

## ***Effluent testing with cell-based In vitro bioassays: use and limitations***



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During the last decade, a range of novel, cost-effective bioanalytical methods (bioassays) have been developed for dioxins, dioxin-like compounds and potential endocrine modulators. These *in vitro* screening tests are designed to give rapid, cost-effective estimates of the toxicity of single compounds or mixtures. Whilst they are able to indicate the presence of certain groups of substances in well-understood media based on a toxic response, caution is needed in broadening their application to complex media such as effluents. *In vitro* screening tests should be validated for appropriateness in effluent testing, and the relevance of results carefully interpreted. At their current stage of development, such tests are suitable only for screening purposes. *In vivo* tests are more appropriate to assess direct toxicity and should preferably be used for risk assessment.

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### **What are *in vitro* bioassays and how do they work?**

An example is the Chemically Activated Luciferase Expression or CALUX\* reporter gene bioassay used to detect dioxins and compounds with a similar toxic mode of action. The cells used in the DR-CALUX (Dioxin Receptor-CALUX) bioassay originate from humans and have been tailored to produce light in a dose-responsive way when exposed to dioxins and dioxin-like chemicals. This means that when more of the substance is present, more light is emitted. The switch that turns on the light is sensitive and somewhat specific to these compounds, making the test selective and relevant for specific biological responses.

The toxicity of polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs), and some polychlorinated biphenyls (PCBs) results from a common mode of action involving binding to a specific molecule in a cell known as the aryl hydrocarbon (Ah) receptor. Binding to this receptor is followed by the transportation of the substance-receptor complex into the cell nucleus and subsequent specific binding to DNA (the genetic material in cells). These binding sites on the DNA are called dioxin-responsive elements (DREs). Binding of the complex to the DRE triggers the expression of DRE-associated genes, and in the case of DR-CALUX, gene expression is in the form of light that can be measured.

The same principle can be applied to other modes of toxic action where it is possible to build in specific receptors into assay cells. An example is the oestrogen receptor, which reacts to substances that behave similarly to this reproductive hormone (a so-called hormone-disrupting substance or endocrine modulator).

Another type of bioassay is the Carp Hepatocyte assay, based on fish liver cells from the common carp, *Cyprinus carpio*. It involves exposure of hepatocytes isolated from fish to the various test chemicals for up to eight days. The cells are not genetically modified. The Carp Hepatocyte assay also detects dioxin-like substances via the Ah receptor based on the induction of cytochrome P450 enzymes. One of these enzymes (EROD [7-ethoxyresorufin-O-deethylase]) reacts with a substrate to form a stable and fluorescent compound in a dose-responsive way which can easily be measured using a fluorimeter.

The assay can also assess the oestrogenic potencies of chemicals by measuring the specific egg yolk protein vitellogenin.

\*DR-CALUX™ is marketed by BioDetection Systems ([www.biodetectionsystems.com](http://www.biodetectionsystems.com))

### **Effluent testing: limited experience to date**

Effluents are usually complex mixtures of diverse substances, including particles. Scientists have gained extensive experience of acute toxicity testing - and to a lesser extent chronic toxicity testing - of effluents and how to interpret results. Nevertheless, many practical limitations are still encountered in applying standard toxicity tests to effluents. With current experience and an understanding of the effect of confounding factors such as acidity, salinity or ammonia, WET (Whole Effluent Testing) testing can give useful results in certain cases.

For effluent testing, the new DR-CALUX and similar cell-based *in vitro* tests demonstrate promising advantages such as speed, low cost and the ability to give an indication of specific toxicity that usually is not expressed in acute toxicity tests. However, they have to date only been used to a limited extent on effluents, making interpretation of test results difficult or in some cases impossible. Additional experience will be essential to improve the interpretation of test results and their relationship to actual environmental impacts. At present, bioassays like the DR-CALUX assay are only suitable as an initial screening step to prioritise effluents or effluent fractions for further study.

### **Current applications for foods and sediments**

The DR-CALUX method applied with an acid clean-up step has been validated extensively and meets criteria specified by the EU Food Directive (2001). It can successfully screen for dioxins and dioxin-like PCBs in accidental food contamination incidents and in regulatory screening of food and animal materials. Any positive screening results still need to be confirmed by specific chemical analysis.

In The Netherlands, the DR-CALUX method has also been tested for screening harbour dredgings. Together with several other tests it is included in the new regulatory framework for evaluation of offshore disposal of harbour sludge.

### **False positives and negatives**

Bioassays can give both false negative and false positive results. False negative results may fail to highlight real health or environmental risks; false positives may imply health or environmental risks where in fact there are none. Due to the high sensitivity of these tests, false positives are likely when applied to complex mixtures like effluents. For example, it is known that, even with an acid clean-up, DR-CALUX will respond to various substances without dioxin-like toxicity, so careful interpretation of results is needed.

### **Conclusions**

Methods are now available that detect tiny quantities of chemicals which may potentially be dioxin-like or endocrine-disrupting chemicals. However, questions remain about which chemicals are responsible when positive results are obtained from drinking water, wastewater, freshwater and seawater, soil, mud or any other sample. For effluents, it is a challenge that samples generally contain many compounds, with the result that false positives are frequently obtained. In the case of a positive response the sample may be split up and analytical methods used to try and identify the responsive chemicals.

Since these tests are highly sensitive and specific to the cell type used, the relevance of positive results for other species, living animals and longer-term exposures is the subject of ongoing studies. Consequently, a positive assay result should always be complemented with an *in vivo* assay and/or analytical detection to confirm the response. Only additional studies - coupled with a proper risk analysis, taking exposure into account - can confirm if responses indicate a genuine environmental risk.