Human cell based screening tests (CALUX) for the quantitative analysis of plastic additives and complex mixtures of food packaging materials

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Introduction

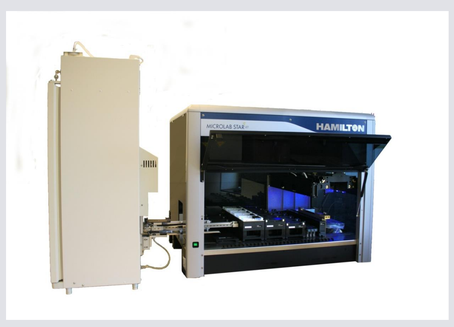
According to EC Framework Regulation 1935/2004, food packaging materials should not endanger human health. Constituents that can migrate from packaging plastics into food include a variety of endocrine disrupting chemicals (EDCs), such as the plasticizers bisphenol A and phthalate. A novel approach to analyze endocrine disrupting effects of constituents of plastic packaging materials is by using effect-based human in vitro reportergene assays such as the CALUX (Chemically Activated LUciferase gene eXpression) panel. This panel can be used to measure effects caused by either single compounds or complex mixtures.

Methods and materials

Various packaging plastics were extracted and measured in a panel of CALUX reporter gene assays to determine the activity profile of the extracts.

- endocrine activity was determined using the ERα-, anti-AR-, anti-GR, anti-PR and TRβ-CALUX
- AhR-receptor activation was determined using the PAH CALUX
- obesogens were detected using the PPARα/δ/γ CALUX

In short, CALUX cells were seeded in 96- or 384-wells plates and exposed to a dilution series of the plastic extracts or reference compounds using a liquid handling robot (Hamilton). After 24 hrs. incubation at 37°C and 5% CO_2, the cells were lysed, and the luciferase formed was measured in a luminometer equipped with a stacker (Berthold).



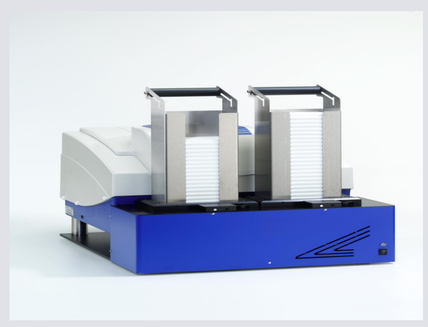


Figure 1: Automated CALUX testing using a robot and a luminometer (+ stacker)

Results

In table 1, a summary of the CALUX analysis results obtained for typical plastic additives and food migration materials. As expected of these known endocrine disrupting chemicals, the compounds showed estrogenic , anti-androgenic, PPAR, anti-PR, ant-GR and BaP-like activities.

Compounds	ERα CALUX	anti-AR CALUX	PPARα CALUX	PPARγ CALUX	anti-PR CALUX	anti-GR CALUX	PAH CALUX
Bisphenol A	**-7.3**	**-6.8**	>-4.0	>-4.0	**-5.5**	**-4.5**	>-4.0
Terephthalic acid	**-5.6**	>-3.0	>-3.0	>-3.0		>-3.0	>-3.0
Orthophthalic acid	**-5.4**	>-3.0	>-3.0	>-3.0		>-3.0	>-3.0
Metaphthalic acid	**-5.7**	>-3.0	>-3.0	>-3.0		>-3.0	>-3.0
Diisobutylpthtalate	**-5.5**	**-5.5**	>-4.5	>-4.5	-6.0	>-4.5	>-4.5
Butylbenzylphthalate	**-5.5**	**-6.1**	>-3.9	>-3.9	**-5.8**	>-3.9	**-4.0**
Di(n-hexyl)phthalate	**-5.4**	**-5.0**	>-3.5	>-3.5	**-6.5**	>-3.5	>-3.5
Dibutylphthalate	**-5.6**	**-5.5**	>-4.5	>-4.5	**-5.5**	>-4.5	>-4.5
Diethylphthalate	**-4.5**	**-5.0**	>-3.5	>-3.5	>-3.5	>-3.5	>-3.5
Nonylphenol	**-5.4**	>-4.9	>-4.9	>-4.9	>-4.9	>-4.9	>-4.9
PFOA/PFOS	n.a.	n.a.	**3.1E-05**	**3.6E-05**	n.a.	n.a.	n.a.

Table 1: Activity of several plastic additives in the CALUX panel (EC_{10} in LOG M). PFOA/PFOS are reported compared to GW7674 (PPARα) and Rosiglitazone (PPARγ).

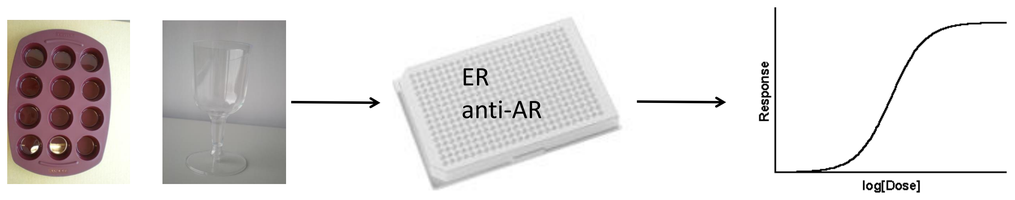
References

1 Behnisch (2012). Hidden cocktails uncovered. FoodLab International, Nr 2, 29-33.
2 WDR Markt TV Show 2009.
3 Brüschweiler, B., Kunz, P. (2011) Hormonaktive Substanzen in abgepacktem Mineralwasser? Bundesamt für Gesundheit, Bulletin 14/11, 311-316 .
4http://www.bds.nl/1/doc/4%20Narbonne%20Drinking%20Water%20ER%20CALUX%20YES%20Oct%202009%20short.pdf

The CALUX panel has already been used successfully in several international studies regarding food packaging, drinking water and plastic migration[1]:

- **Germany:** Testing of migration of plastic materials from baby food packaging in plastic (NaturNes) compared to glass materials (Hipp):
- *No difference observed between plastics and glass (ERα, anti-ERα, anti-AR, TRβ and PAH CALUX)[2].*

- **Belgium:** Food packaging extracts were analysed for (anti)-estrogenic and (anti)-androgenic activity
- *Estrogenic activity was found in all flexible elastomers.*
- *Anti-androgenic activity was found in 2 out of 3 polycarbonate samples.*



- **Switzerland:** Drinking water in PET- and glass bottles was analysed for estrogenic activity
- *50% of the samples slightly exceeded the limit of 3.6 pg EEQ/l water, but there was no significant difference between PET- and glass bottles[3]*

- **France:** Drinking water in PET bottles was analysed for estrogenic activity
- *All samples were below the detection limit of the CALUX assays (80 pg EEQ/ l water)[4]*

Perspectives

The CALUX panel for the detection of endocrine disruptors can also be applied in other areas. One example is the detection of estrogens in milk (cow's-, soya- or mother's milk) and other diary products.

Also obesogens such as PFOA, PFOS, endrine, methoxychlor or TBT are easily detected using the PPAR CALUX panel approach (see also table 1).

Conclusions

The human cell based CALUX bioassay is a useful tool to evaluate the safety of packaging materials and plastic additives. Moreover, the described panel can be used to determine the extent of migration from plastic to food.

In addition to endocrine disruption, the CALUX panel is able to analyze a variety of other toxicologically relevant endpoints such as DNA damage, AhR receptor activation, hypoxia , obesity and oxidative stress.

The CALUX assay is cost-effective, fast, animal-free, effect-based and quantitative, and is therefore a valuable addition to the existing hazard prediction methods such as chemical analysis and animal testing.

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