APPLYING AN EFFECT-DIRECTED STRATEGY TO THE SEARCH FOR UNRECOGNIZED TOXIC CHEMICALS

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Effect-directed analysis (or *bioassay-directed* analysis) combines analytical and bioassay methods:

- **Method goes back to 1970s-1980s; now being applied primarily in Germany**
- **Mainly recognized as a method for analyzing individual samples**
- **This work treats it as a broader method for *chemical screening* – less emphasis on analysis of individual samples**

EARLY USE OF EFFECT-DIRECTED ANALYSIS

and

BASIC PRINCIPLES

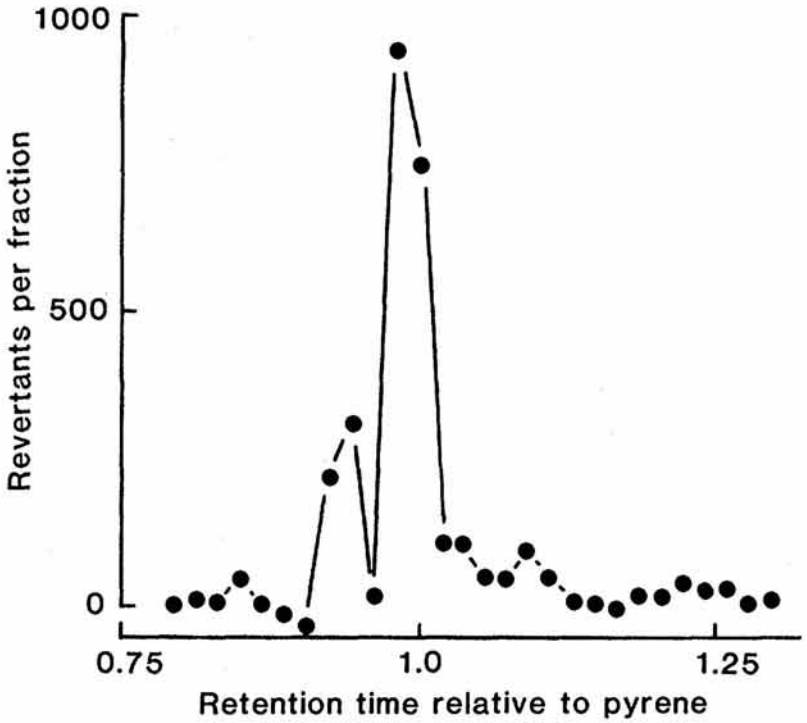
Rosenkranz and others (from New York Medical Institute, Cabot Corporation, and Xerox Corporation) found that:

“By combining HPLC fractionation with the microbial assay [Ames test] we were able to focus quickly on the most active fractions.”

Rosenkranz et al., *Science* **209**, 1039 (1980)

Same project as Rosenkranz et al.: Each dot represents a different HPLC fraction; vertical axis is bioassay response:

Fig. 1. Mutagenic response of fractions from a reverse-phase HPLC separation of the aromatic fraction of an extract from copies from a 4a machine. The sample, 50 μl containing activity corresponding to about 4000 revertants, was applied to a Spherisorb S5-ODS column (220 by 4.6 mm, internal diameter) with aqueous methanol as eluent, 2 ml per minute (gradient 38 percent methanol to 80 percent methanol for 10 minutes, then 80 percent methanol). Fractions (0.5 ml, 15 seconds) were collected and duplicate samples of 0.2 ml of each fraction were assayed for mutagenicity with TA98. The mutagenicity, expressed as net revertants above the spontaneous background per 0.5-ml fraction, is plotted against retention time relative to pyrene.



Lofroth et al., *Science* **209**, 1037 (1980)

Schuetzle (Ford Motor Company) and Lewtas (EPA) recognized that "…bioassays could be used in combination with chemical fractionation to greatly simplify the process of identifying significant mutagens in complex environmental samples…

"The use of short-term bioassays in conjunction with analytical measurements constitutes a powerful tool for identifying environmental contaminants."

Schuetzle & Lewtas, *Analytical Chemistry* **58**, 1060A (1986)

Rosenkranz, Lofroth, Schuetzle & Lewtas:

- ***Sample fractionation*** **by liquid chromatography (HPLC)**
- ***Bioassay*** **by Ames test**
- **See which sample fractions show *high bioassay response*; do further analysis of those fractions**
- ***Subfractionate*** **the active samples (at smaller intervals of HPLC retention time); perform bioassays and analyses on these subfractions**

What are liquid chromatography (HPLC)

and

retention time?

HPLC is *High-Pressure Liquid Chromatography* or *High-Performance Liquid Chromatography*.

It's a process that sorts or fractionates chemical mixtures

- **Different chemical compounds in a given sample are *retarded* or *delayed* by different amounts as they move through the HPLC process.**
- **The sample is dissolved in a solvent and pumped at high pressure through a specially designed *column* (usually a tube packed with small round particles with a certain surface chemistry).**

- **The amount of retardation (delay time in the column) depends on the compound and also on the column material and the solvent being used.**
- **The time at which a specific compound comes out of the end of the HPLC column is called the *retention time* and is considered a reasonably unique identifying characteristic of a given compound.**

Going into the column / into the race

All bunched up

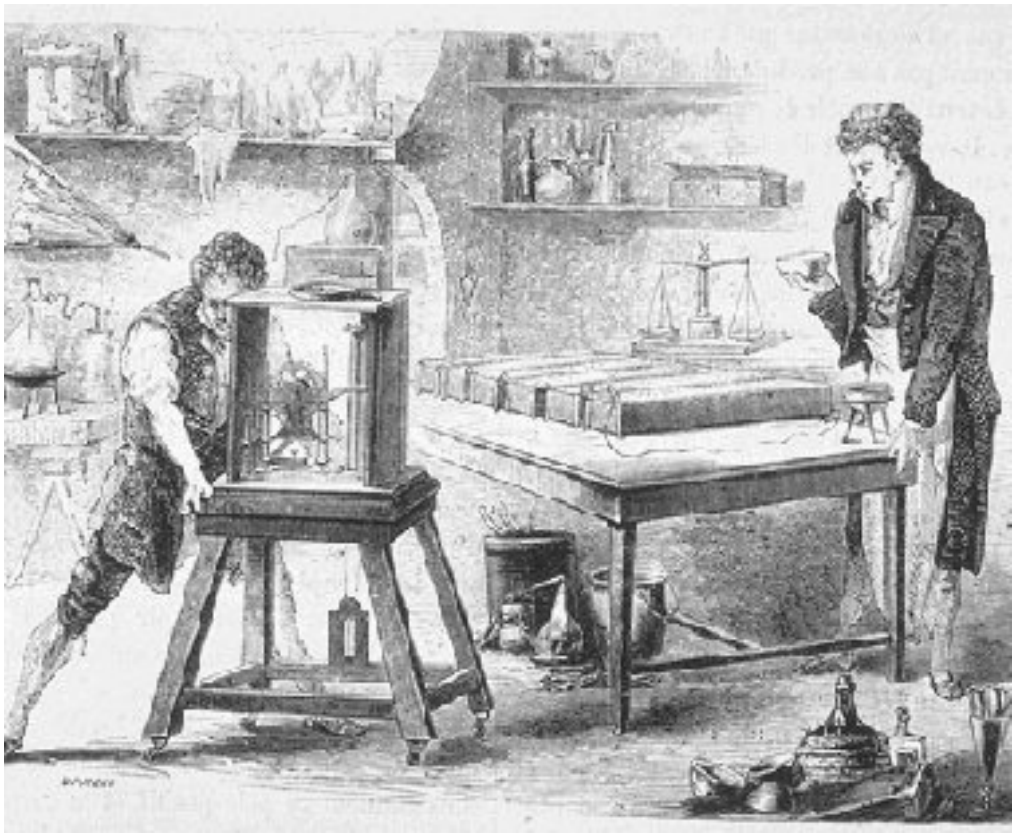
Coming out of the column / end of race

Well-sorted by “retention time”

- **Compounds that emerge from the HPLC column between retention times X and Y are one *fraction* of the sample and can be put automatically into a vial (using a fraction collector). Likewise for fraction between times Y and Z, etc.**
- **Bioassays and analytical tests can be done on the contents of these vials that contain different sequential fractions.**

What is a bioassay?

Usually not this…



…but usually not as highly automated as HPLC and other analytical methods

A bioassay is a short-duration test (e.g., several hours) in which:

- **Chemicals in an unknown sample interact with biological materials (e.g., a live cell broth in a 96-well plate)**
- **The interaction *correlates reasonably well with an accepted measure of toxicity* (not too many false positives or false negatives)**

- **The test yields a *quantitative measure* of the chemical interaction or toxicity**

- **The test usually responds broadly to certain categories or groups of chemicals in a sample; it usually cannot distinguish individual compounds but 'integrates' all responsive compounds**

- **The test can be performed on standard samples for calibration**

EXAMPLES OF BIOASSAYS:

Old: **Ames test (mutagens)**

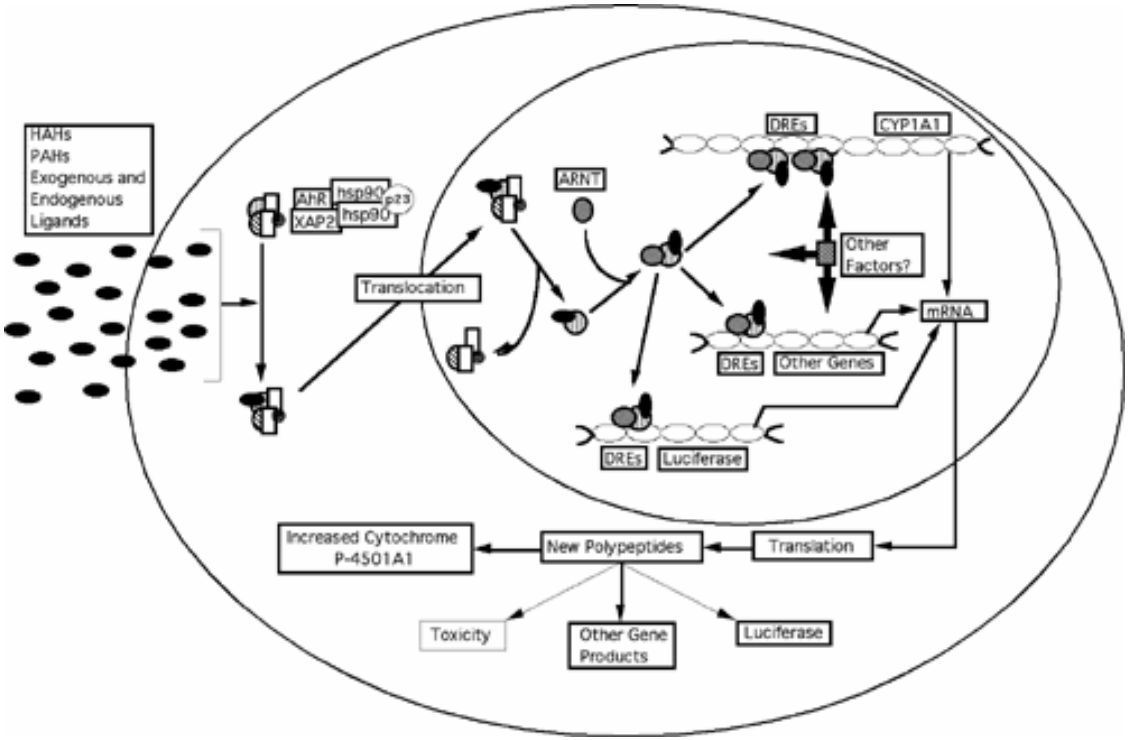
Current: **CALUX and other reporter-gene bioassays (dioxin-like compounds)**

EROD (dioxin-like compounds)

ER-CALUX (estrogenic compounds/endocrine disruptors)

Emerging: **Long-Term Potentiation??**

CALUX bioassay: Dioxin-like compounds interact with Ah-receptor.



Light emission is quantitative response.

For effect-directed analysis to be truly useful for chemical screening, additional bioassays are needed.

Additional bioassays should correlate with additional toxic effects or health outcomes.

CURRENT USE OF EFFECT-DIRECTED ANALYSIS

in

GERMANY

HELMHOLTZ CENTRE FOR ENVIRONMENTAL RESEARCH - UFZ

Research for the environment!

deutsch search

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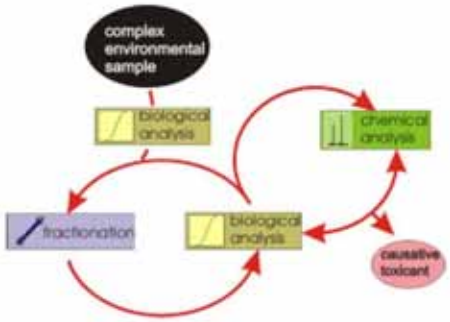

Department Effect-Directed Analysis

Potentially hazardous environmental pollution often occurs as a complex mixture of toxicants together with non-toxic natural and anthropogenic compounds.
Effect-directed analysis (EDA) focuses on the identification and assessment of major toxicants in complex environmental mixtures on the basis of adverse effects and exposure. The major goal is to unravel cause-effect relationships for a reliable risk assessment of environmental contamination.

Major topics of our research activities are:

- the development of integrated chemical and biological tools for the isolation of toxicants e.g. in sediments, ground- and surface waters,
- the chemical and ecotoxicological analysis of transformation processes in the environment including the identification of toxic metabolites,
- the analysis and evaluation of toxicant chemodynamics and bioavailability in effect assessment studies,
- the assessment of risks of complex contaminations in aquatic ecosystems.

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News

open PhD position "Integrated Analytical and Computer tools for Toxicant Identification in Effect Directed Analysis"

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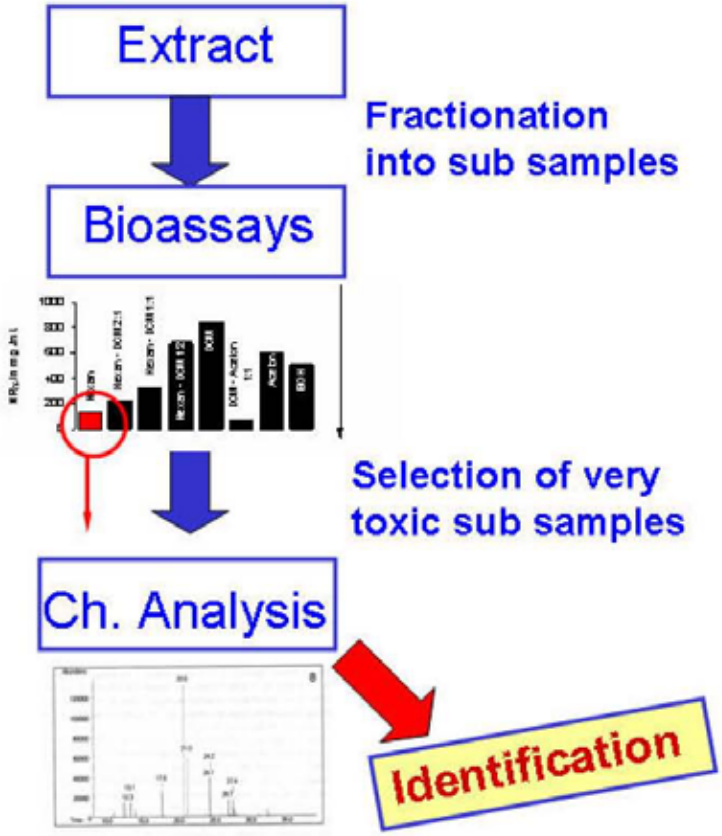
Bioassay-directed fractionation procedures

In general, sediments are contaminated with thousands of chemicals. These include heavy metals, pesticides,
pharmaceuticals, detergents, byproducts of industrial production, and incineration products as well as innumerable metabolites thereof. A complete chemical analysis of these compounds is impossible. Therefore, the assessment of the chemical quality of aquatic environments according to the EU Water Framework Directive is based on a limited number of priority pollutants. Unknown and unexpected compounds are not considered. However, using bioassays mostly only a minority of the biological effectiveness of the samples could be explained by the substances analyzed chemically. Thus, non-analyzed compounds are causing the biological effects.
Therefore, reliable hazard and risk assessment require both the detection of adverse effects and the identification of the chemicals causing the effects. This can be done by combining biological testing, physicochemical fractionation and chemical analysis. One approach is the bioassay-directed fractionation. This is done by the sequential reduction of the complexity of environmental mixtures eventually to individual toxicants. The sediment extracts are tested for biological effects and subjected t

one or several fractionation procedures. After each separation step the fractions are biotested for selection
of active fractions for further investigation. When the complexity of the mixture is reduced to a few individual compounds, the fractions are subjected to chemical identification and quantification.



Recent projects: Bioassays and effect-directed fractionations to evaluate the ecotoxicological hazard of suspended matter during flood events

REVIEW OF *STANDARD* AND *NEW* COMPONENTS OF AN EFFECT-DIRECTED STRATEGY

Effect-directed analysis: Standard step 1:

Environmental samples are fractionated, then each fraction is tested by bioassay(s) for one or more toxic effects.

Standard step 2:

Any bioassay-responsive fraction is separated into finer fractions, and these in turn may undergo further fractionation based on their bioassay responses.

Standard step 3:

After bioassay-informed fractionation has been carried to a practical limit, each bioassay-responsive fraction is tested by analytical methods to isolate/identify the compound(s) that produced the response.

New, additional part of the strategy: Proportional response

Isolation and identification are aided by the *proportionality* between analytical peaks and bioassay responses in *many different samples*.

The task of correlating high bioassay responses to analytical results (to isolate and identify toxic compounds) is made more difficult when two or more bioassay-responsive compounds end up in the same fraction or subfraction (how to find what produced response?)

A single sample, or several similar samples, won't help much

PROPORTIONAL RESPONSE in many different samples:

- **Different environmental samples (e.g., from entirely different contaminated sites) tend to contain *different proportions* of bioassay-responsive compounds**

- **Zero bioassay response = zero analytical detection (no peak)**
- **Low bioassay response = low analytical detection (small peak)**
- **High bioassay response = high analytical detection (large peak)**
- **Thus: Proportional response**

- **Can write and solve simultaneous equations AX = B for a *given fraction* of several *different* samples, where**

B=known bioassay responses of samples

A=known analytical results for peaks and associated compounds

X=unknown relative potencies of cpds.

The task of correlating analytical peaks with bioassay responses to identify toxic compounds may be complicated, but should not defeated, by:

- **Not-entirely-linear dose-response in bioassay (producing an *approximately* proportional response)**
- **Synergism and antagonism (important to recognize and understand in any case)**

SUMMARY

EFFECT-DIRECTED ANALYSIS

- **provides an efficient strategy for finding and cataloging toxic compounds in the environment**

- **is a useful complement to the lists of toxic chemicals in commerce that are being compiled by Environment Canada, EPA, and others**

EFFECT-DIRECTED ANALYSIS

- **focuses on *chemicals in the environment* rather than chemicals in commerce (so lists may be somewhat different due to weathering, metabolic processes, etc.)**

EFFECT-DIRECTED ANALYSIS

- **should be conducted on the widest possible variety of environmental samples, including samples from highly contaminated sites**
- **will require (and will help promote?) the development of additional bioassay methods**

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