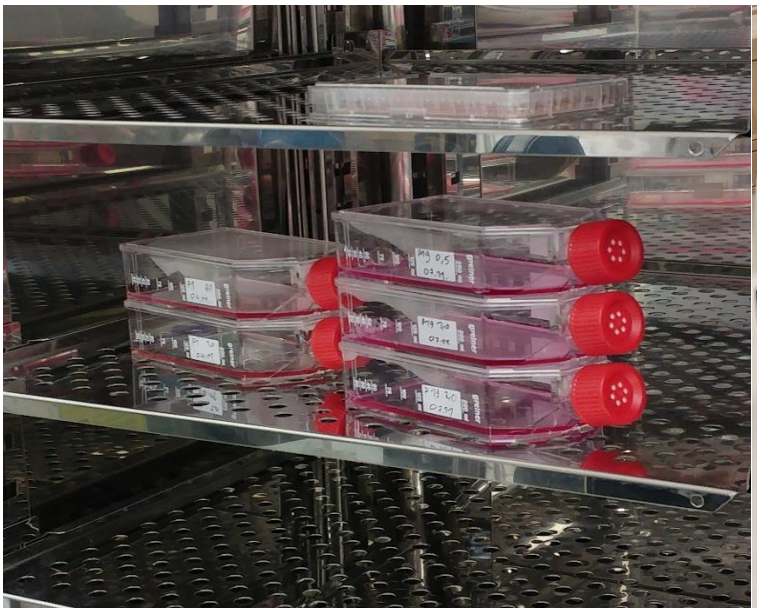
An *in vitro* bioassay for the detection of dioxins in foodstuffs and routine use in the Hessian State Laboratory



Dioxins, furans and dioxin-like polychlorinated biphenyls (dl-PCB) belong to a group of particularly toxic and persistent environmental pollutants. These pollutants arise as by-products during the production of organochlorine compounds or during the combustion of organic materials containing chlorine (Fig. 1). In comparison, dl-PCB has been added in the past as so-called "technical mixtures" for plastics, as heat exchangers in transformers, as dielectrics in condensers, as well as paints, joint seals and hydraulic fluids. In the past, large quantities of dioxins, furans and dl-PCB were released into the environment and food chains via the various manufacturing processes and areas of application. Due to their good fat solubility and longevity, these toxins accumulate in the fatty tissue of organisms at the end of the food chain. Some compounds of these substance classes can develop a broad spectrum of harmful effects in animal experiments in very low concentrations.

Dioxins and furans

- Combustion processes: Household and industrial waste, natural fires
- By-products during production of organochlorine compounds
- Metallurgical processes

Dioxin-like polychlorinated biphenyls

- 1930-1985: >1 million tons worldwide
- Insulating media in transformers
- Paints, varnishes, corrosion inhibitors
- Elastic joint seals etc.

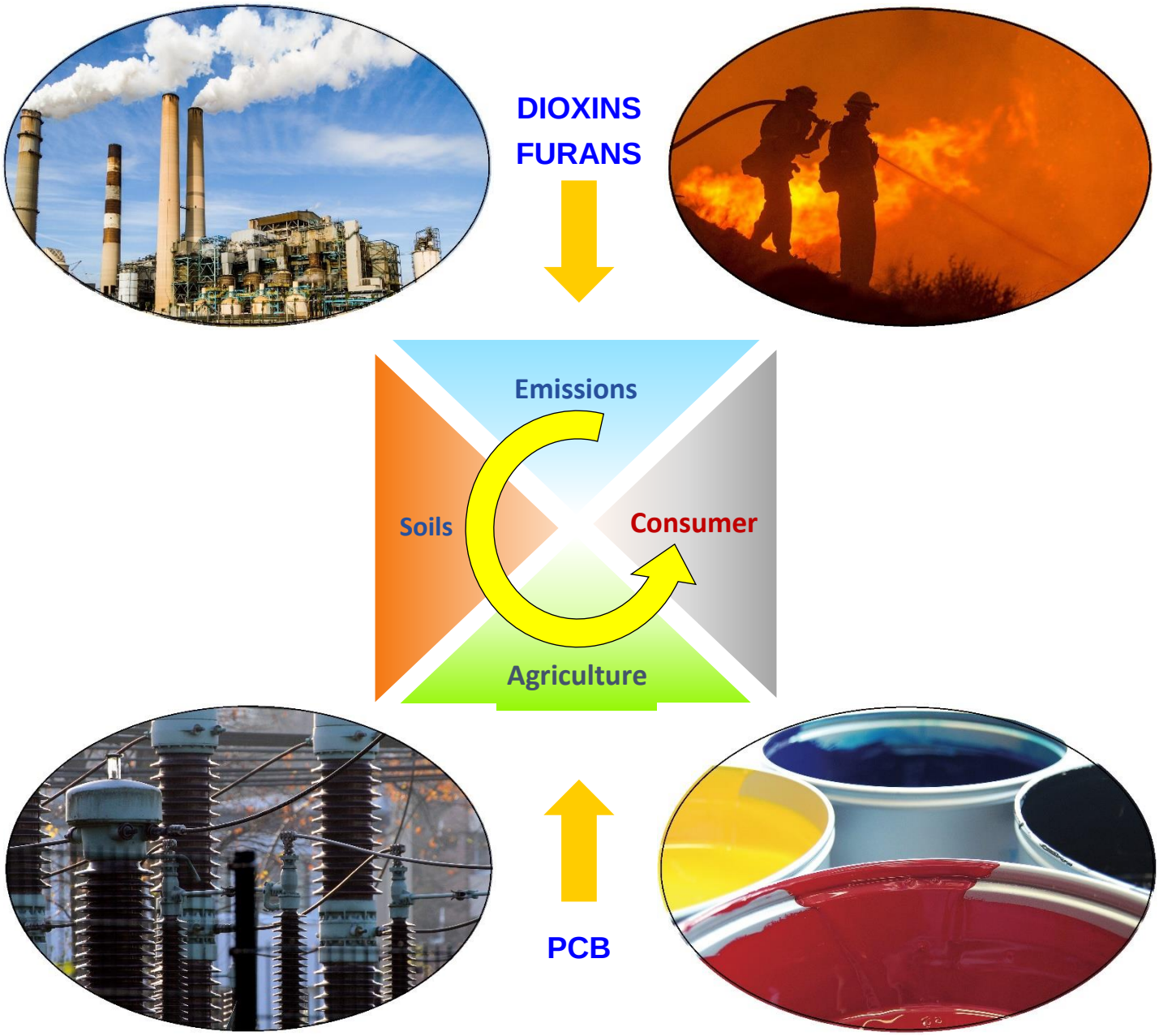
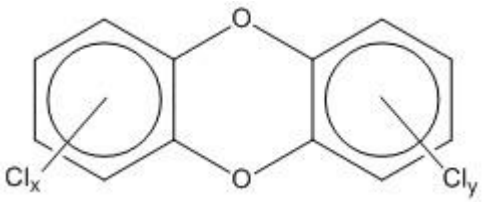


Figure 1. Predominantly past sources of contamination for dioxins, furans and dl-PCB.

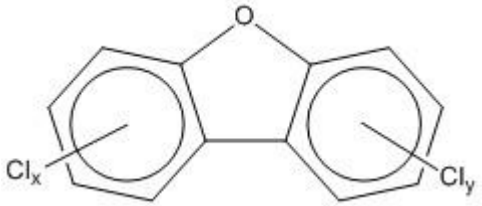
What classes of substances are involved?

Figure 2 shows the basic chemical structures of the 3 substance groups which are detected with the bioassay. In principle, a cell receptor inside the cell reacts with all polycyclic hydrocarbons. Chlorine-containing dioxins, furans and dl-PCB with the highest toxicity bind particularly effectively.

Polychlorinated Dioxins (PCDD)



Polychlorinated Furans (PCDF)



Polychlorinated Biphenyls (PCB)

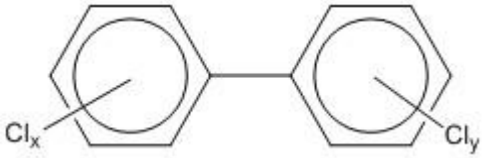


Figure 2. General structural formulae of chlorinated dioxins, furans and PCBs

How the "dioxin bioassay" works

The method presented here, using a cell-based *in vitro* bioassay (Murk et al. 1996), follows the approach of "effect-related analysis". In contrast to chemical analysis, which determines the concentration of a single substance, the effect of a sample on biological target structures is determined in its entirety and thus the effect of the entire "dioxin, furan or PCB cocktail" is recorded. The molecular reaction principle of the assay runs via a specific receptor in the cell interior, which binds to dioxins, furans and PCBs and subsequently induces the formation of the glowworm enzyme luciferase via a complex signal chain (Fig. 3). The biological response or the toxicity of a chemical compound correlates with the binding strength to the receptor and the resulting amount of the glowworm enzyme. The activity of the enzyme, represented by bioluminescence, is then determined in a measuring device called a luminometer.

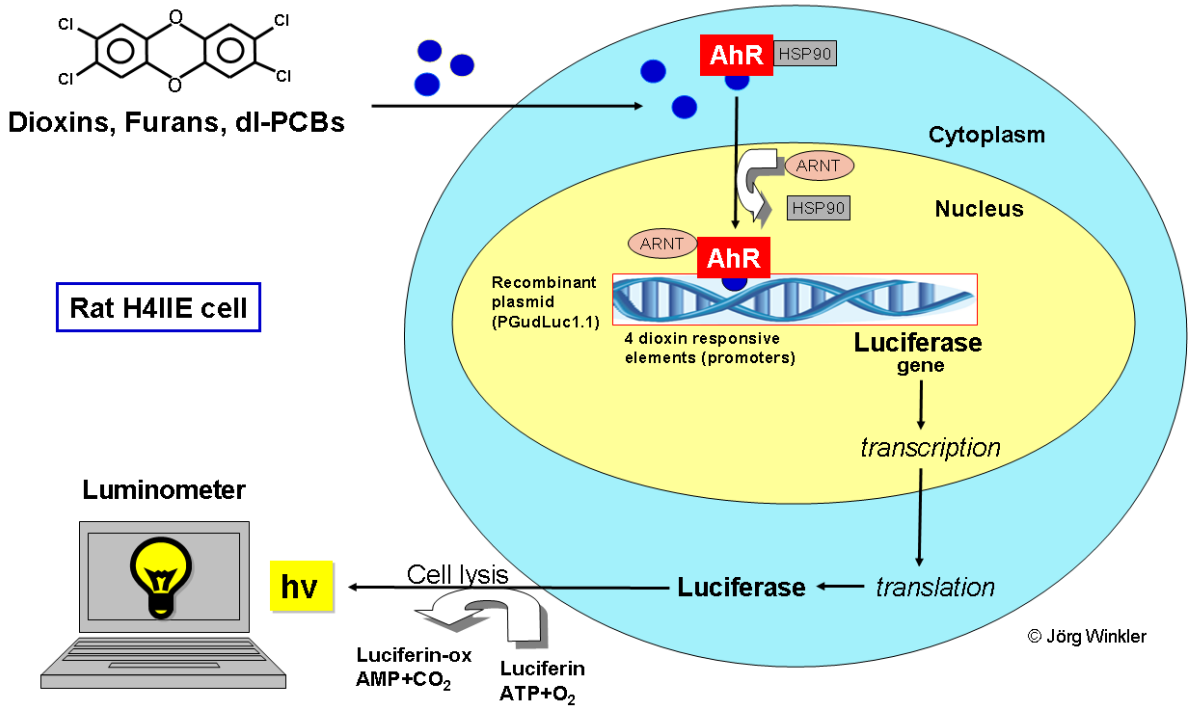


Figure 3. Molecular principle of action of the "dioxin bioassay". AhR = aryl hydrocarbon receptor, HSP = heat shock protein, ARNT = aryl hydrocarbon receptor nuclear translocator, dl-PCB = dioxin-like polychlorinated biphenyls, Rat H4IIE = genetically modified rat liver cells. Environmental toxins such as dioxins, furans and dl-PCB are absorbed by the cells and lead via a signal chain to the formation of the glowworm enzyme, luciferase. By adding luciferin, the substrate of the enzyme, the cell extract begins to luminesce. The concentration of environmental toxins correlates with the strength of luminescence.

Implementation of the bioassay

First, the fats in which the dioxins, furans and PCBs accumulate are extracted from the food and enriched via a silica gel column chromatography (Fig. 4). After further processing, the concentrated extracts are transferred to a culture of genetically modified rat liver cells which are able to form the glowworm enzyme called luciferase upon contact with environmental toxins such as dioxins. Finally, the liver cells are destroyed and the glowworm enzyme is released from the cells. By adding luciferin, the enzyme-specific substrate, the extract begins to glow. The intensity of this luminescence is proportional to the concentrations of harmful dioxins, furans and dl-PCB.

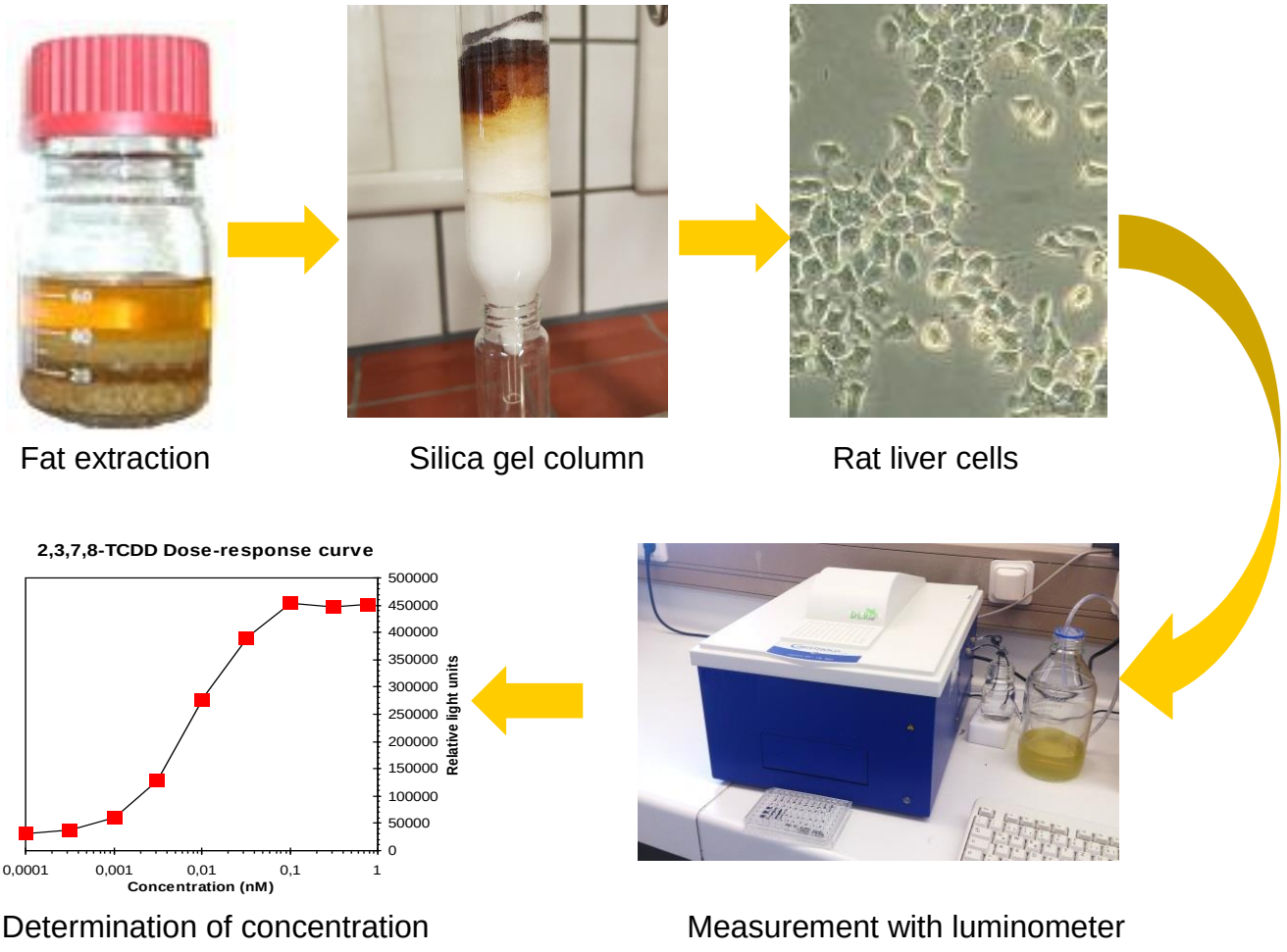


Figure 4. Methodological sequence of the dioxin bioassay

Use of the bioassay for safe foodstuffs

For the dioxin analysis in food and feed, there is an EU-wide set of regulations that defines the examinations of these harmful substances. Since the majority of foodstuffs are uncritical with regard to their dioxin content, the bioassay is a comparatively fast method for the initial analysis of samples. Only a small percentage of samples with conspicuously high contents are subsequently re-examined using complex and expensive chemical-analytical methods. Since a bioassay can also be used to detect other dioxin-like compounds such as dioxin analogues containing bromine or nitrogen without additional effort, these substances, which are harmful to human health, can also be excluded from inconspicuous food samples.

A combination of the "dioxin bioassay" presented here and time consuming chemical analysis enables a significantly higher sample throughput and, in view of future dioxin crises, a quicker clarification of the causes and therefore greater safety for the consumer (Winkler, 2014 and 2015).

References

Murk AJ, Legler J, Denison MS, Giesy JP, van de Guchte C, Brouwer A. Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water. Fundam. Appl. Toxicol. 1996; 33:149-60.

Winkler J. High levels of dioxin-like PCBs found in organic-farmed eggs caused by coating materials of asbestos-cement fiber plates: a case study. Environ. Int. 2015; 80: 72-78.

Winkler J. Nachweis von Dioxinen und dioxinähnlichen Verbindungen in Lebensmitteln mit dem DR-CALUX®-Bioassay. 43. Deutscher Lebensmittelchemikertag, September 2014, Gießen.