An in vitro model for screening estrogen activity of environmental samples after metabolism

Naima Chahbane[1], Karl-Werner Schramm[1], Antonius Kettrup[2]

[1]GSF research center for Environment and Health-Institute od ecological Chemistry, Neuherberg
[2]Institut of ecolgical chemistry TUM, Munich

1. Introduction

For a few years, yeast estrogen assay (YES) was accepted as a reliable and economic model for screening of environmental estrogens [1]. Though the chemicals directly act with estrogen receptor (ER) can be filtered out by this model, there are still chemicals act with ER only after metabolism [2,3,4] and some chemicals eliminate their estrogen activities after metabolism [5]. That is to say, their metabolites exert or have stronger estrogen activities than themselves, which can be called bio-activation. In this case, for the lack of the metabolism enzyme system as human and other animals, only the assay with recombinant yeast cells is insufficient. So, it is necessary to combine the YES with metabolism procedure to evaluate the estrogen activities of these chemicals.

The most common method used currently for *in vitro* metabolic activation in mutagenicity testing and also be applied to the estrogen screening field is S-9 mixture [6,7]. Also, there is an attempt to develop a chemical model for cytochrome P450 as a bio-mimetic metabolic activation system [8]. All these methods can be used as *in vitro* models for metabolism. Compare with these models, using whole H4II E cells for metabolism is an alternative and with superiorities. It has the excellence of short experiment period as all other *in vitro* models, but is much more close to the real surroundings as *in vivo*. Furthermore, the activity of 7-ethoxyresorufin-O-deethylase (EROD) can be easily measured during the whole incubation period for us to discuss the metabolic activities in a quantitative foundation, not only in qualitative.

Methoxychlor is one of the chemicals with bio-activation ability. When directly used in the YES, it shows weak estrogen activity. But a main metabolite of methoxychlor, 2,2-bis (p-hydroxyphenyl) -1,1,1-trichloroethane (HPTE) is a known estrogen mimic [9]. For the long time using methoxychlor as a pesticide and its clear background [10], it is an ideal chemical to establish this in vitro system.

2. Materials and Methods

Metabolic activation procedure:

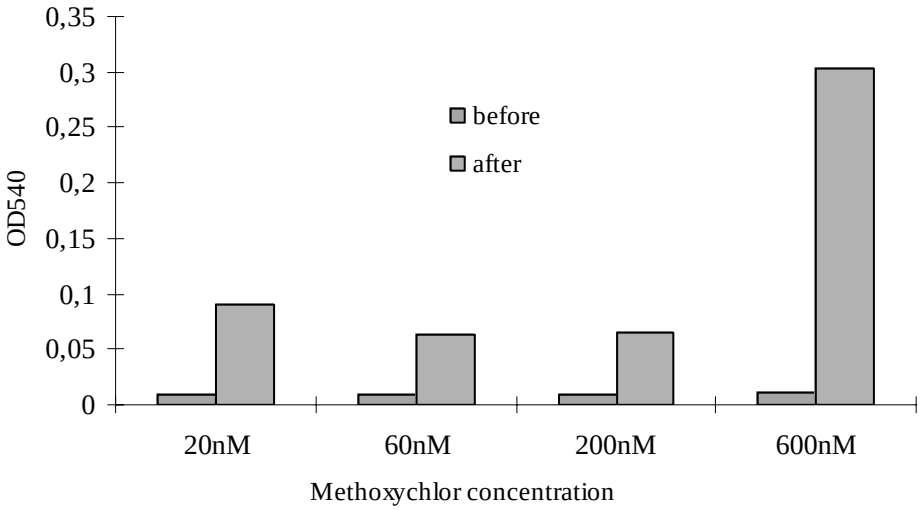
The H4IIE rat hepatoma cells was cultured into a 25 cm^2 flask for 24 hours, then incubated with methoxychlor (99%) for 72 hours at 37°C, the mixture was after extracted with dichloromethane, the solvent was removed by flushing with nitrogen gas. The residue was dissolved into DMSO: isopropanol (v/v=4:1) and the sample solution was transferred to a well of microtiter plate for recombinant yeast estrogenicity assay. This essay is conducted as described by Routeladge and Sumpter [11].

3. Results and discussion

The increasing the estrogen activity of methoxychlor after treatment with H4IIE cells

It is clear in the Fig. 3 that the treatment of H4IIE cell line increased the estrogen activity of methoxychlor to a large extent. Furthermore, the cell model does not only contain the metabolic enzyme, but also other control factors which may act in this process. Thus, it is a better model than other *in vitro* models in simulating a real environment as *in vivo*. In conclusion, using the cells as the metabolism medium is feasible and reliable.

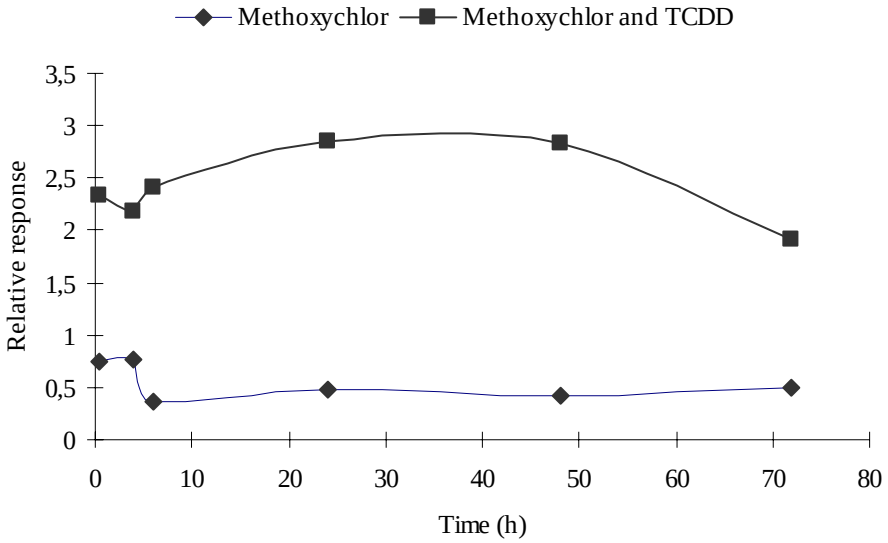
Figure 3 Comparison of estrogenic activities before and after the treatment by cells



The possibility of using 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as an active regent and stable reagent.

The 7-ethoxyresorufin-O-deethylase essay (EROD) was also used to elaborate the enzyme activity during the incubation process. Incubate the cells with methoxychlor only or incubate the cells with TCDD and methoxychlor together, and measure the EROD activity after different time point.

Figure 5 Relative response of EROD test



The result [Fig. 5] showed that the relative response of the EROD test in both cases is stable, and the relative response of TCDD and methoxychlor together is much higher and than that of pure methoxychlor. This indicates that TCDD can be used as a persistent reagent for the activation of the system.

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