When cells are exposed to xenobiotic compounds, including carcinogens, the physiology of the cell may change. This can vary from subtle alterations, such as on metabolic capacity, cell-cell interaction, proliferation, to the most severe effect: cell death. Many of these changes will be accompanied by alterations in the expression of genes, either as a direct/initial or an indirect/secondary response. Ligand-activated nuclear receptors, for instance the Aryl hydrocarbon receptor, will, upon activation directly act as transcription factors and enhance the expression of specific genes. Many non-genotoxic carcinogens, but also some genotoxic carcinogens, appear to act according to this mechanism. The DNA damage response induced by many genotoxic carcinogens, may be an example of an indirect/secondary response, since first several succeeding events are required (e.g. activation of the carcinogen by metabolic enzymes, formation of sufficient DNA damages to trigger a signalling pathway, the successive steps in that pathway [many by protein kinases]), ultimately leading to the activation of transcription factors.

Here, the aim is to discuss the dose response relations and potential thresholds on modulation of gene expression due to exposure of cells to carcinogens, with a focus on the dioxin TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) as model for non-genotoxic carcinogens, and polycyclic aromatic hydrocarbons (especially benzo[a]pyrene, B[a]P) for genotoxic carcinogens. Furthermore, where feasible, the overview is restricted to studies on effects in animal models, with a special attention for those using DNA microarrays as the method for measuring gene expression, and with investigation of at least 3 different doses.

The most comprehensive in vivo study on TCDD-induced gene expression changes, was recently published by Boverhof et al. [1]. Dose-response analyses were performed on hepatic tissue from immature ovariectomized C57BL/6 mice treated with TCDD (0, 0.001, 0.01, 0.1, 1, 10, 100, or 300 µg/kg) and sacrificed after 24 h. Gene expression profiles were monitored using cDNA microarrays containing ca. 13,000 genes. The data indicate that TCDD elicits gene specific dose-dependent responses as the ED50 values may vary drastically. This may be due to gene-specific upper thresholds (saturation of gene expression), differential temporal regulation, or differing basal expression levels, which would affect the dose at which transcriptional regulation may be initiated or detected. Whether thresholds exist below which no expression changes occur, is not described. However, taking into account the large dose-steps and the variation in observations, it appears not feasible to establish the existence of such thresholds for most genes.
In general, the gene expression responses preceded or paralleled the observed histopathology for the various functional categories. This suggests that apparent thresholds for histopathological parameters, may not be confirmed at gene expression level.

Another study addressing the effects of TCDD on gene expression in vivo, was undertaken during murine cardiovascular development in C57Bl/6N pregnant mice dosed with 1.5, 3.0, or 6.0 µg TCDD/kg [2]. Although a few genes showed typical dose-related effects on expression, many more of the genes showed significant alterations in expression only at the highest dose or only at the lower doses.

Numerous studies have been conducted in many mouse and rat organs, on TCDD-induced effects on the expression CYP450 genes by RT-PCR methods. In a dose–response study on CYP1B1, CYP1A1, and CYP1A2 in the liver of female rats following chronic exposure to TCDD, results showed that while the liver concentration of TCDD required for half-maximal induction was similar, the shaping parameter of the dose–response curve for CYP1B1 was significantly higher than that for CYP1A1 or CYP1A2 [3]. Even at the lowest dose (3.5 ng/kg/day), however, all genes were clearly induced. Another study in the liver of female mice 24 h following a single exposure, however, showed that for CYP1A1 a steeper dose-response was observed than for CYP1B1 [4]. Furthermore, it appears that a threshold for induction of gene expression may exist, although differences between mouse strains are evident. In organs from the immune system of the rat, this thresholded effect on CYP1A1 induction is not that clear following acute [5] or chronic treatments [6].

For the genotoxic class of carcinogens of polycyclic aromatic hydrocarbons, including B[a]P, much less data are available. Despite that several gene expression studies on B[a]P have been described, few of the microarray or RT-PCR based gene expression studies apply at least 3 dose levels or are conducted in animals. To our knowledge, the only in vivo study fulfilling our selection criteria was from Bartosiewicz et al. [7]. In this study, DNA arrays containing 148 genes for relevant toxic processes were used to examine gene expression patterns in the liver of mice in dose-related response to B[a]P. Administration of B[a]P up-regulated only CYP1A1 and CYP1A2 and produced no significant increases in any of the stress response genes or any of the DNA repair genes present on the array. Whether or not a threshold occurs at low doses is not apparent from the current data.

Due to the lack of in vivo data, in vitro studies using cell lines or primary cells have also been reviewed. Surprisingly few studies on multiple doses, genotoxicants, and gene expression by microarray or RT-PCR methods (focussing on CYPs, p21/Cip1/Waf1 and GADD45) were retrieved. In one study, the human TK6 cell line was used as an in vitro model system, and reactive metabolites of human carcinogens, N-hydroxy-4-acetylaminobiphenyl (N-OH-AABP) and benzo[a]pyrene diol epoxide (BPDE), were used as model compounds [8]. Each compound was tested at low, medium, and high toxicities (5, 15, and 40%) and analysed for mutagenic activity and gene expression modulation using microarrays comprising approximately 18,000 genes. The microarray data from the treatment groups were compared using self-organising map clustering algorithms, as well as a statistical regression modelling approach. Gene clusters were identified that showed differential
Dose response and potential thresholds in gene expression
dose-dependent responses to BPDE and N-OH-AABP, such as a continuous dose-
dependent increase or decrease, saturation at the two high doses, or possible thresholded
effects at low doses. Whether these differential dose-dependent responses are linked to
specific functional processes or pathways is unclear. Another study in TK6 cells using
BPDE showed that DNA adduct formation was the most sensitive indicator of DNA
damage [9]. DNA adduct formation was clearly evident at low doses, where the number
of genes with significantly altered expression was minimal. Alterations in gene expression
were more robust at doses associated with cellular toxicity and induction of mutations.
For both of these studies it is not evident whether thresholded effects on gene expression
occur. For RT-PCR based studies, no examples were found that fulfilled all criteria.
Summarising, both for the non-genotoxic and genotoxic carcinogens, no firm
conclusion can be given whether their effects on gene expression in target tissue \textit{in vivo}
or in cells \textit{in vitro} show a threshold or not at low doses. Besides some conflicting data,
most studies simply do not have the power for conclusive statements.

**Conclusion and perspectives**

As indicated above, most dose-response studies on modulation of gene expression by non-
genotoxic and genotoxic carcinogens in target tissue \textit{in vivo} or in cells \textit{in vitro}, simply do
not have the power for firm conclusions whether or not thresholds for induced effects
exist at low dosages. The main problem is to discriminate true thresholds from apparent
thresholds due to technical limitations, mostly the observed variations for the
investigated effect parameters. Obviously, the only answer lies in large-scale studies, with
many dose levels with small intercepts, lots of biological repeats, combined with
appropriate statistics that can significantly discriminate thresholded dose-response curves
from linear dose-response curves.

For microarray studies on gene expression changes where thousands of genes are
investigated, an additional problem appears: namely the chance of false positive
conclusions. With such a huge number of genes, it is likely that statistical tests will
always — by chance — come up with some significant thresholded responses. This
specification of the multiple testing problem, raises the question of how to discriminate
true thresholded responses from false positive responses. Possibly, new statistical
approaches are required to solve this problem for dose-response analyses. Also in this case,
the answer lies in large-scale studies, with many dose levels with small intercepts, and
lots of biological repeats. The application of smaller arrays rather then whole-genome
arrays (e.g. after selection of the affected genes by limited preliminary studies), may
reduce the otherwise huge costs for microarray analyses. Furthermore, the verification of
array data with independent methodologies, such as quantitative RT-PCR, will aid to
discriminate true thresholded responses from false positive responses.
References


