CONTRIBUTION OF PAHs AND PERSISTENT DIOXIN-LIKE COMPOUNDS TO OVERALL AhR-MEDIATED ACTIVITIES IN ABIOTIC SAMPLES

Miroslav Machala, Kateřina Pěnčíková, Miroslav Ciganek, Jiří Neča, Jan Vondráček[1]

Veterinary Research Institute, Brno, Czech Republic
[1] Institute of Biophysics, AS CR, Brno, Czech Republic

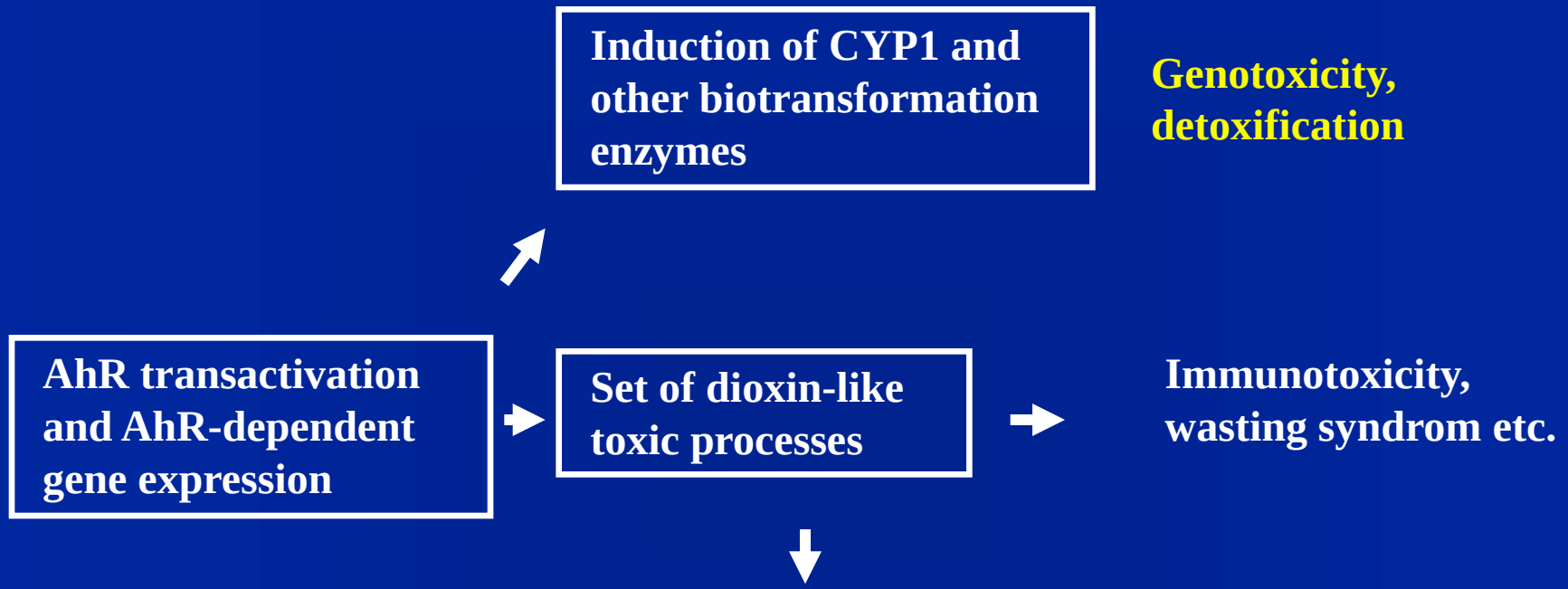
DIOXIN-LIKE TOXICITY AND TOXIC EQUIVALENCY FACTORS ARE DEFINED ONLY FOR PERSISTENT ORGANIC POLLUTANTS WHICH ARE AGONISTS OF AhR (van den Berg et al., 1998; 2006)

NON-PERSISTENT AhR AGONISTS?

Many other chemicals, including PAHs, their derivatives and heterocyclic aromatic hydrocarbons, have been reported to transactivate AhR. However, they elicit also additional AhR-dependent events associated with developmental toxicity and tumor promotion.

PAHs = less persistent vs. chronic exposure

What represents AhR-mediated activity?



THE APPLICATION OF DR-CALUX ASSAY

1. Determination of relative effective potencies of individual compounds.

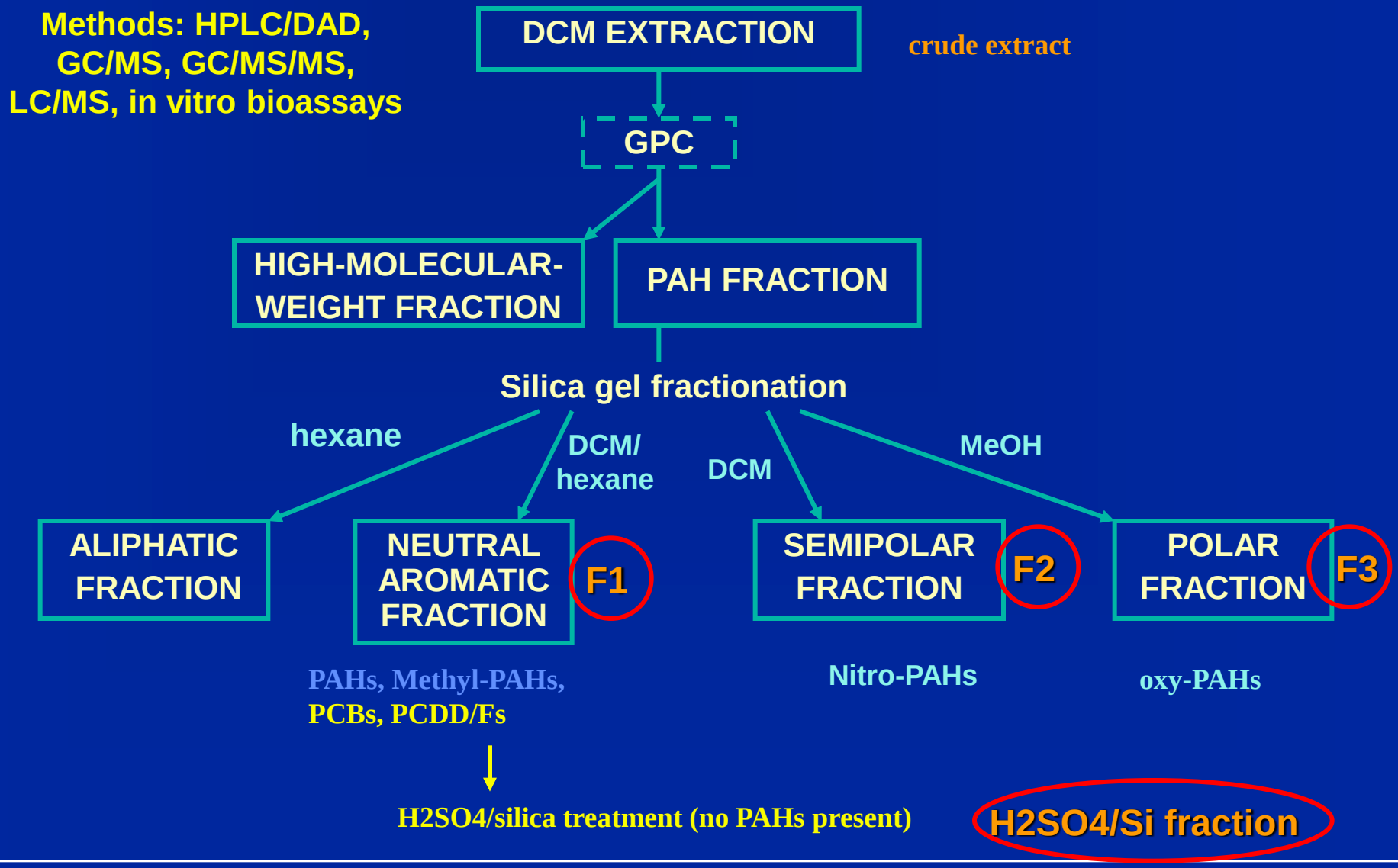
2. Chemical analysis of more than 100 PAHs, PAH derivatives and selected heterocyclic aromatic compounds in crude extracts and LC fractions from river sediment and airborne-particle samples; comparison of the concentration data with the AhR-mediated activities of the fractions, including effect-directed analysis – estimation of AhR-mediated activity of a large series of chromatographic fractions; chemical identification of key contributors of the activity.

3. Relevance of PAH-induced AhR-dependent gene expression (global gene expression and RT-PCR data) to "dioxin-like" activity of PAHs in CALUX assays?

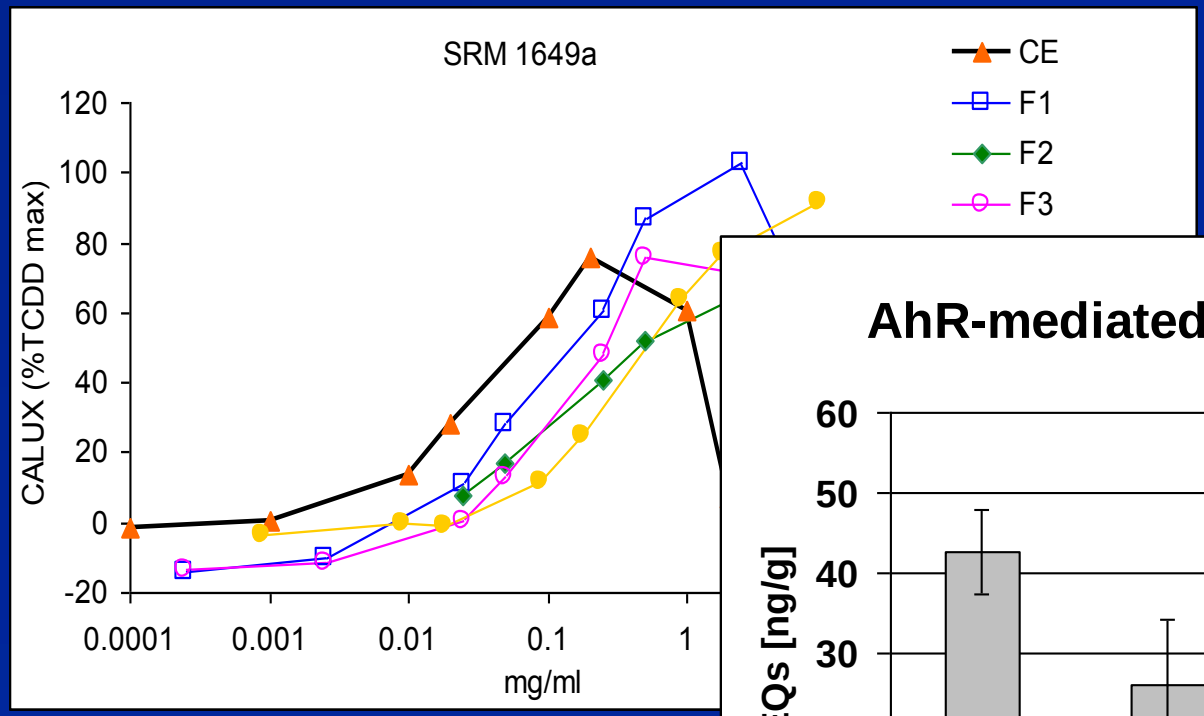
AhR-MEDIATED ACTIVITY AND RELATED EFFECTS OF ENVIRONMENTAL MIXTURES

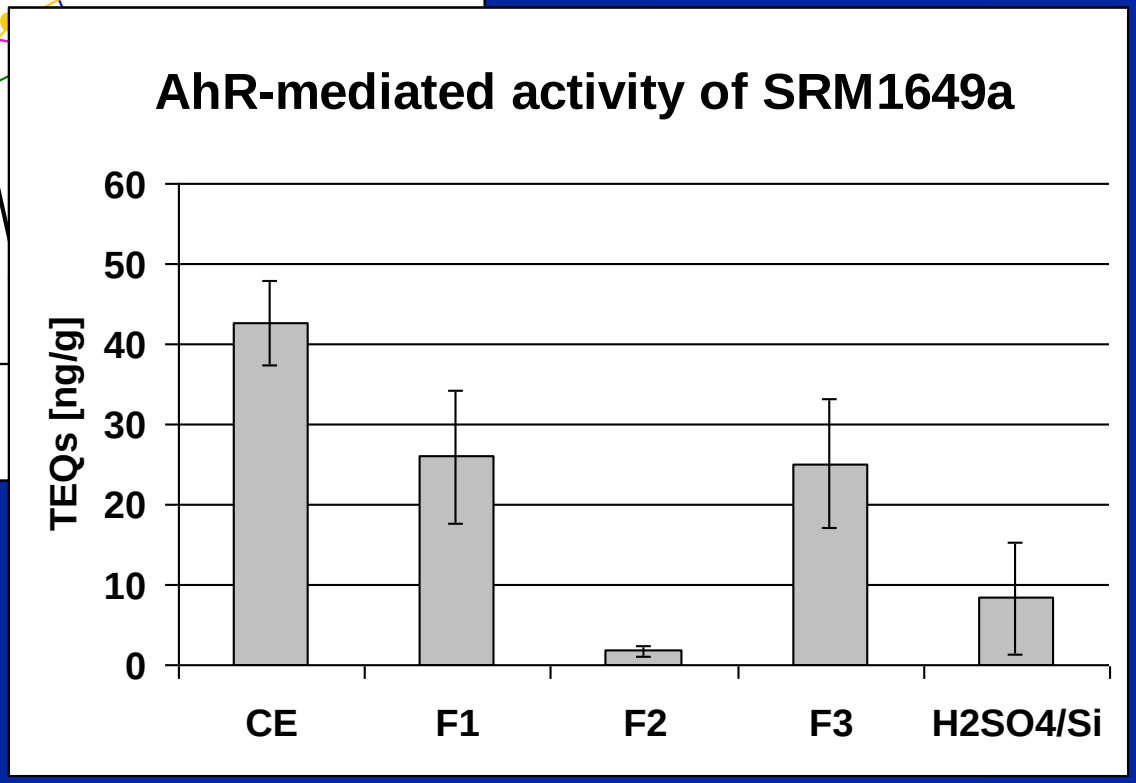
Veterinary Research Institute, Brno, Czech Republic

FRACTIONATION PROTOCOL AND ANALYSIS

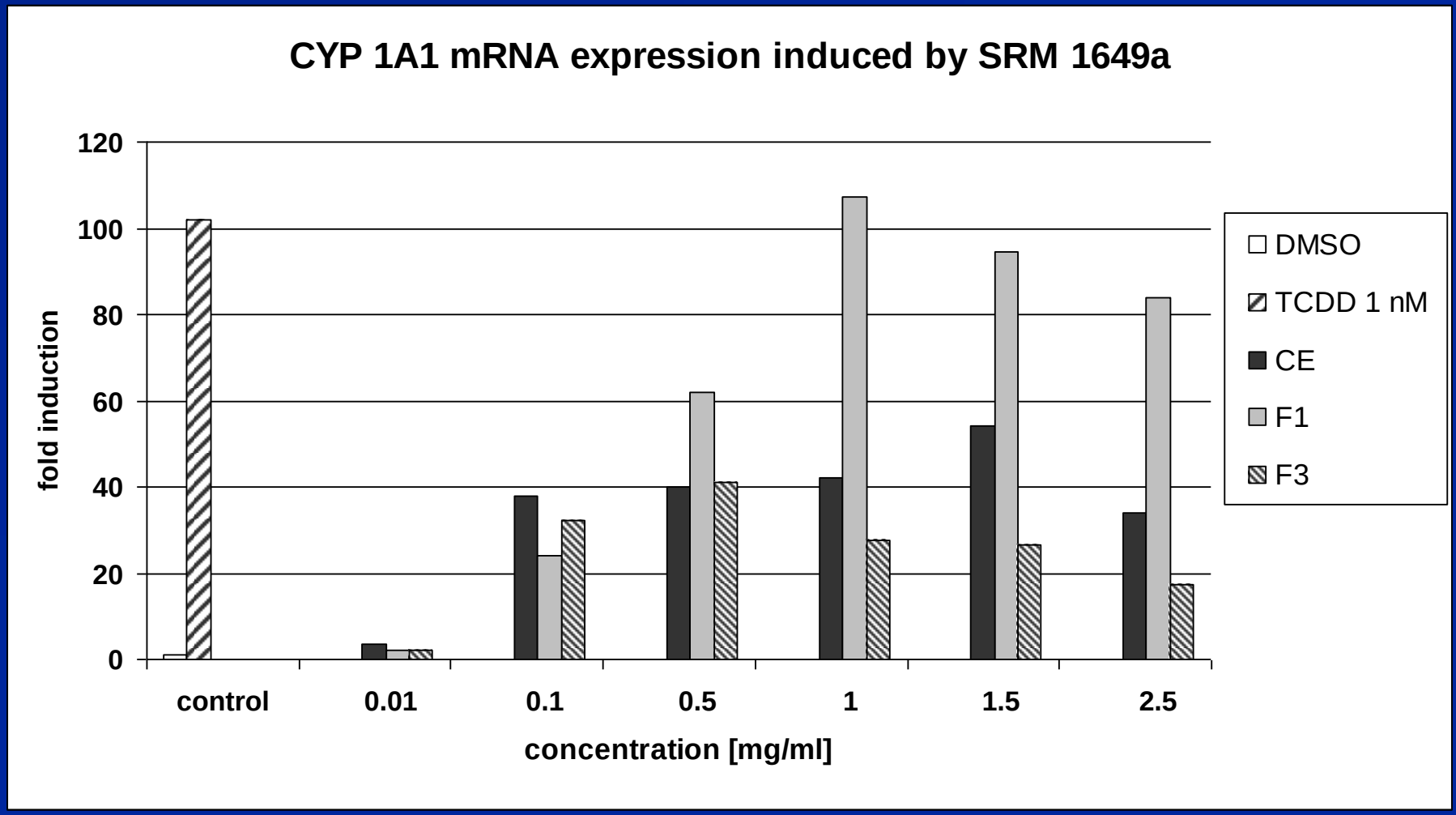


Ah receptor-mediated activity of SRM1649a extract (DR-CALUX)



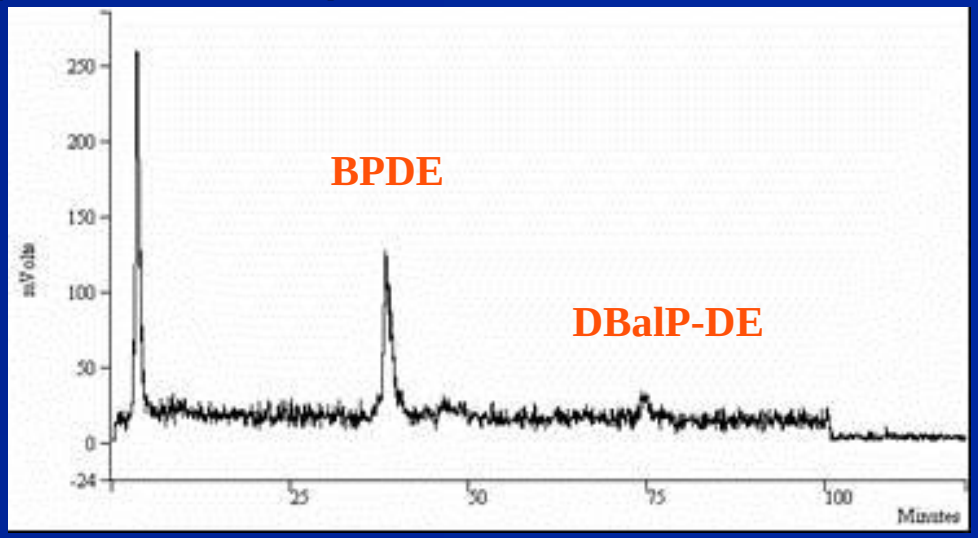


Ah receptor-dependent induction of CYP1A1mRNA in rat liver epithelial WB-F344 cell line

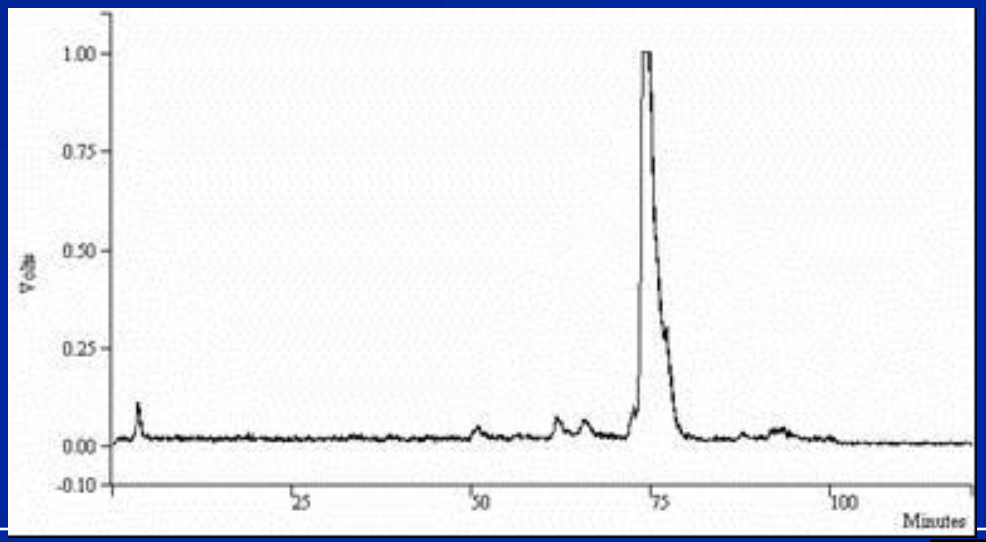


Production of PAH-DNA adducts in rat liver epithelial WB-F344 cells exposed to crude extract of airborne particles (SRM1649a)

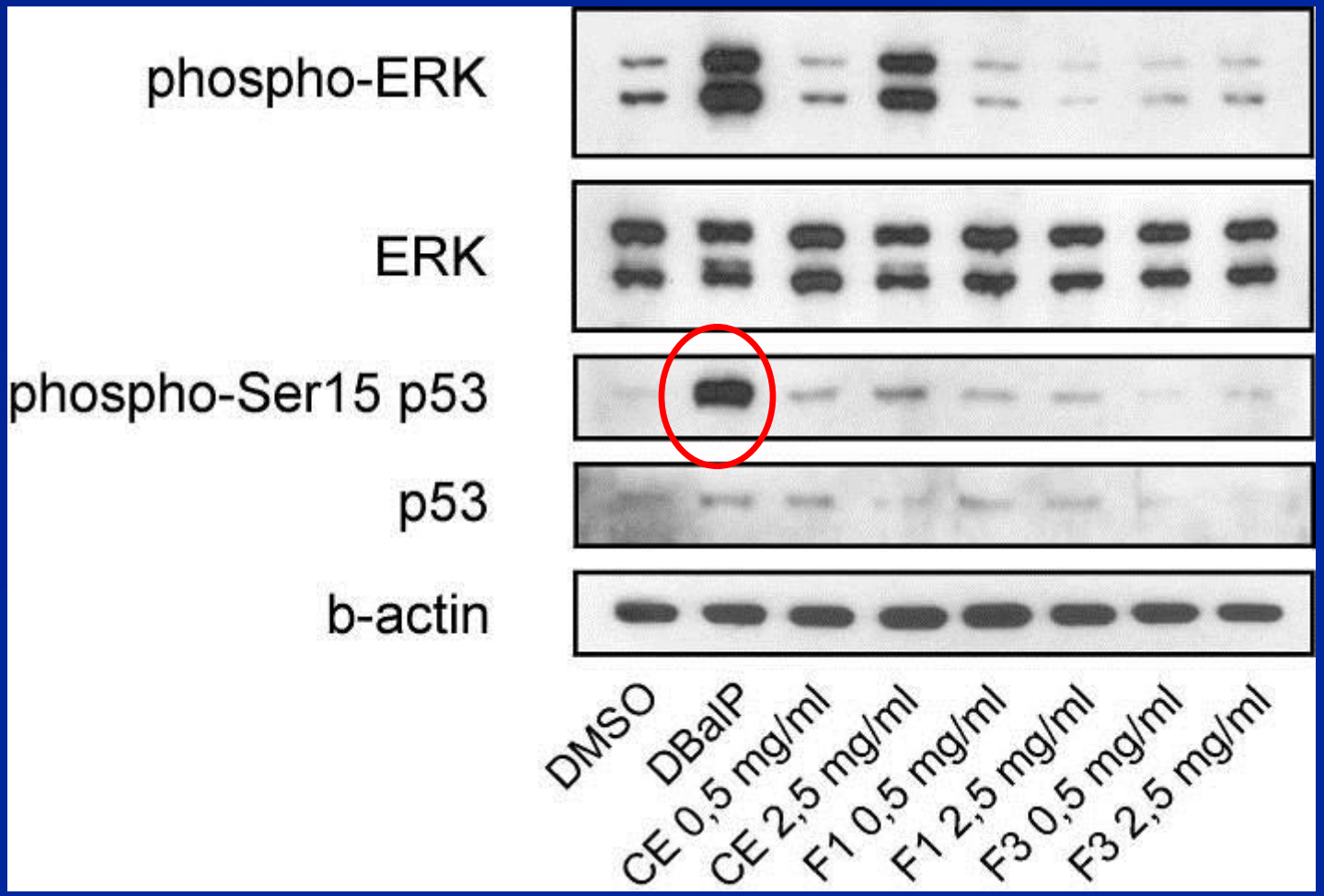
equivalent of 2.5 mg / ml
^{33}P-postlabeling/HPLC



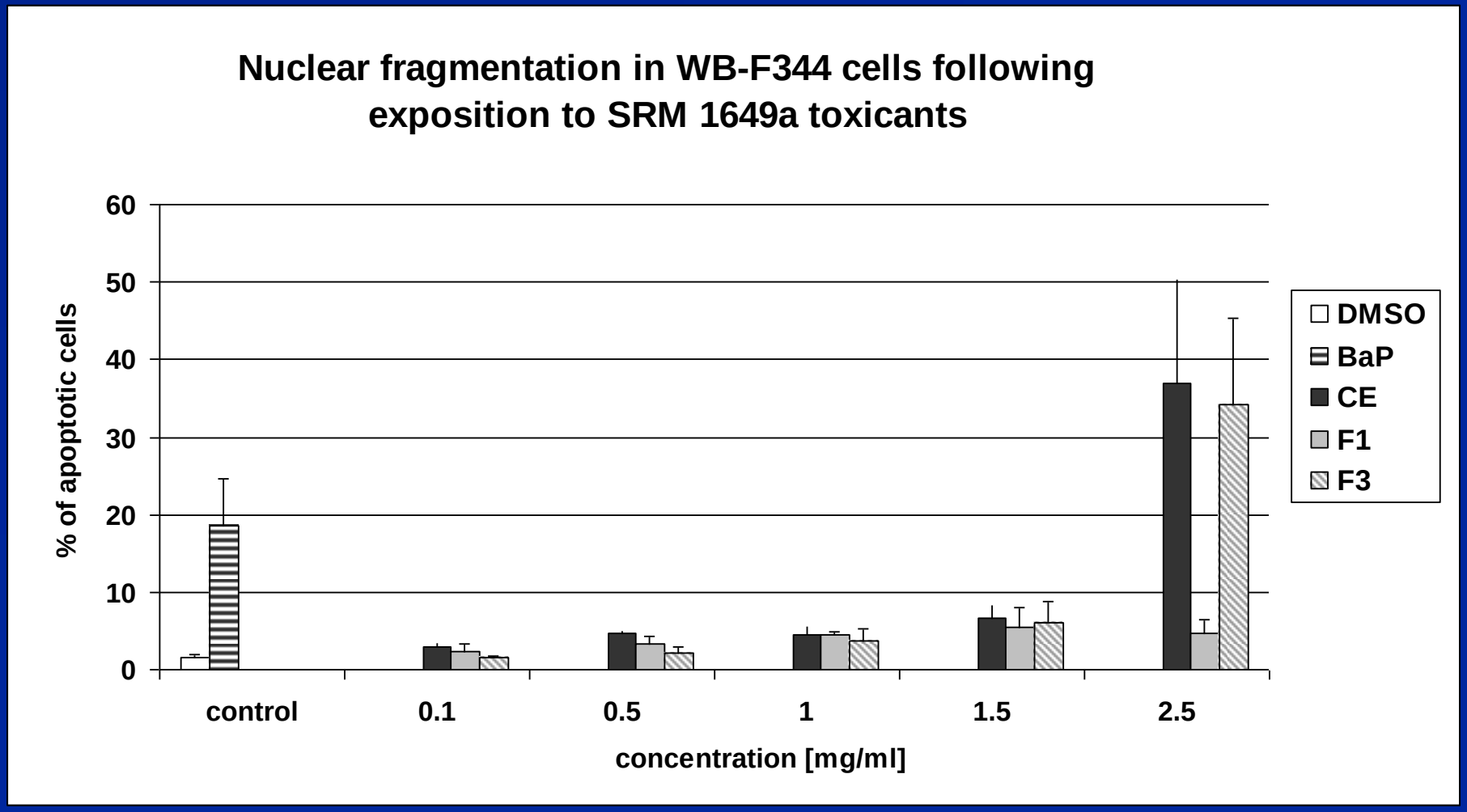
100 nM DBalP



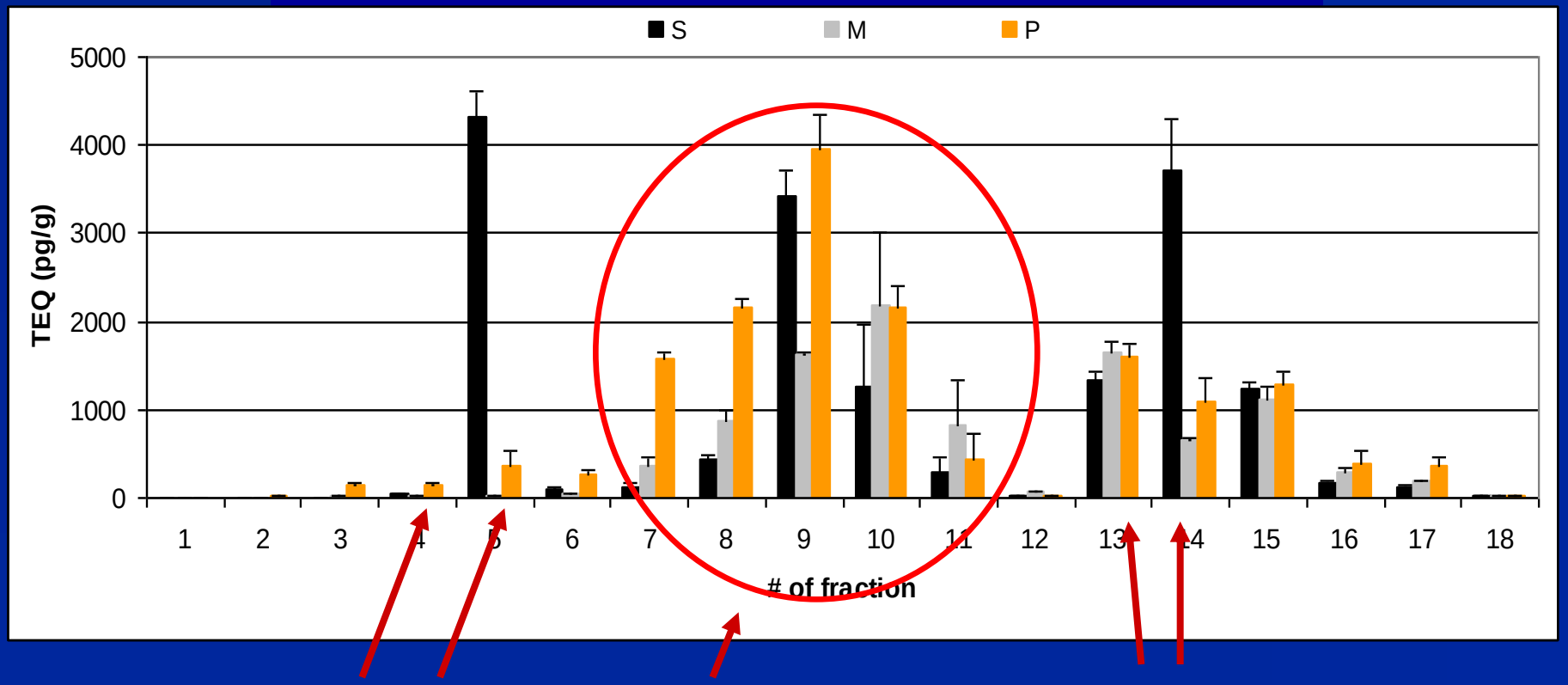
Detection of genotoxic effects in WB-F344 cells (phosphorylation of p53 protein) after exposure to SRM1649a extract



Weak induction of apoptosis in WB-F344 cells exposed to the SRM1649a (method: DAPI staining)



DR-CALUX ASSAY USED IN THE EFFECT-DIRECTED ANALYSIS OF RIVER SEDIMENTS (THREE HOT-SPOTS FOUND IN THE ELBE RIVER BASIN)



Low dioxin-like activity of POPs fraction (PCDD/Fs, PCBs etc.)

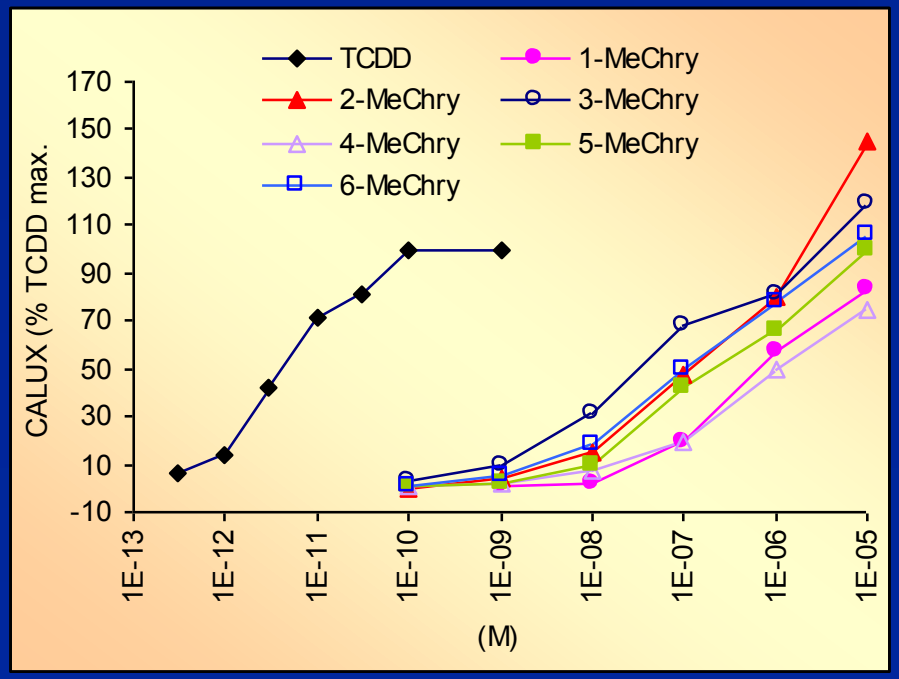
High AhR-mediated activity of PAH fractions

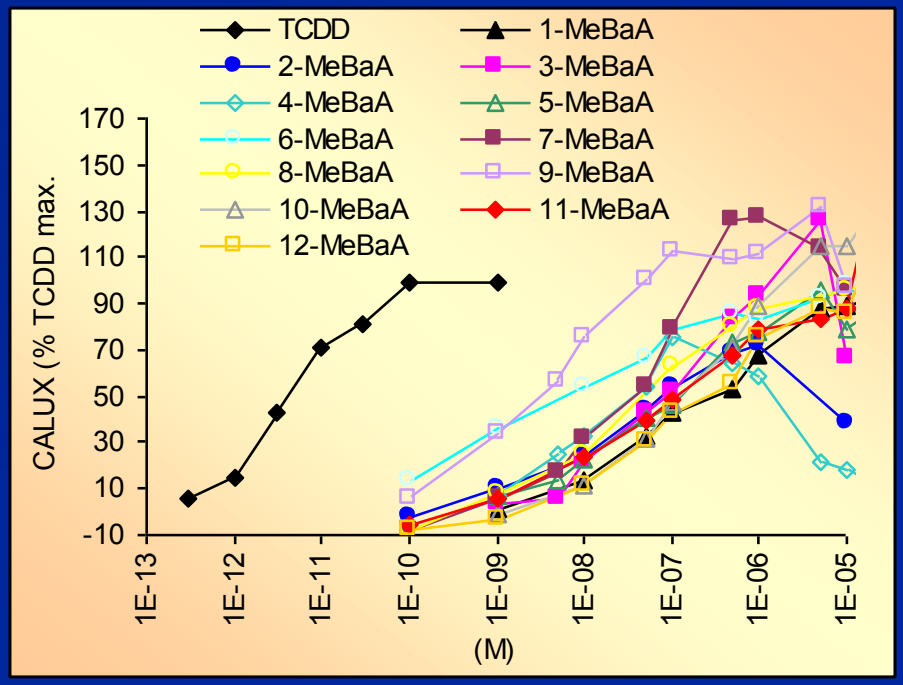
High AhR-mediated activity of polar compounds

AhR-MEDIATED ACTIVITY OF INDIVIDUAL POLYCYCLIC AROMATIC HYDROCARBONS AND RELATED COMPOUNDS

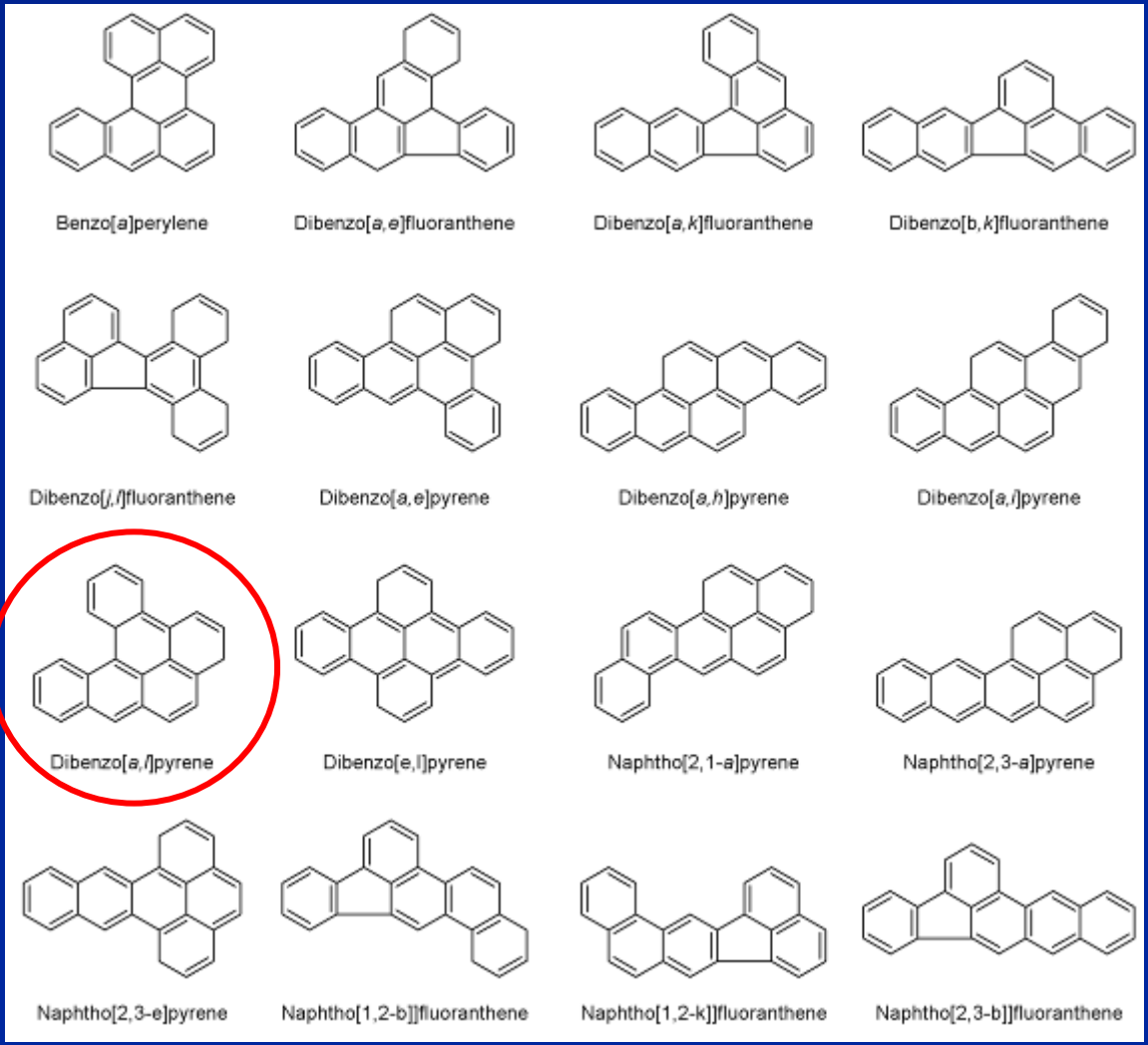
Veterinary Research Institute, Brno, Czech Republic

APPLICATION OF DR-CALUX: RELATIVE POTENCIES OF METHYL-PAHs: (EC50 values of methylated benzanthracenes and chrysenes ranged from 2 to 230 nM)

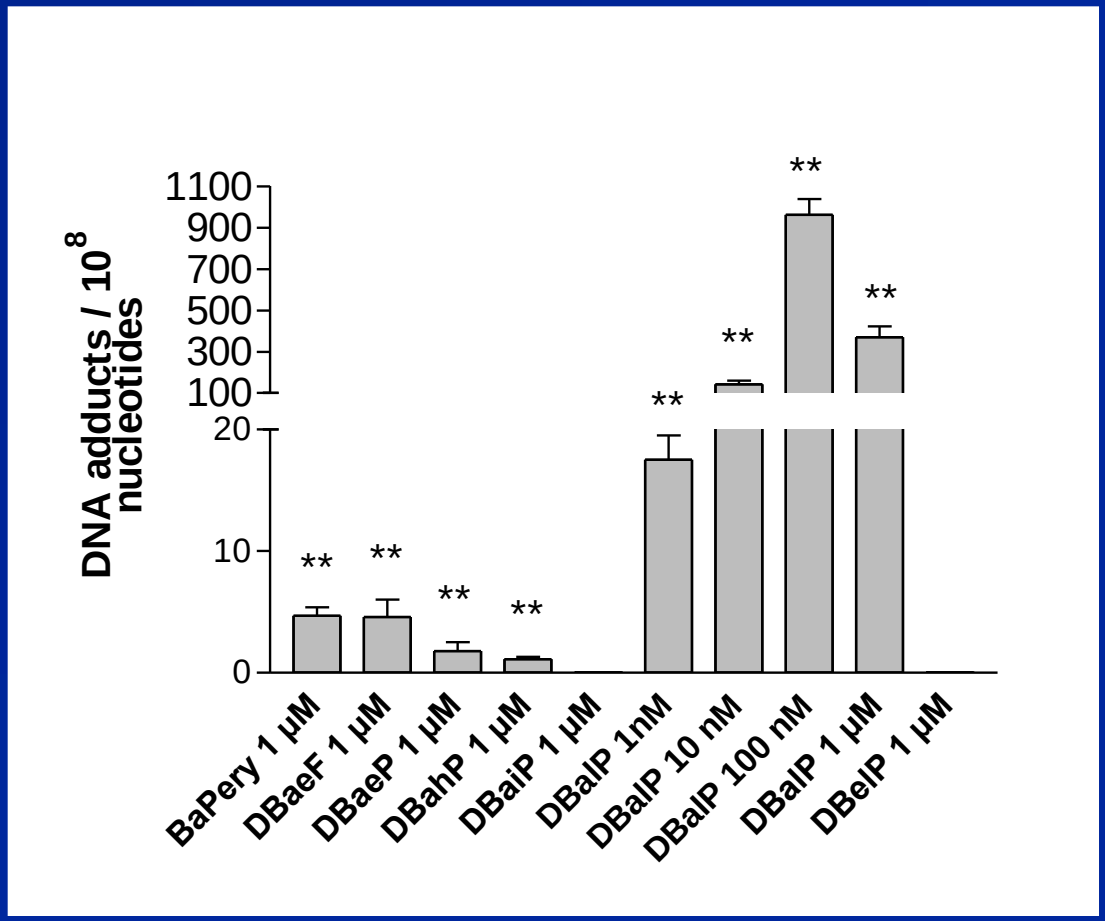




Effects of PAHs with molecular weight 302 (nongenotoxic effects >> genotoxicity, cell death)



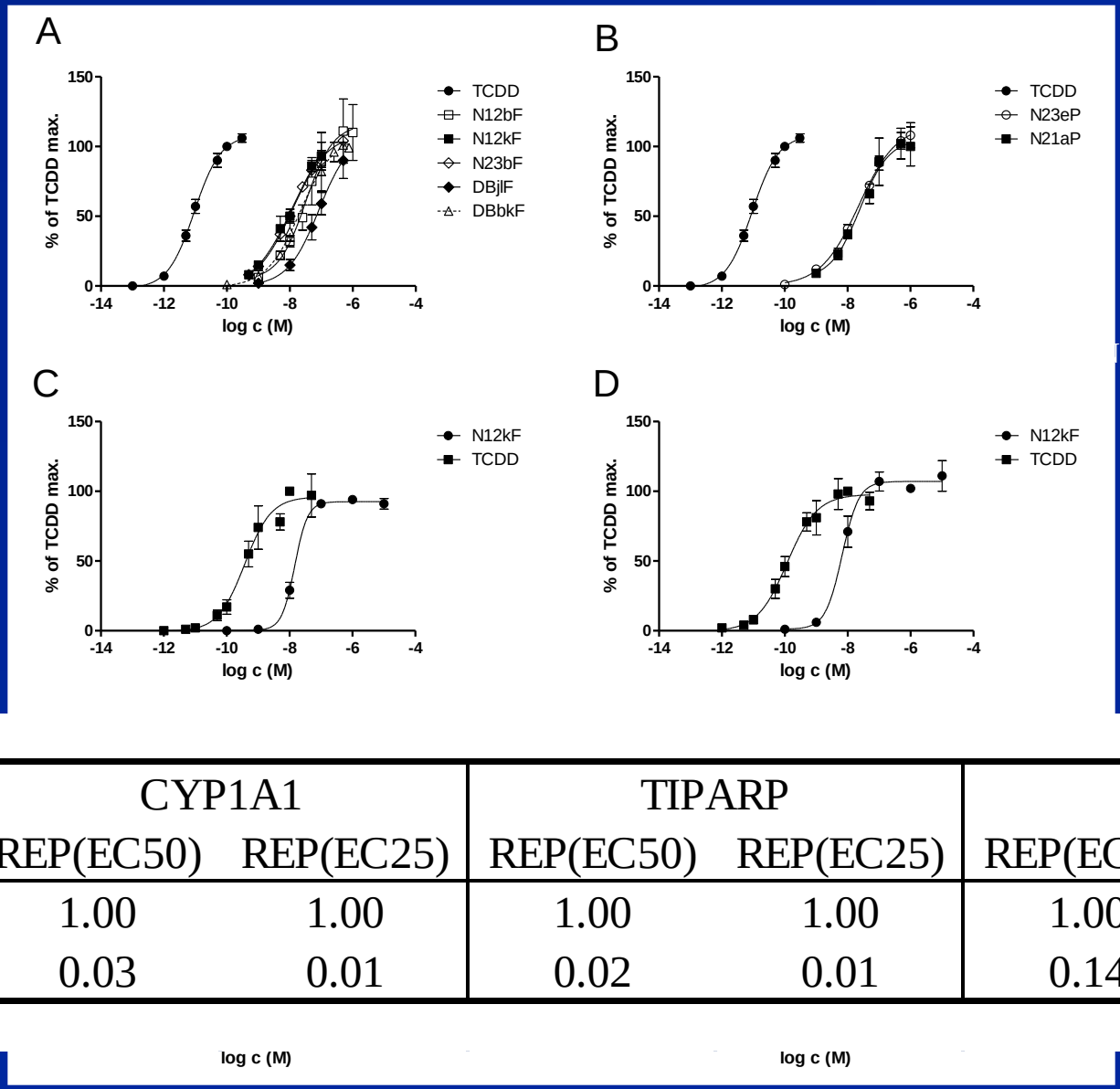
Effects of PAHs with molecular weight 302 (nongenotoxic effects >> genotoxicity, cell death)



Effects of PAHs with molecular weight 302 (similar REPs in H4IIE.Gud.luc and A549 cells)

DR-CALUX

CYP1A1



DR-CALUX

TIPARP

12kF.

	CYP1A1		TIPARP		AXIN2	
	REP(EC50)	REP(EC25)	REP(EC50)	REP(EC25)	REP(EC50)	REP(EC25)
TCDD	1.00	1.00	1.00	1.00	1.00	1.00
N12kF	0.03	0.01	0.02	0.01	0.14	0.07

AhR-mediated activity of measured compounds in the DR-CALUX assay after 24 h of exposure

PAHs_302	Abbr.	CAS	IEF (DR CALUX) 24 h		reference	LOEC_NRU [c]
			IEF (EC50)	IEF (EC25)		
Benzo[*a*]perylene	BaPery	191-85-5	6.19E-06	6.28E-06	a	> 10 µM
Dibenzo[*a,e*]fluoranthene	DBaeF	5385-75-1	9.30E-06	1.18E-05	a	> 10 µM
Dibenzo[*a,k*]fluoranthene	DBakF	84030-79-5	1.23E-03	1.37E-03	a	> 10 µM
Dibenzo[*b,k*]fluoranthene	DBbkF	205-97-0	4.97E-04	7.08E-04	b	> 10 µM
Dibenzo[*j,l*]fluoranthene	DBjlF	203-18-9	1.34E-04	1.43E-04	b	> 10 µM
Dibenzo[*a,e*]pyrene	DBaeP	192-65-4	1.80E-05	3.90E-05	a	> 10 µM
Dibenzo[*a,h*]pyrene	DBahP	189-64-0	7.14E-05	3.70E-04	a	> 10 µM
Dibenzo[*a,i*]pyrene	DBaiP	189-55-9	1.65E-04	4.41E-04	a	> 10 µM
Dibenzo[*a,l*]pyrene	DBalP	191-30-0	4.90E-06	1.13E-06	a	> 10 µM
Dibenzo[*e,l*]pyrene	DBelP	192-51-8	n.i.	ni	b	10 µM
Naphtho[2,1-*a*]pyrene	N21aP	189-96-8	3.63E-04	4.10E-04	b	> 10 µM
Naphtho[2,3-*a*]pyrene	N23aP	196-42-9	2.05E-04	3.83E-04	a	> 10 µM
Naphtho[2,3-*e*]pyrene	N23eP	193-09-9	4.24E-04	5.86E-04	b	> 10 µM
Naphto[1,2-*b*]fluoranthene	N12bF	5385-22-8	2.75E-04	3.05E-04	b	> 10 µM
Naphtho[1,2-*k*]fluoranthene	N12kF	238-04-0	9.91E-04	8.09E-04	b	> 10 µM
Naphtho[2,3-*b*]fluoranthene	N23bF	206-06-4	7.73E-04	9.84E-04	b	> 10 µM

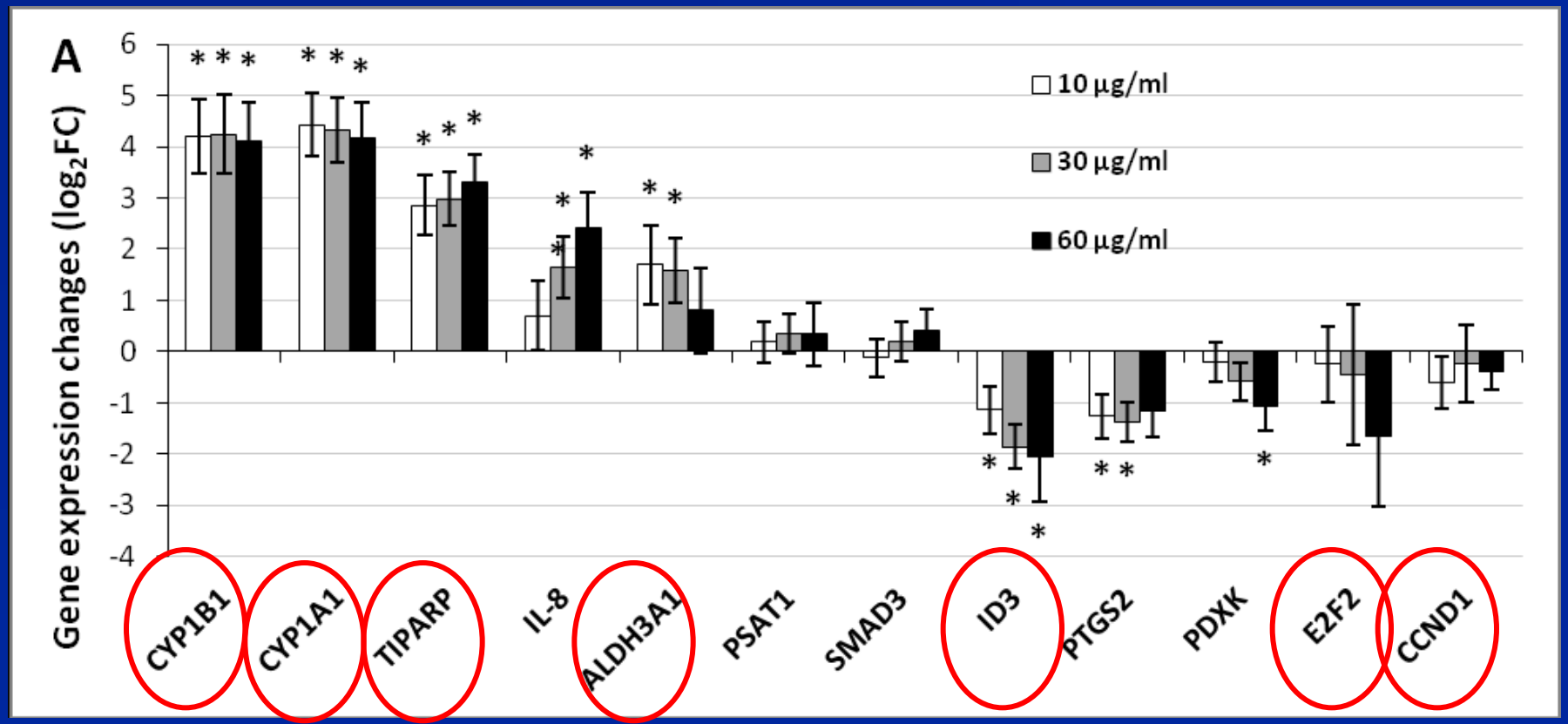
SIMILAR PATTERN OF EFFECTS OF AhR INDUCERS (and mixtures containing PAHs)

- Very low DNA adduct formation, low percentage of apoptotic cells (e.g. DAPI staining), no accumulation of phosphorylated p53 protein
- Induction of AhR-dependent gene expression (CYP1A1/2, CYP1B1 or luciferase reporter gene)
- Global gene expression, cell population studies: PAHs exhibited effects similar to TCDD

TEQs / IEQs values derived from chemical data (REP x conc) identification of major contributors of AhR-mediated activity

	SRM 1649 a	Sed. - Site 1	Sed. - Site 2	Sed. - Site 3	Sed. - Site 4
BkF	3206	430	638	784	950
9-MeBaA	190	227	126	252	23
IcdP	898	143	189	271	131
BjF	523	87	141	158	87
Chry	295	50	71	74	59
DBahA	289	39	36	80	35
BaP	209	36	48	62	30

Global gene expression in A549 treated with airborne particle extract, TCDD and BaP



Comparison of REPs of PAHs determined by DR-CALUX assay (in rat hepatoma H4IIE cells) and AZ-AhR assay (in human hepatoma HepG2 cells)

		CALUX assay		
		H4IIE.Luc	AZ-AhR (HepG2.AhR)	
PAHs_302	PAH	IEF(50)	IEF(25)	IEF(50)
Dibenzo[a,k]fluoranthene	**DBakF**	1,23E-03	1,77E-03	3,76E-03
Dibenzo[j,l]fluoranthene	**DBjlF**	1,36E-04	w.i.	n.i.
Dibenzo[b,k]fluoranthene	**DBbkF**	4,07E-04	3,99E-03	7,98E-03
Dibenzo[a,e]fluoranthene	**DBaeF**	9,30E-06	n.i.	n.i.
Dibenzo[a,e]pyrene	**DBaeP**	1,80E-05	n.i.	n.i.
Dibenzo[a,i]pyrene	**DBaiP**	1,65E-04	8,80E-04	1,52E-03
Dibenzo[a,h]pyrene	**DBahP**	7,14E-05	3,93E-05	n.i.
Dibenzo[a,l]pyrene	**DBalP**	4,90E-06	n.i.	n.i.
Dibenzo[e,l]pyrene	**DBelP**	n.i.		
Naphtho[1,2-k]fluoranthene	**N12kF**	6,79E-04	1,09E-03	1,87E-03
Naphtho[2,3-b]fluoranthene	**N23bF**	7,36E-04	1,00E-02	1,61E-02
Naphto[1,2-b]fluoranthene	**N12bF**	2,75E-04	1,93E-03	4,38E-03
Naphtho[2,3-e]pyrene	**N23eP**	3,85E-04	3,65E-03	7,59E-03
Naphtho[2,1-a]pyrene	**N21aP**	3,54E-04	3,64E-03	8,39E-03
Naphtho[2,3-a]pyrene	**N23aP**	2,05E-04	3,42E-03	5,54E-03
Benzo[a]perylene	**BaPery**	6,19E-06		

CONCLUSIONS

PAHs under study exhibited effects similar to TCDD and other persistent dioxin-like compounds and they had only a limited genotoxic potencial.

The PAH fractions represented major AhR-mediated activity in CALUX assays; significant activity of persistent dioxin-like compounds was found only in several particular sediment samples. A significant portion of AhR-mediated activity was found in polar aromatic fractions of both river sediment and airborne samples, however, principal contributors were not identified to this time.

DR-CALUX data showed good correlation with other AhR-dependent adverse effects after exposure to both individual PAHs and neutral and polar fractions of abiotic environmental samples.

DR-CALUX is a key test system for risk assessment of PAHs and other less persistent aromatic compounds.

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