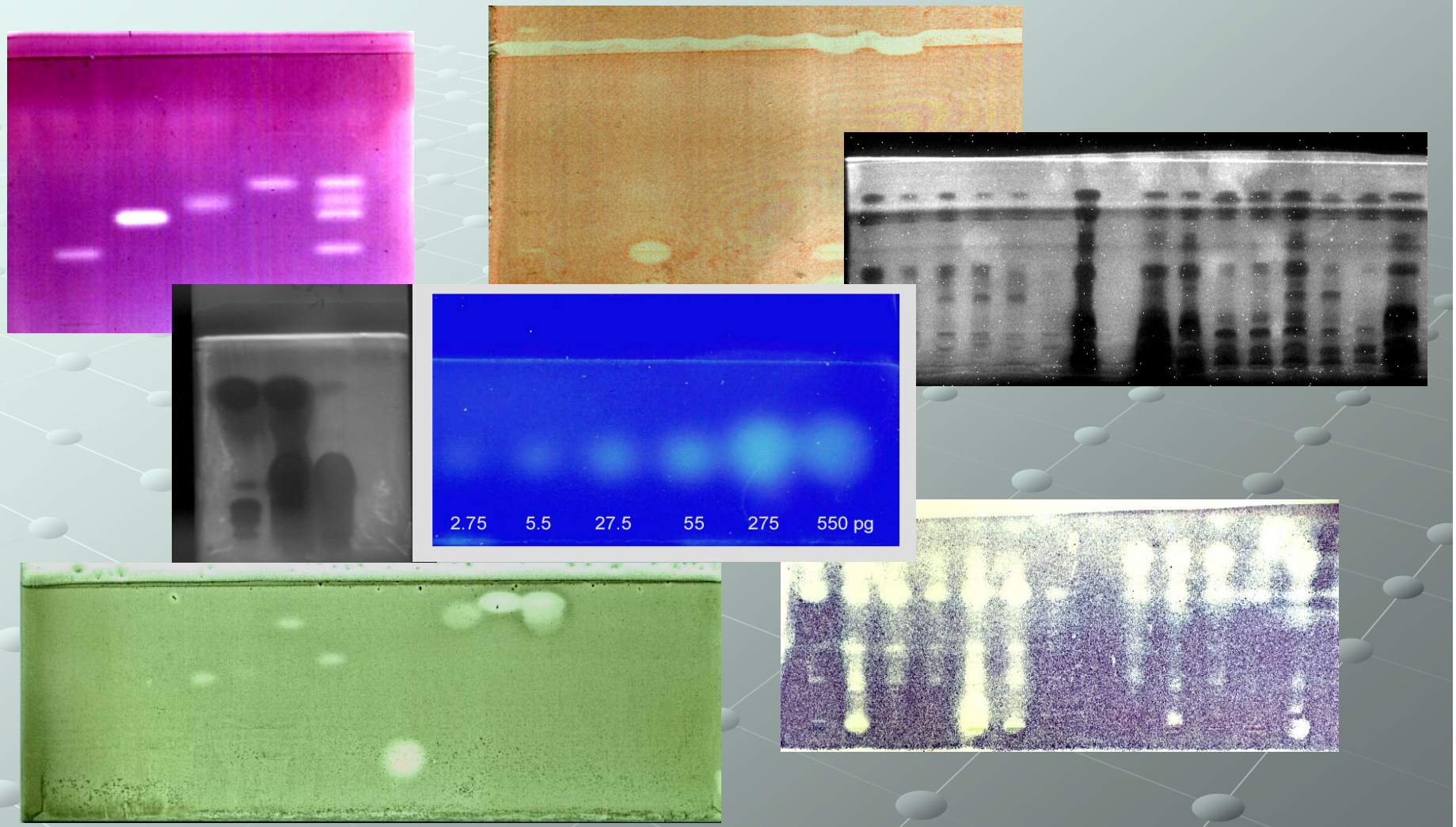
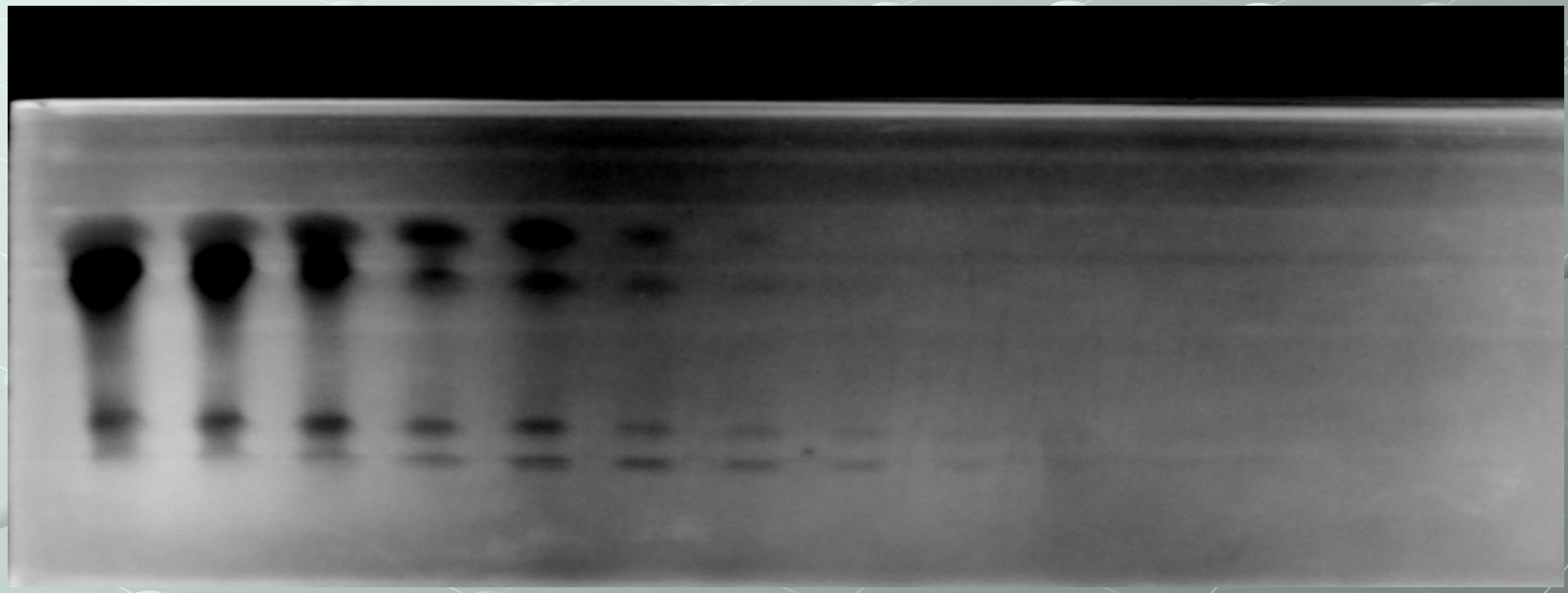


Verification of reference substances

Detection of inhibitors of cholinesterase in a solution of methiocarb



600 400 100 60 20 10 6 2 1 ng

Methiocarb 600 – 1 ng

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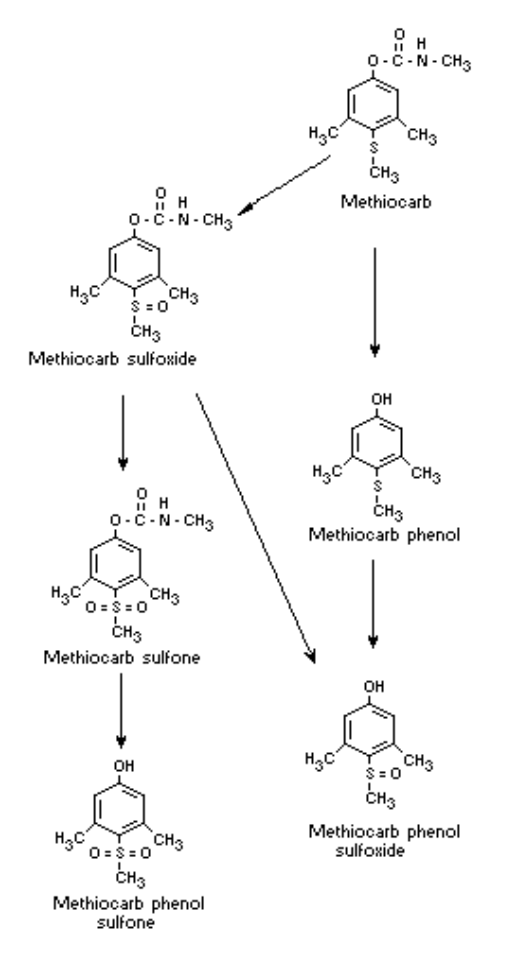

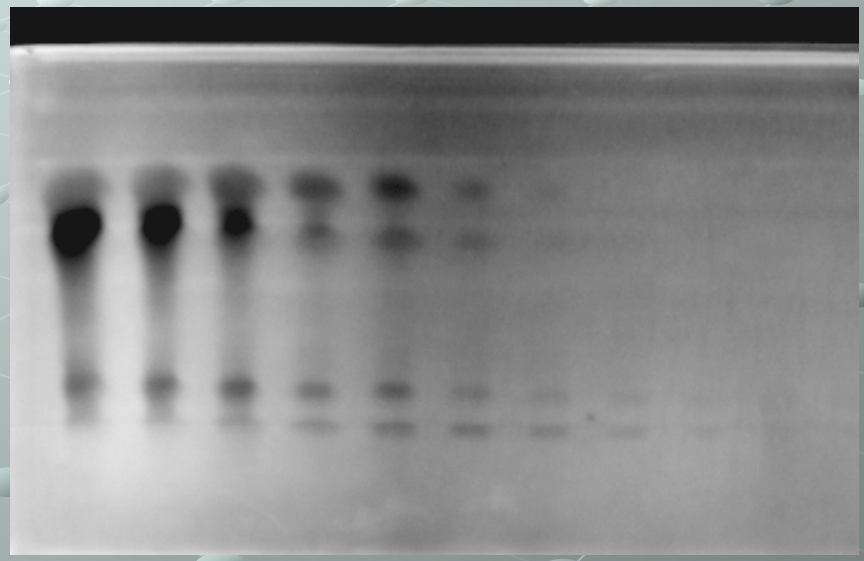

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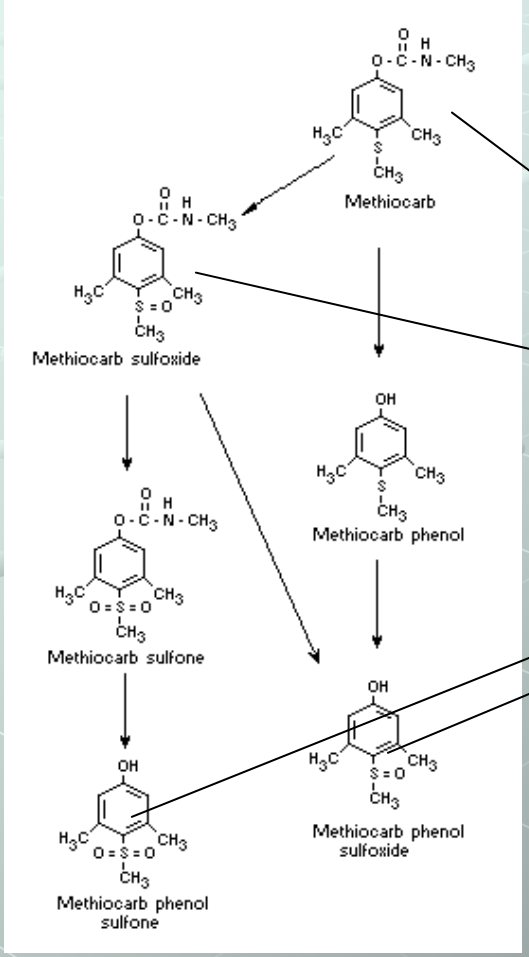
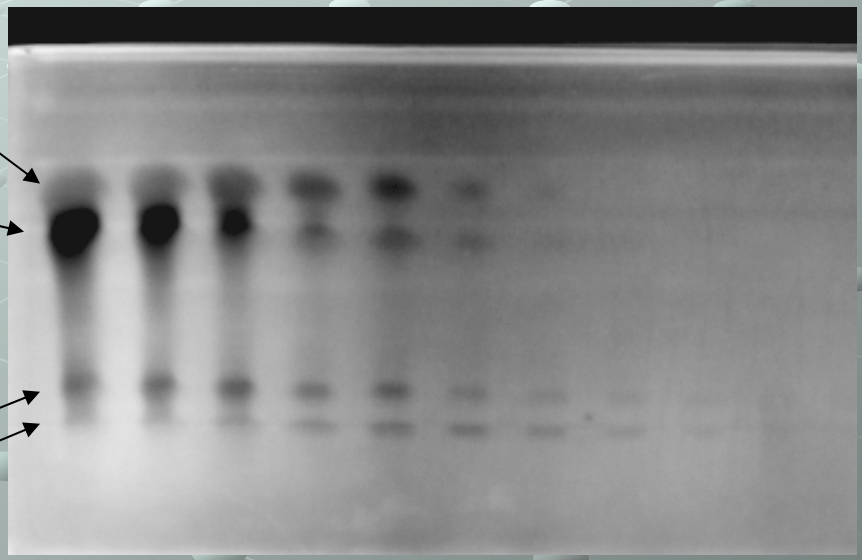
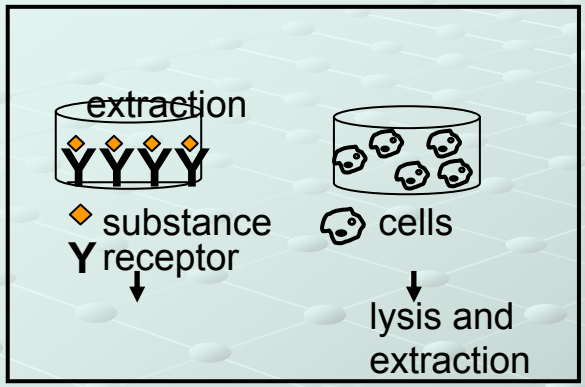




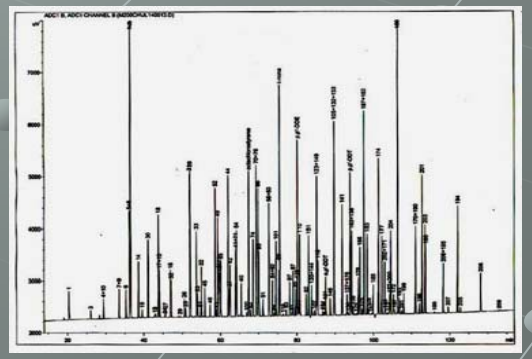
Outlook:
Bioactivity based analysis and ist future



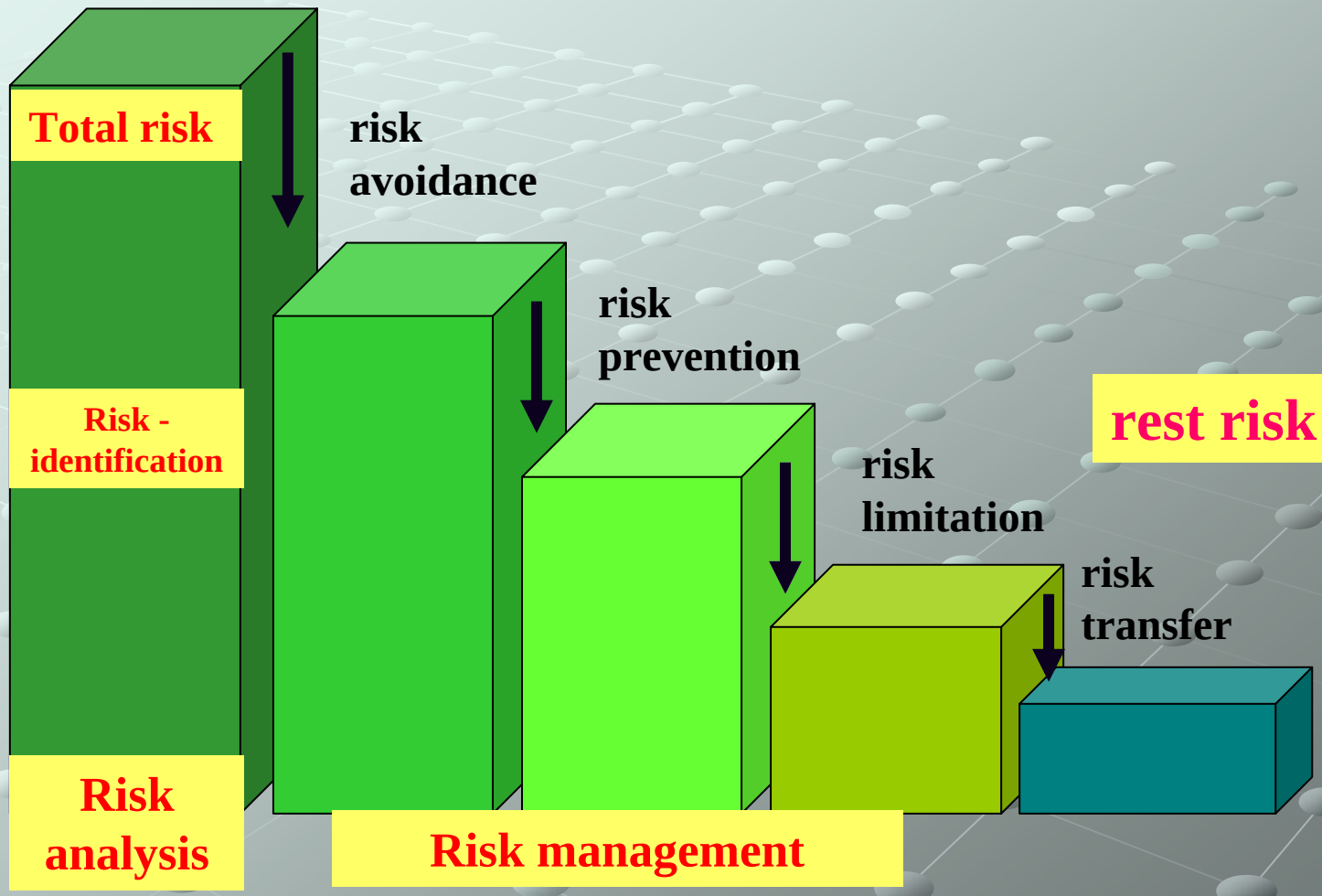
Sample preparation by biological recognition

biological tests in situ

identification

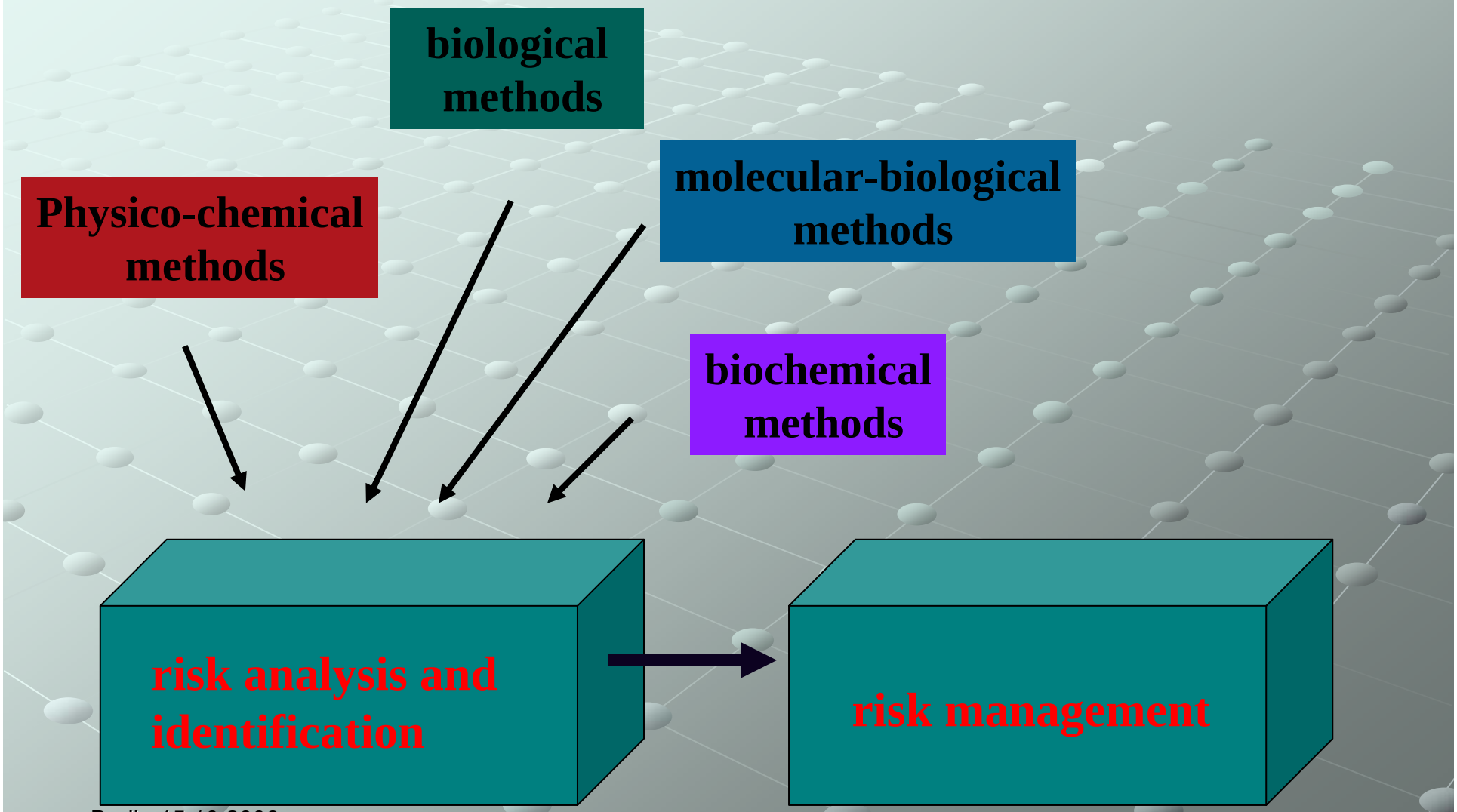


General Aspects of Risk assessment*



* Lecture about Information-management
of Prof. Dr. Dr. h.c. mult. Peter Mertens, Universitäty Erlangen-Nürnberg

Bioactivity based analysis a mandatory of risk analysis



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Berlin 15.10.2006

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YES