Xenobiotic metabolising capabilities of cell preparations used in KE1, KE2 and KE3 tests

Franz Oesch
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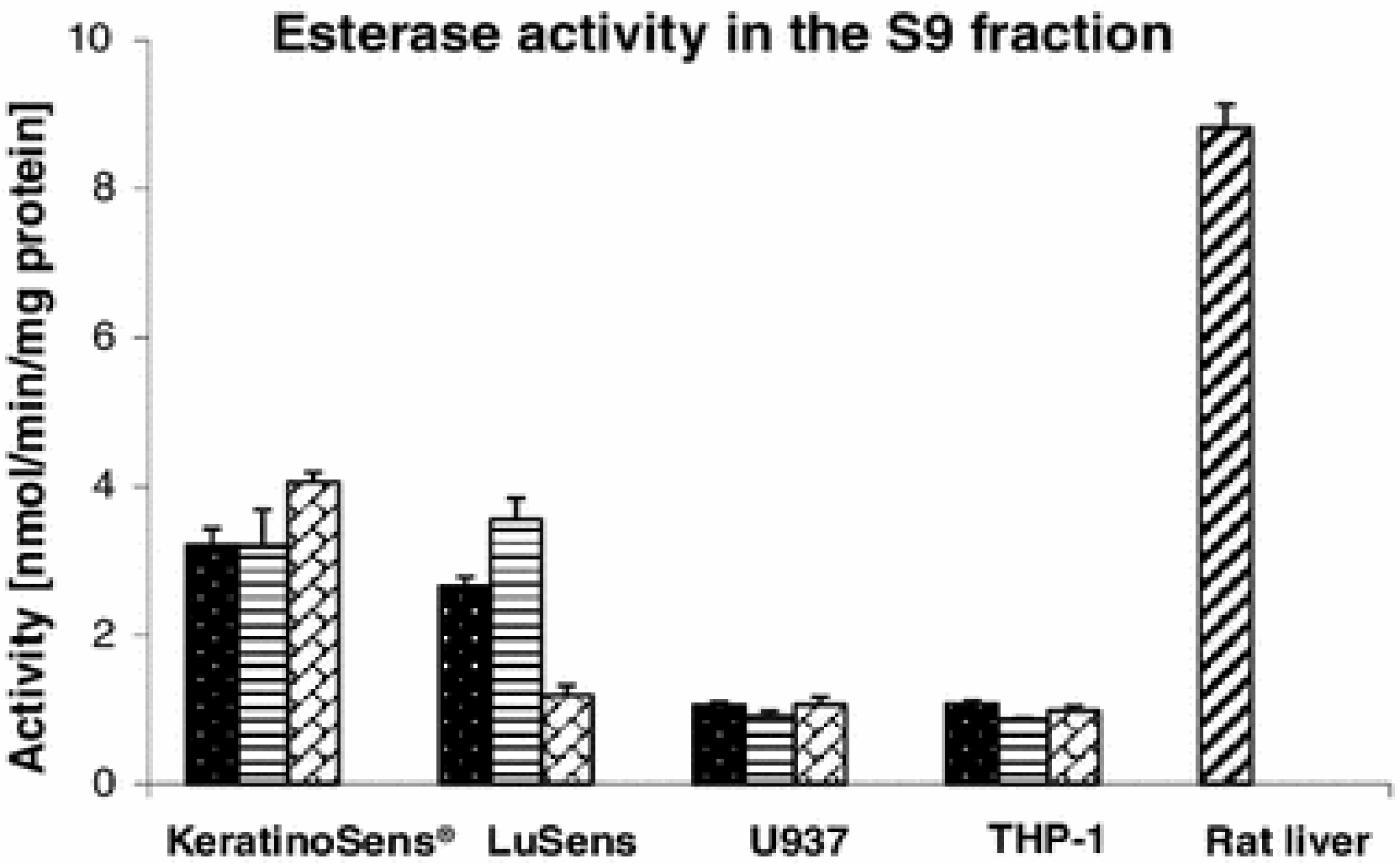
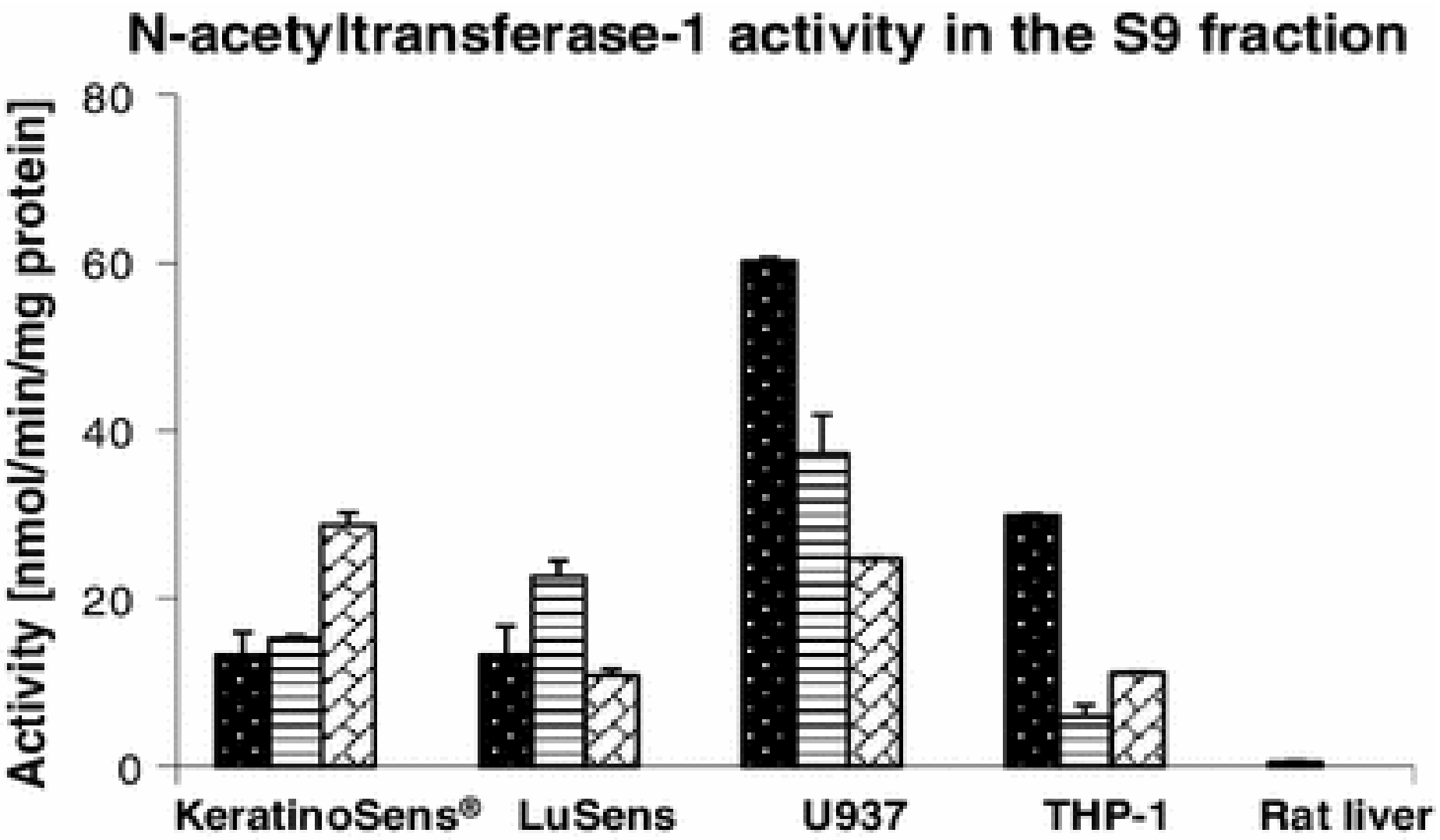


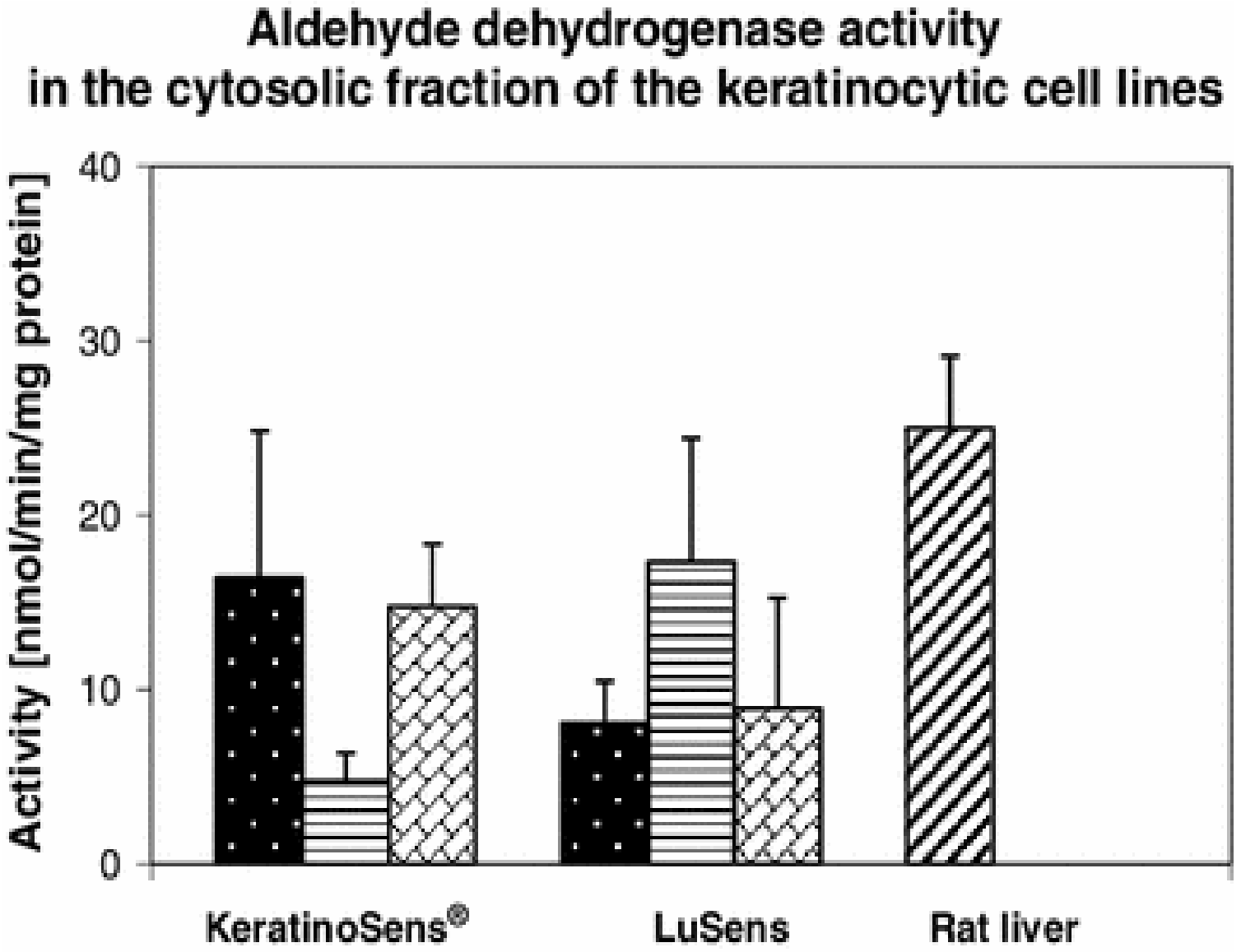
First bar 5th passage, *second bar* 7th passage, *third bar* 8th passage
Level of detection 22.7 pmol product/min/mg protein

Fabian et al. Arch Toxicol (2013) 87:1683-1696



First bar 5th passage, *second bar* 7th passage, *third bar* 8th passage
Level of detection 0.667 pmol product/min/mg protein

Fabian et al. Arch Toxicol (2013) 87:1683-1696



First bar 5th passage, *second bar* 7th passage, *third bar* 8th passage

Fabian et al. Arch Toxicol (2013) 87:1683-1696

Aldehyde dehydrogenase activities in the cytosolic fractions of dendritic cell lines

Cell line	Passage	Activities[a] (nmol/ min/mg protein)
U937	7	<LOD
	8	<LOD
	9	<LOD
THP-1	7	<LOQ
	8	<LOD
	10	<LOQ
Rat liver		25.0 ± 4.0

LOD, level of detection: 1.96
LOQ, level of quantification: 3.91

Fabian et al. Arch Toxicol (2013) 87:1683-1696

Alcohol dehydrogenase activities in the cytosolic fractions				
Cell line	Passage	Activities (nmol/min/mg protein)	LOD (nmol/min/mg protein)	LOQ (nmol/min/mg protein)
KeratinoSens®	5	<LOD	16.1	32.3
	7	<LOD		
	8	<LOD		
LuSens	5	<LOQ	6.40	12.8
	6	<LOQ		
	7	<LOQ		
U937	7	<LOD	23.3	46.5
	8	<LOD		
	9	<LOD		
THP-1	7	30.1 ± 8.4	3.77	7.55
	8	<LOD		
	10	<LOQ		
Rat liver		13.5 ± 2.1	1.73	3.45

LOD, level of detection; LOQ, level of quantification

Fabian et al. Arch Toxicol (2013) 87:1683-1696

Matsunaga et al. Anti-Cancer Drugs 2014, 25:868–877 reported aldo keto reductase activity in U937 cells

Cytochrome P450 (CYP), flavin-containing monooxygenase (FMO) and UDP glucuronosyltransferase (UGT) activities[a] in the microsomal fractions						
	Cytochrome P450			FMO[c]	UGT	
	EROD[c]	PROD[c]	BROD[c]		UGT-1[c]	UGT-2[c]
Cell lines[b]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Rat liver	1,200 ± 72	241 ± 7	436 ± 70	11.02 ± 1.46	12.6 ± 0.2	251,000 ± 32,000
LOD[c]	2.36	7.00	0.954	0.420	0.173	2,510
LOQ[c]	4.72	14.0	1.91	0.840	0.346	5,020

[a] CYP: pmol/min/mg protein; FMO and UGT-1: nmol/min/mg protein; UGT-2: FU/min/mg protein

[b] KeratinoSens, LuSens, U937, THP-1

[c] EROD, 7-ethylresorufin O-deethylase, PROD; 7-pentylresorufin O-depentylase; BROD, 7-benzylresorufin O-debenzylase; FMO with benzydamine as substrate;
UGT-1 with the planar substrate 4-methylumbelliferone;
UGT-2, with the non-planar substrate 4-hydroxybiphenyl

[d] LOD, level of detection; LOQ, level of quantification

Fabian et al. Arch Toxicol (2013) 87:1683-1696.

Skazik-Voogt et al. ALTEX33, 37-46, 2016: U937 and THP-1 no CYP3A mRNA

Performances of the investigated non-animal test methods and the ‘2 out of 3’ approach in different datasets.

	Bauch et al. (2012)		Natsch et al. (2013)		Urbisch et al. 2015	
	Acc [%]	n	Acc [%]	n	Acc [%]	n
Compared to LLNA data						
Peptide reactivity DPRA	79	54	80	145	75	194
KC activation KeratinoSens™	81	54	77	145	73	188
LuSens	77	54	–	–	73	77
DC activation (m)MUSST	74	54	71	141	73	149
h-CLAT	–	–	–	–	76	166
Compared to human data						
Peptide reactivity DPRA	86	51	–	–	84	102
KC activation KeratinoSens™	80	51	–	–	82	102
LuSens	84	51	–	–	79	61
DC activation (m)MUSST	86	51	–	–	78	85
h-CLAT – – – – 82 98						
Prediction model						
’2 out of 3’ approach (vs. LLNA data)	83	54	81	145	79	180
’2 out of 3’ approach (**vs. human data**)	**94**	51	–	–	**90**	101

Acc, accuracy; n, number of analyzed substances; KC, keratinocyte; DC, dendritic cell; ‘‘–’’, no data available or data not considered

2 out of 3’ prediction model in Bauch et al. (2012): DPRA, LuSens, mMUSST); in Natsch et al. (2013): DPRA, KeratinoSens™, MUSST; in Urbisch et al. 2015: DPRA, KeratinoSens™, h-CLAT.

Urbisch et al. Regul Toxicol Pharmacol, 71:337-351, 2015

“2 out of 3” Performance vs. LLNA Data

Accuracy (%)	77.1
Sensitivity (%)	75.3
Specificity (%)	85.0
Balanced Accuracy (%)	**80.2**

Accuracy: Correct classification rate
Sensitivity: True positive rate
Specificity: True negative rate
Balanced accuracy: Average of sensitivity and specificity

OECD Supporting document for evaluation and review of draft Guideline for Defined Approaches for Skin Sensitisation September 2019

Similar performance was demonstrated using the LuSens for KE2:
GD 256; Urbisch et al. Regul Toxicol Pharmacol, 71:337-351, 2015

The 2 out of 3 DA achieved accuracies equivalent to the LLNA and performance exceeding that of the LLNA when compared to human data

Draft OECD Guideline Defined Approaches for Skin Sensitisation September 2019

Pre- and Pro-haptens and the Results of Nonanimal Tests as Well as the 2 out of 3 WoE Approach

TP = true positive

FN = false negativ

‘+’ = positive result in vivo

sensitivity = [TP/(TP + FN)]

Urbisch et al. Chem. Res. Toxicol. 2016, 29, 901−913

No.	Name	LLNA final	Human final	DPRA (Cys+Lys) (HPLC)	Cys-Peptide (LC-MS)	Keratino-Sens™	h-CLAT	'2 out of 3' WoE Approach
1	5-Amino-2-methylphenol	+		TP	Adduct	TP	no data	TP
2	Ethylenediamine	+	+	TP	No adduct	TP	TP	TP
3	4-Amino-m -cresol	+		TP	Adduct	TP	no data	TP
4	Isoeugenol	+	+	TP	Adduct	TP	FN	TP
5	1,4-Phenylene diamine	+	+	TP	Adduct	TP	TP	TP
6	Hydroquinone	+	+	TP	Adduct	TP	TP	TP
7	4-Allylanisole	+		TP	Adduct	inconclusive	TP	TP
8	Propyl gallate	+	+	TP	No adduct	TP	TP	TP
9	Eugenol	+	+	TP	Adduct	inconclusive	TP	TP
10	3-Methylcatechol	+		TP	Adduct	TP	no data	TP
11	2-Nitro-1,4-phenylendiamine	+	+	TP	Adduct	TP	TP	TP
12	4-(Methylamino) phenol sulfate (Metol)	+	+	TP	Adduct	TP	no data	TP
13	2,5-Diaminotoluene sulfate (PTD)	+	+	TP	Adduct	TP	TP	TP
14	Abietic acid	+	+	TP	No adduct	TP	FN	TP
15	Lauryl gallate	+	+	TP	No adduct	TP	TP	TP
16	2-Aminophenol	+	+	TP	Adduct	TP	TP	TP
17	Cinnamyl Alcohol	+	+	TP	Adduct	TP	TP	TP
18	Benzo(a)pyrene	+		TP	No adduct	TP	TP	TP
19	2-methoxy-4-methylphenol	+		FN		FN	TP	FN
20	Resorcinol	+	+	FN		FN	TP	FN
21	3-Aminophenol	+		FN		FN	TP	FN
22	Geraniol	+	+	FN		TP	TP	TP
23	Diethylenetriamine	+	+	FN		FN	FN	FN
24	Farnesol	+	+	FN		TP	TP	TP
25	3-Dimethylamino propylamine	+	+	FN		TP	TP	TP
26	N,N-Dibutylaniline	+		FN		FN	FN	FN
27	4-Chloroaniline	+		FN		TP	TP	TP
		Sensitivity [%]:		67		80	83	81

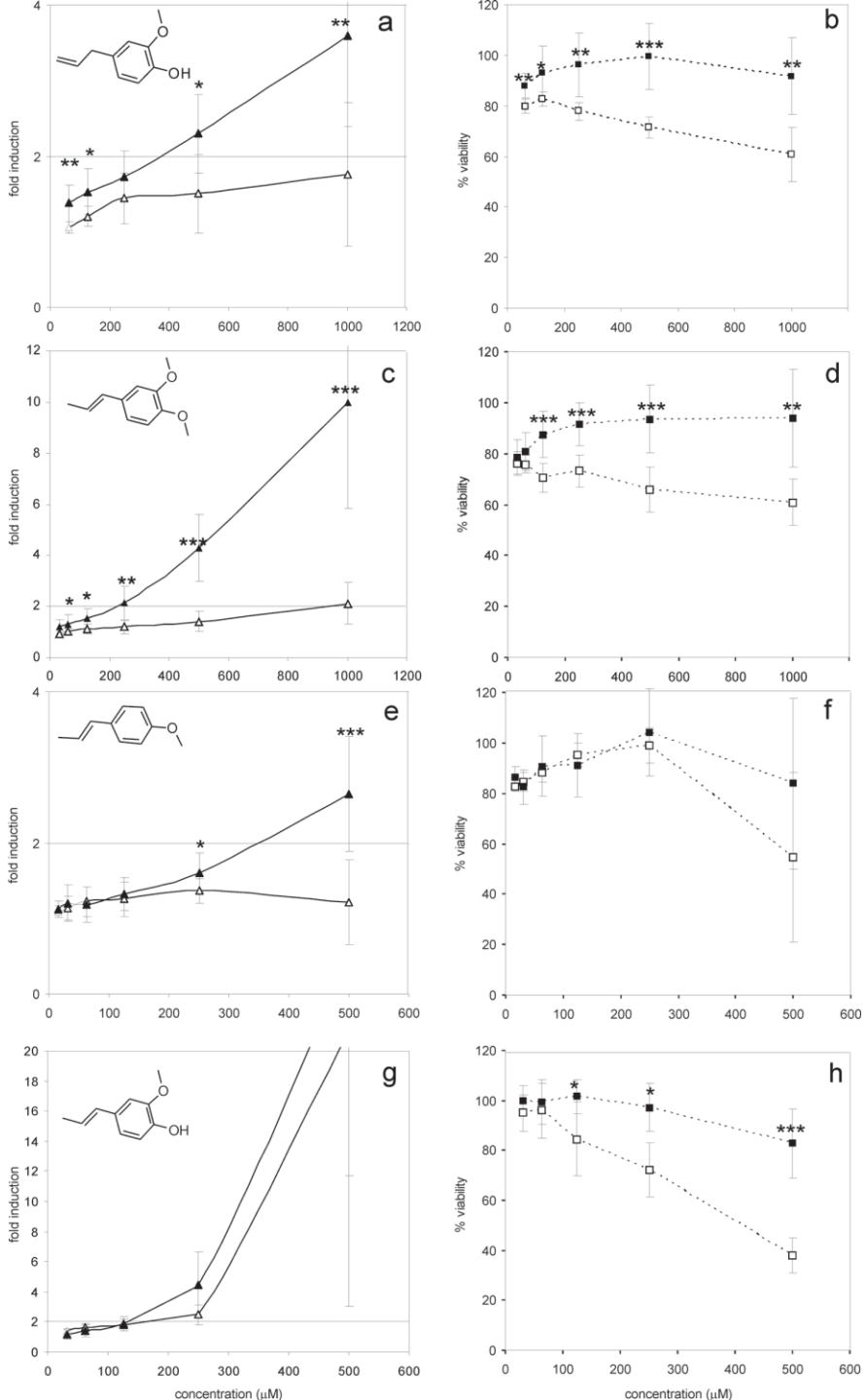
No.	Name	LLNA final	Human final	DPRA (Cys+Lys) (HPLC)	Cys-Peptide (LC-MS)	Keratino-Sens™	h-CLAT	'2 out of 3' WoE Approach
1	5-Amino-2-methylphenol	+		TP	Adduct	TP	no data	TP
17	Cinnamyl Alcohol	+	+	TP	Adduct	TP	TP	TP
18	Benzo(a)pyrene	+		TP	No adduct	TP	TP	TP
19	2-methoxy-4-methylphenol	+		FN		FN	TP	FN
20	Resorcinol	+	+	FN		FN	TP	FN
21	3-Aminophenol	+		FN		FN	TP	FN
22	Geraniol	+	+	FN		TP	TP	TP
23	Diethylenetriamine	+	+	FN		FN	FN	FN
24	Farnesol	+	+	FN		TP	TP	TP
25	3-Dimethylamino propylamine	+	+	FN		TP	TP	TP
26	N,N-Dibutylaniline	+		FN		FN	FN	FN
27	4-Chloroaniline	+		FN		TP	TP	TP
			Sensitivity [%]:	67		80	83	81

TP = true positive, FN = false negative; '+' = positive result in vivo; sensitivity = [TP/(TP + FN)]

Urbisch et al. Chem. Res. Toxicol. 2016, 29, 901−913

KeratinoSens in presence (filled symbols) and absence (open symbols) of metabolic activation by rat liver S9:

Eugenol (a and b), methylisoeugenol (c and d), *trans*-anethole (e and f) and isoeugenol (g and h).



Natsch and Haupt, Toxicol Sci 135, 356–368, 2013

Recombinant human CYP content of skin-like rhCYP cocktail

CYP	content (pmol)
CYP1A1	3.6
CYP1B1	2.0
CYP2B6	0.035
CYP2E1	11
CYP3A5	5.6
Total CYP	22

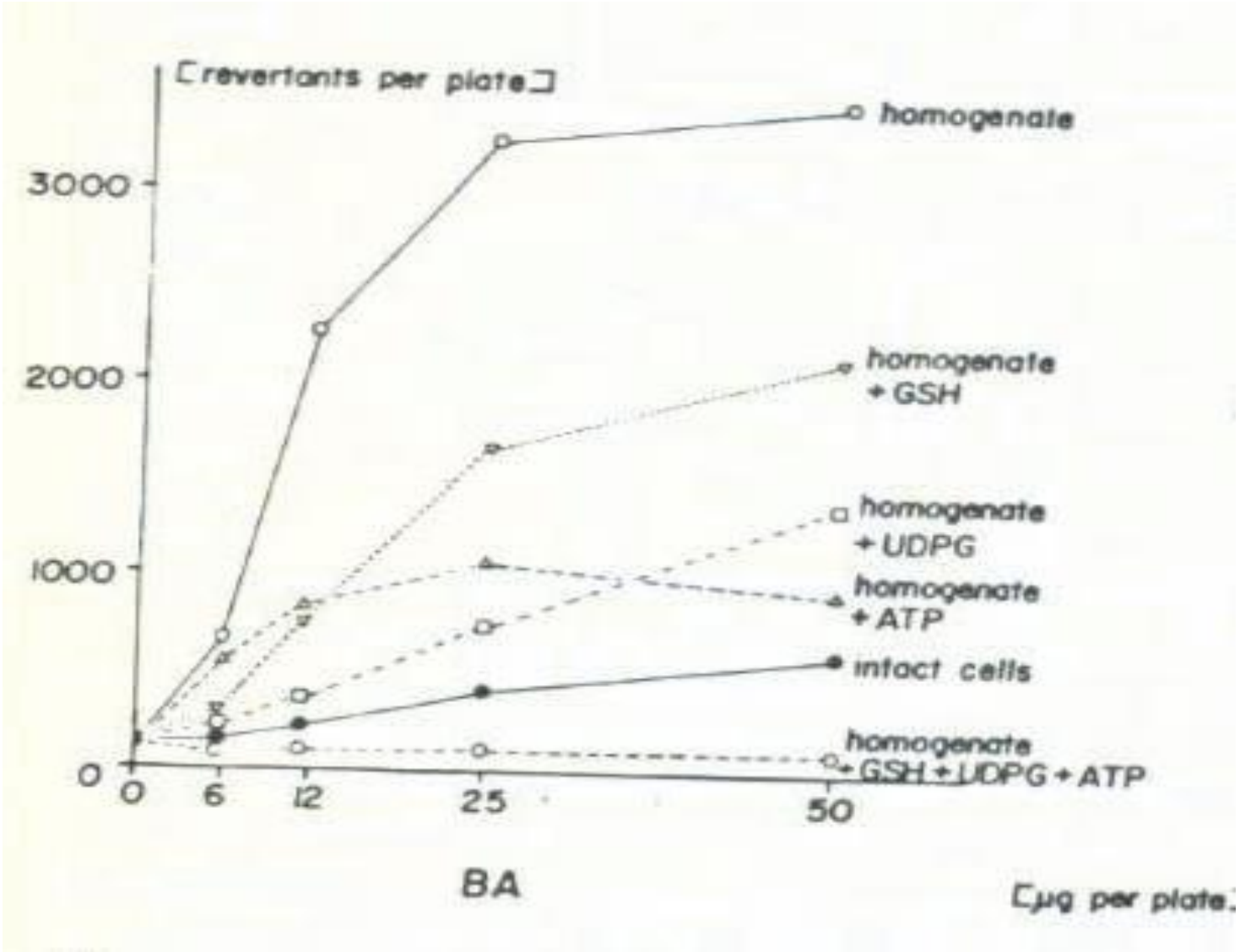
Bergström et al. J Invest Dermatol 127, 1145-1153, 2007

Effect of cofactors for conjugation and of pure epoxide hydrolase on homogenate-mediated mutagenicity

Test compound	No. of revertants above solvent control	Mutagenicity (% of control) in the presence of				
		ATP	UDP-glucuronic acid	Glutathione	ATP + UDP-glucuronic acid + glutathione	Epoxide hydrolase
BP (2 μg)	275, 206, 366	35, 38	31, 37	53, 56	15, 23	102, 105
3-OH-BP (5 μg)	309, 503, 361	16, 46	22, 43	36, 37	0, 18	101, 111
9-OH-BP (5 μg)	1115, 1776, 1895	84, 100	41, 45	70, 85	38, 49	98, 101
BP-7,8-dihydrodiol (0.6–1 μg)	651, 515, 923	148, 166	68, 96	49, 51	55, 65	99, 109
BP-9,10-dihydrodiol (50 μg)	644, 627, 753	106, 120	29, 35	18, 20	8, 14	61, 66

Presence or absence of ATP (10 mM), UDP-glucuronic acid (10 mM), glutathione (5 mM)

Glatt et al. Cancer Res 41, 270-277, 1981



BA, benz[a]anthracene; GSH, 5 mM glutathione; UDPG, 10 mM UDP-glucuronic acid; ATP, 10 mM ATP

Oesch et al. Xenobiotica 18, 35-44, 1988

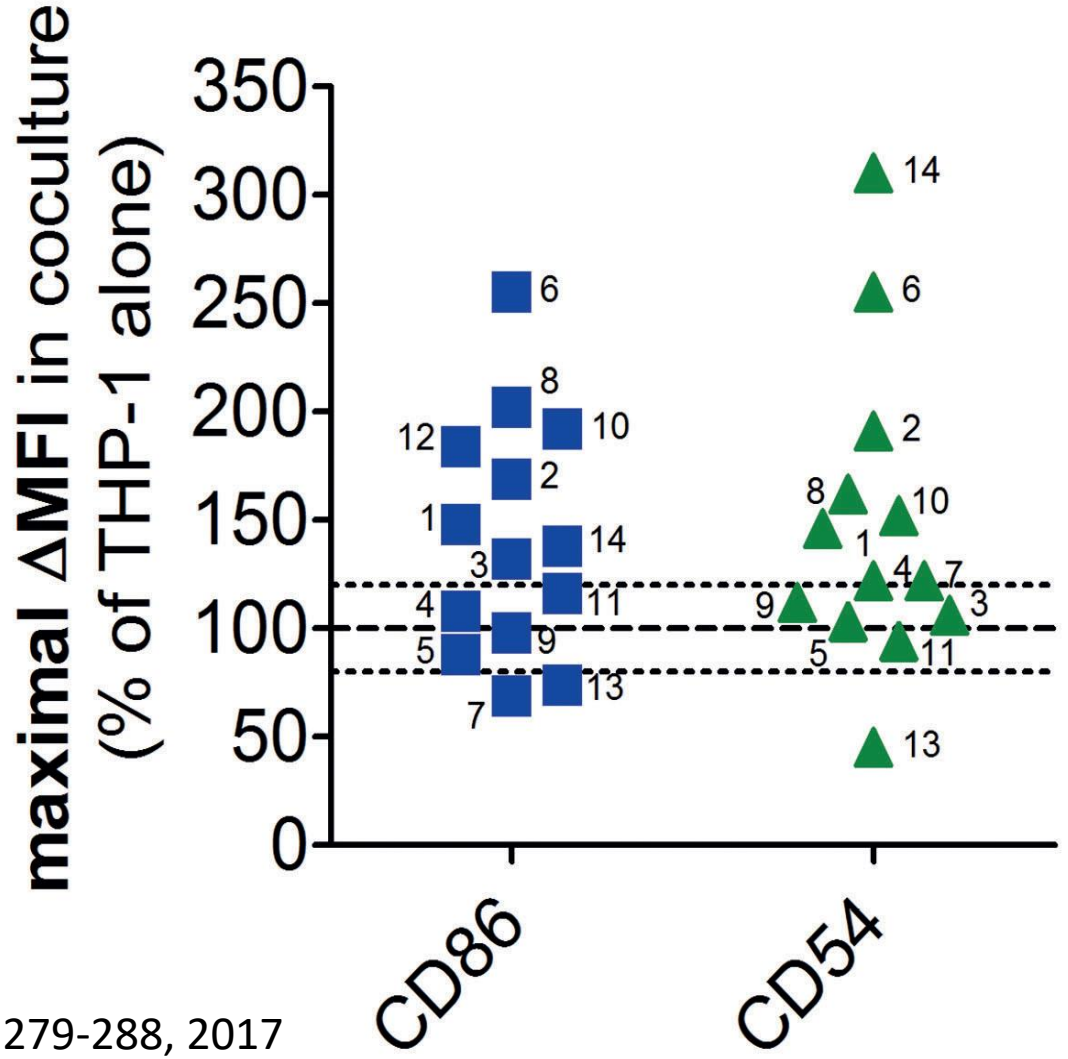
Compound	Carcinogenicity†	Mutagenicity with *S. typhimurium* TA 100			
		Hepatocytes‡		Homogenate‡	
		Potency¶	Max. effect¶	Potency¶	Max. effect¶
DMBA	++++	13·3	1100	35·6	1130
7-MBA	+++	3·0	960	10·8	1810
12-MBA	++	3·0	380	8·3	830
6-MBA	++	1·2	210	11·4	1560
8-MBA	++	1·4	120	16·7	1150
BA	+	3·6	490	50·9	3610
4-MBA	+	1·7	160	10·5	1230
5-MBA	+	3·7	500	12·5	2450
9-MBA	+	1·4	150	8·9	860
10-MBA	+	2·0	210	28·8	1970
11-MBA	+	1·5	320	59·1	3080
1-MBA	±	0·9	240	23·8	2480
2-MBA	±	(0·3)	58	10·9	450
3-MBA	±	(0·2)	35	6·8	430

DMBA, 7,2- dimethylbenz[a]anthracene; MBA, methylbenz[a]anthracene

1-, 2- and 3-MBA produced 3, 1, and 1 tumors in combined carcinogenicity studies ("putative non-carcinogen")

Oesch et al. Xenobiotica 18, 35-44, 1988

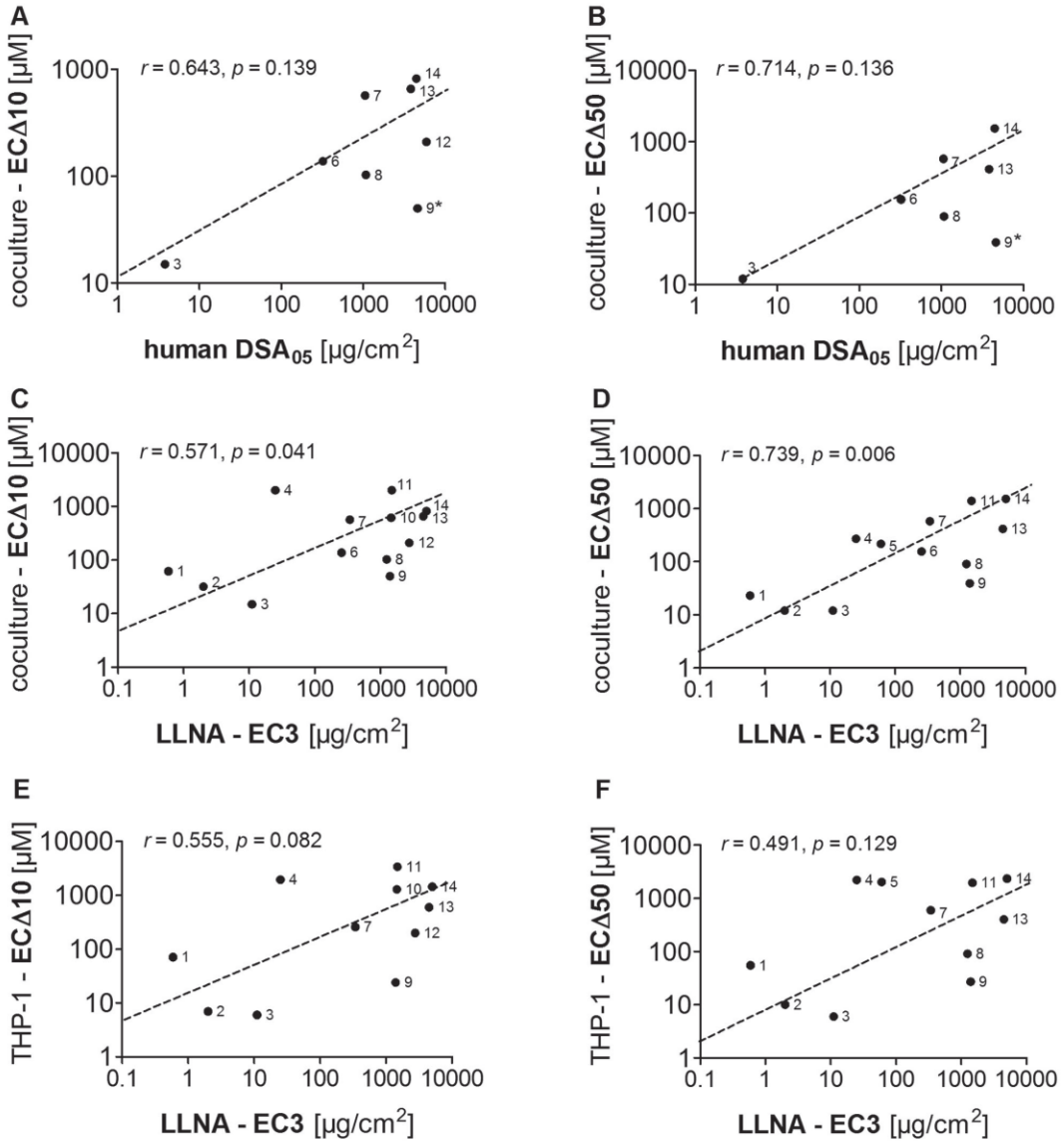
Modulation of maximal upregulation of CD86 and CD54 on THP-1 cells by coculture with HaCaT keratinocytes



1 oxazolone
2 Bandrowski's base
3 2,4-dinitrochlorobenzene
4 acetaminophen
5 3-aminophenol
6 cinnamic aldehyde
7 isoeugenol
8 citral
9 tetramethylthiuram disulfide
10 2-methoxy-4-methylphenol
11 resorcinol
12 eugenol
13 geraniol
14 cinnamic alcohol

Hennen J, Blömeke B. ALTEX. 34, 279-288, 2017

Correlation analysis of ECΔ10 (CD86) and ECΔ 50 (CD54) values obtained in the HaCaT/THP-1 coculture, or in THP-1 monoculture with human DSA_{05} and murine LLNA EC3 values



1 oxazolone
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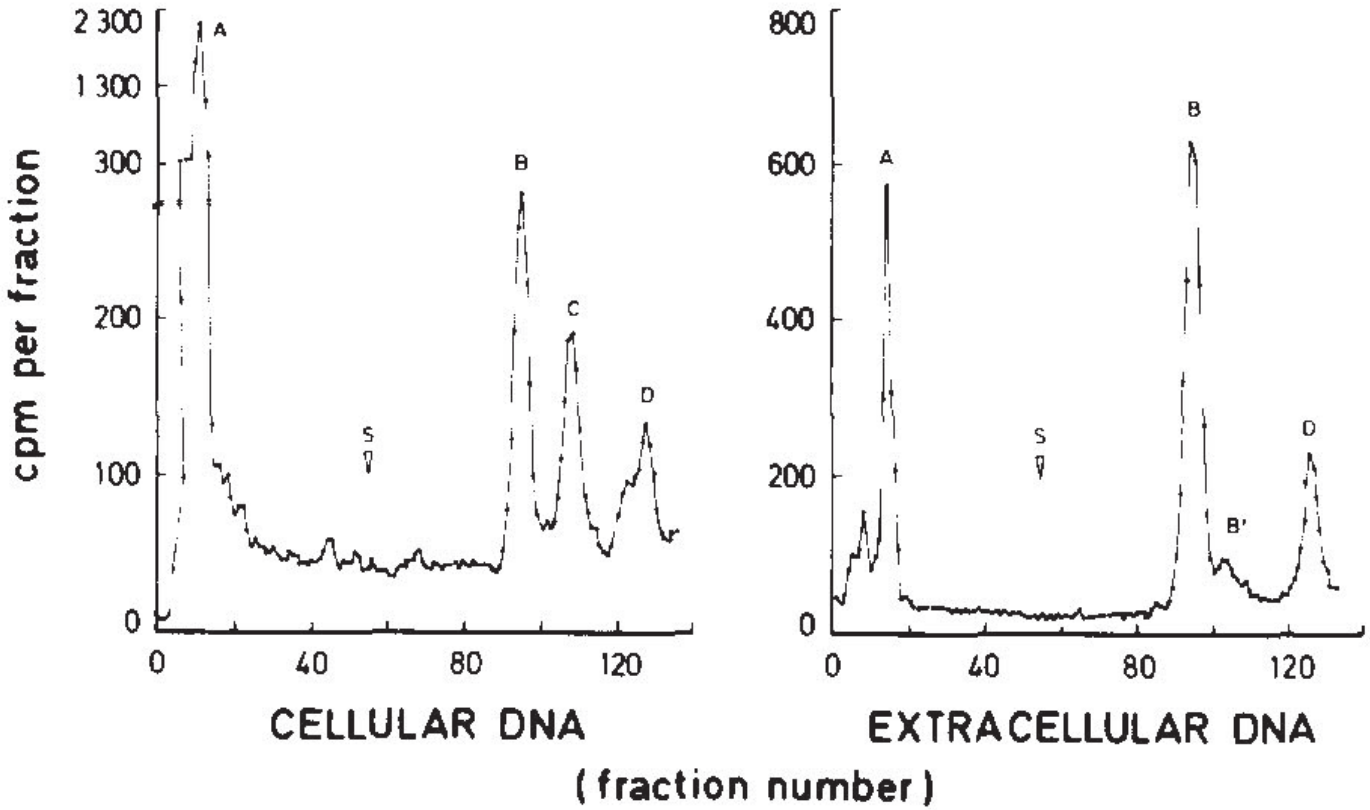
Hennen J, Blömeke B. ALTEX. 34, 279-288, 2017



Hepatocyte-mediated binding of benzo[a]pyrene to cellular DNA (left panel) and exogenously added DNA (right panel).

Turchi et al. Mutat Res 190,31-34, 1987

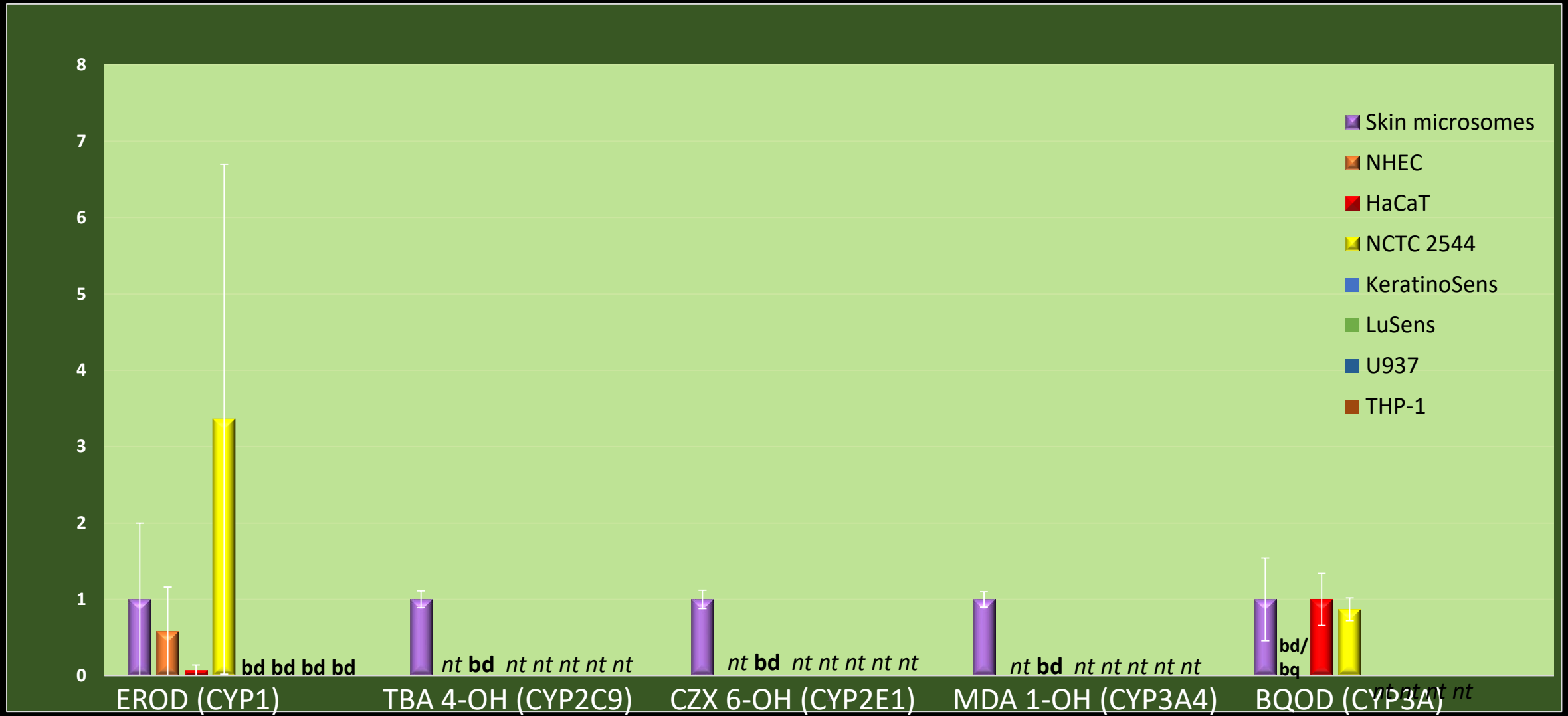
TABLE 1. Activities of Xenobiotic-Metabolizing Enzymes in Cell Lines and in Rat Hepatocytes[a]

Cells	Activity (pmol/mg protein/min)				
	P-450 reductase	Microsomal epoxide hydrolase	Cytosolic epoxide hydrolase	Glutathione transferase	UDP-glucuronosyl transferase
BALB3T3 A31	13,600	157	<2[c]	231,000	n.t.
BHK21 Cl 13	9,400	7	0.6	156,000	9,000
BT_3Ca_f	6,400	25	<1[c]	151,200	8,740
CHEL-1	20,800	72	<1[c]	538,000	9,840
C3H10T1/2	11,600	139	<2[c]	149,000	260
CO-631	11,500	2381	<2[c]	421,000	180
CO-6-SI	6,500	1022	<2[c]	317,000	<50[c]
CO-60	16,100	1352	<2[c]	335,000	980
FRH	22,800	20	n.t.	29,000	n.t.
HepG2	45,900	265	13	47,100	n.t.
HKZ	84,100	1240	n.t.	327,000	n.t.
HM-1	25,700	114	2.1	41,000	<50[c]
HuFoe-15	10,600	135	4.2	3,200	<50[c]
IEC-17	8,400	37	1.5	47,400	11,820
IEC-18	8,600	83	3.0	31,800	4,630
REL-1	4,900	16	n.t.	16,600	n.t.
Reuber H4-II-E	31,000	106	<2[c]	340,400	11,990
V79	3,200	118	<2[c]	637,000	<50[c]
Hepatocytes[b]	42,800	5310	23[d]	440,000	21,000

[a]Activities were determined in cell homogenates. Cytochrome c, benzo[a]pyrene 4,5-oxide, *trans*-stilbene oxide, 1-chloro-2,4-dinitrobenzene and 1-naphthol, respectively, were used as the substrate. n.t., Not tested.
[b]Freshly isolated by the collagenase-perfusion technique from adult male Sprague-Dawley rat.
[c]Detection limit.

Glatt et al. Mol Toxicol 1, 313-334, 1987

Cytochrome P450 activities in cultured human skin cell (lines) compared with human skin

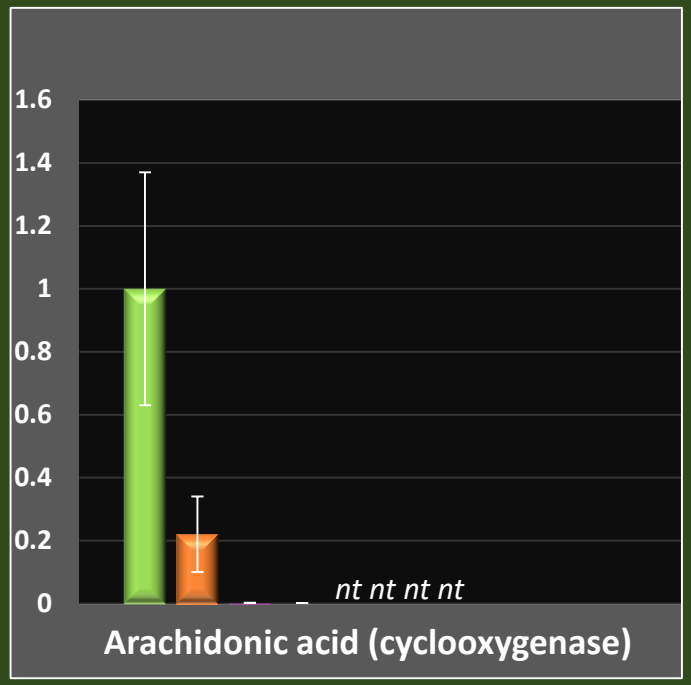


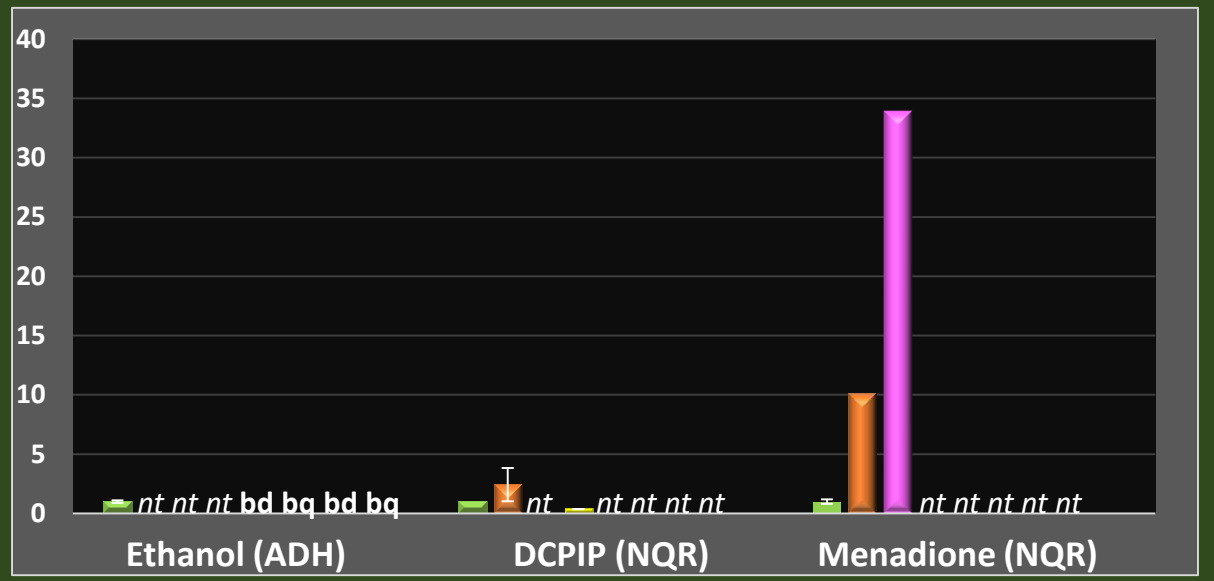
Abbreviations: EROD, 7-Ethoxyresorufin O-deethylase; TBA 4-OH, Tolbutamide 4-hydroxylation; CZX 6-OH, Chlorzoxazone 6-hydroxylation; MDA 1-OH, Midazolam 1-hydroxylation; BQOD, benzyloxyquinoline O-dealkylase; NHEC, Normal Human Epithelial Keratinocytes, ; *nt,* not tested; **bd**, below detection; **bq**, below quantitation

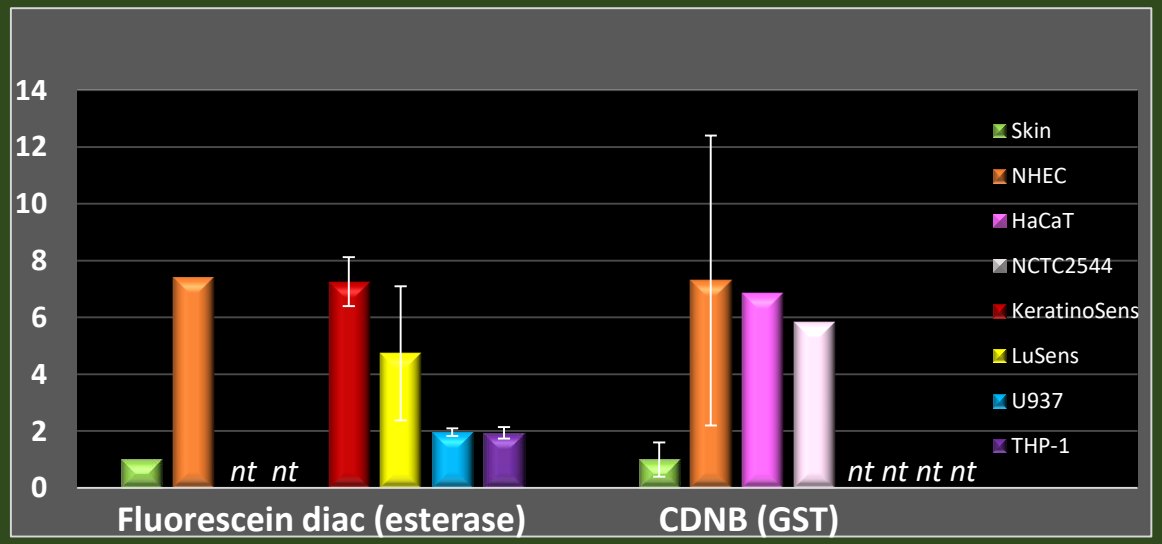
Oesch et al. Arch Toxicol 92, 2411-2456, 2018

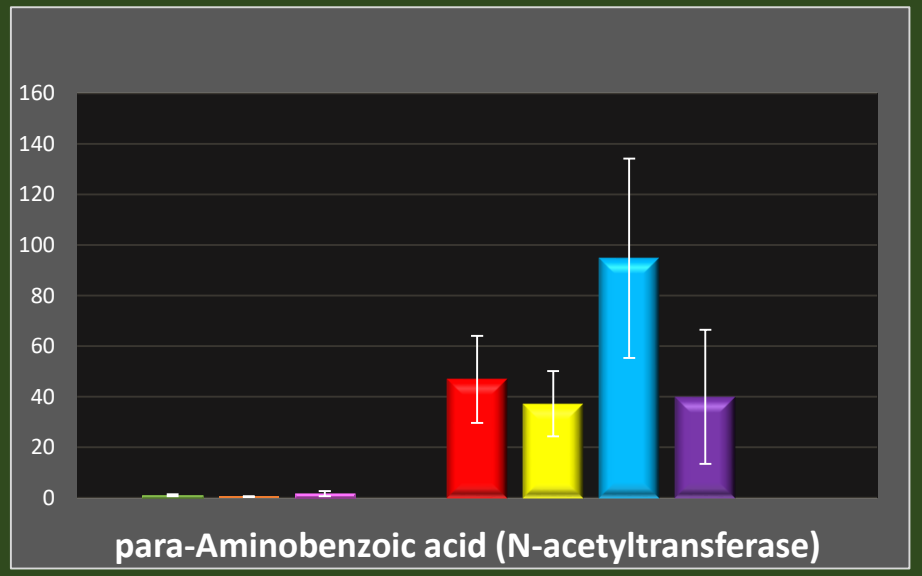
Non-CYP xenobiotica-metabolizing enzyme activities in cultured human skin cell (lines) compared with human skin

x-fold activity compared with human skin









Abbreviations:

ADH, alcohol dehydrogenase;
DCPIP, 2,6-dichlorophenol indophenol;
NQR, NADH/NADPH quinone reductase;
diac, diacetate;
CDNB, 1-chloro-2,4-dinitro benzene;
GST, glutathione S-transferase**;**
nt, not tested;
bd, below detection
bq, below quantitation

Oesch et al. Arch Toxicol 92, 2411-2456, 2018

RELATIVE SUITABILITY OF CULTURED HUMAN SKIN CELL (LINES) COMPARED WITH HUMAN SKIN

Very tentative because of paucity of data

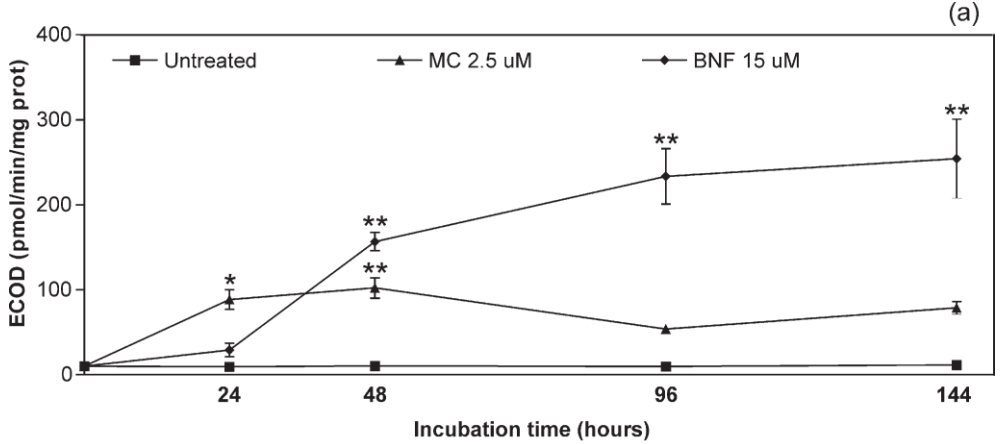
Arbitrary: Compared with human skin: 1-2x: Excellent; >2-3x: Good; >3-10x: Marginally acceptable; >10x: Too distant

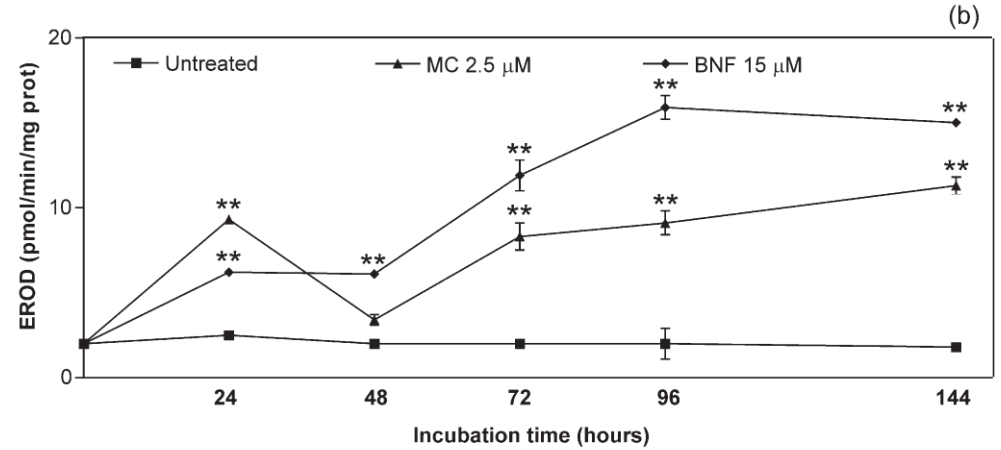
Enzyme	NHEC	HaCaT	NCTC 2544	Keratino Sens®	LuSens	U937	THP-1
Cytochrome P450	vlt	too distant	**good, but vlt**	too distant	too distant	too distant	too distant
Cyclooxygenase	marg accept	too distant	too distant	*nt*	*nt*	*nt*	*nt*
ADH	*nt*	*nt*	*nt*	too distant	too distant	too distant	too distant
NQR	marg accept	too distant	**good, but vlt**	*nt*	*nt*	*nt*	*nt*
Esterase	marg accept	*nt*	*nt*	marg accept	marg accept	**exc, but vlt**	**exc, but vlt**
GST	marg accept	marg accept	marg accept	*nt*	*nt*	*nt*	*nt*
N-Acetyltransferase	**exc**	**exc**	*nt*	too distant	too distant	too distant	too distant

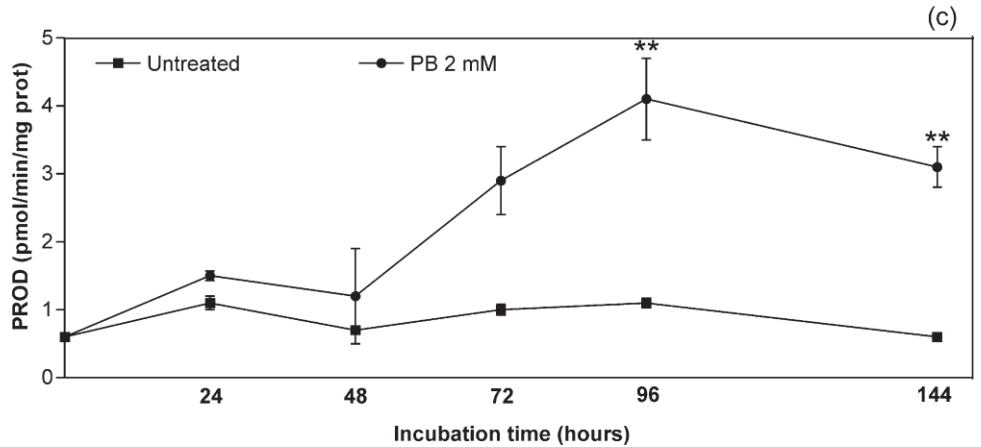
Abbreviations: **vlt, very little tested**; marg accept, marginally acceptable; *nt*, not tested; **exc, excellent;** ADH, alcohol dehydrogenase; NQR, NADH/NADPH quinone reductase; GST, glutathione S-transferase; mEH, microsomal epoxide hydrolase; UDP-glucuronyltrasferase; SULT, sulfotransferase

!NO comparable data on mEH, UGT, SULT! *Oesch et al. Arch Toxicol 92, 2411-2456, 2018*

Phase I xenobiotica-metabolizing enzyme activities in the human keratinocyte cell line NCTC 2544

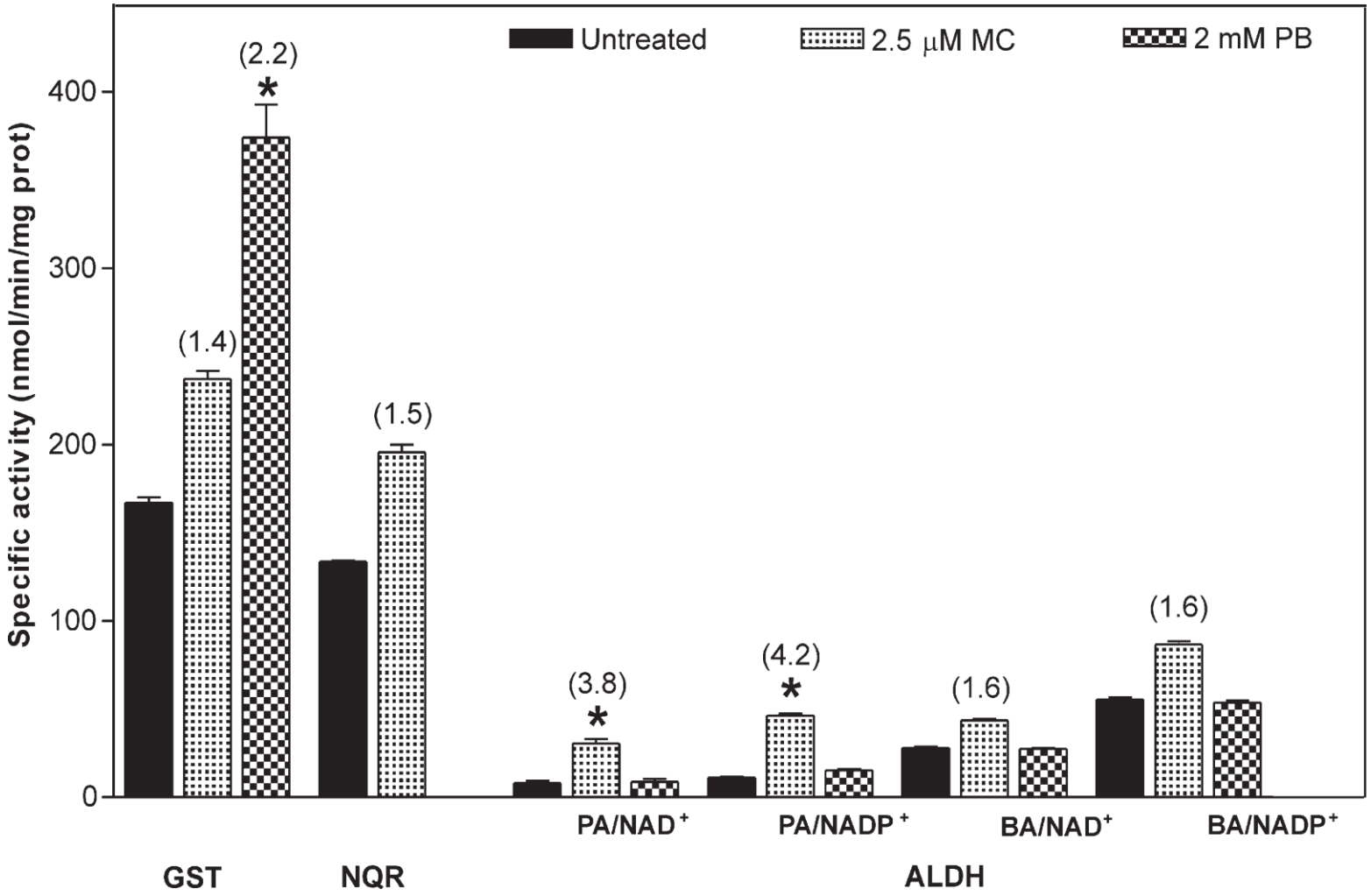






Gelardi et al. Toxicology in Vitro 15 (2001) 701–711

Phase II xenobiotica-metabolizing enzyme activities in the human keratinocyte cell line NCTC 2544



GST: glutathione *S*-transferase; NQR: quinone reductase; ALDH: aldehyde dehydrogenase, substrates: propionaldehyde (PA) and benzaldehyde (BA)

Gelardi et al. Toxicology in Vitro 15 (2001) 701–711

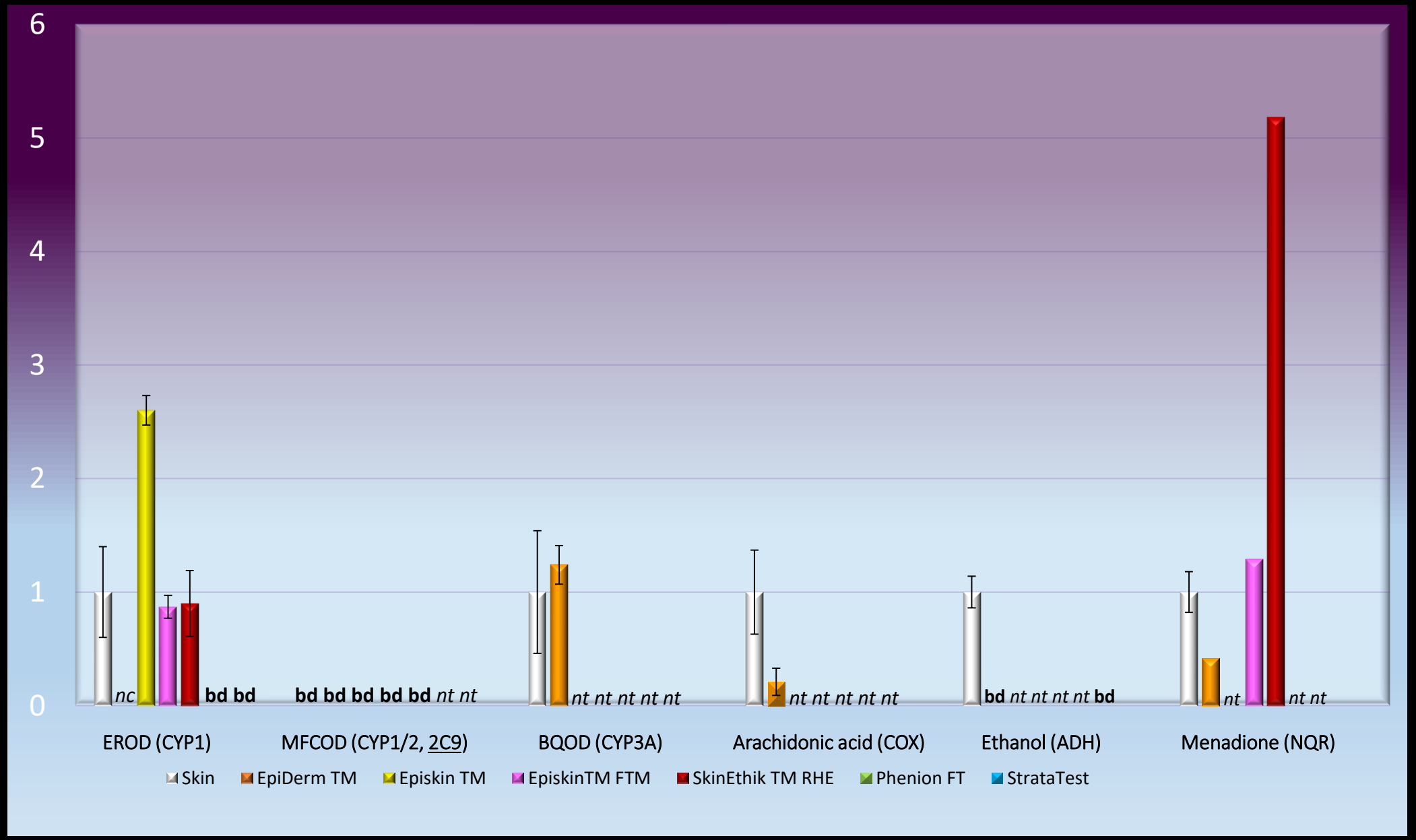
Table IV. Protein content and activities of GST isoenzymes in cultures of rat liver NEC[a]

	NEC	NEC + butyrate
Protein (mg/10^6 cells)	0.253	0.448
1-Chloro-2,4-dinitrobenzene	7.50	66.3
4-Hydroxynon-2-enal	5.00	20.0
trans-4-Phenyl-3-buten-2-one	0.180	0.749
Ethacrynic acid	4.75	22.0

[a] **NEC, nonparenchymal epithelial cells** (from rat liver), grown in the absence or presence of 3.75 mM sodium butyrate.
Enzyme activities expressed as nmol product/min/10^6 cells.

Utesch et al. Carcinogenesis 14, 457-462, 1993

Oxidoreductase activities in human 3D skin models compared with human skin



Abbreviations:

EROD, 7-ethoxyresorufin O-dealkyase;

MFCOD, 7-methoxy-4 trifluoromethyl coumarin;

BQOD, benzyloxy quinoline O-dealkylase;

COX, cyclooxygenase;

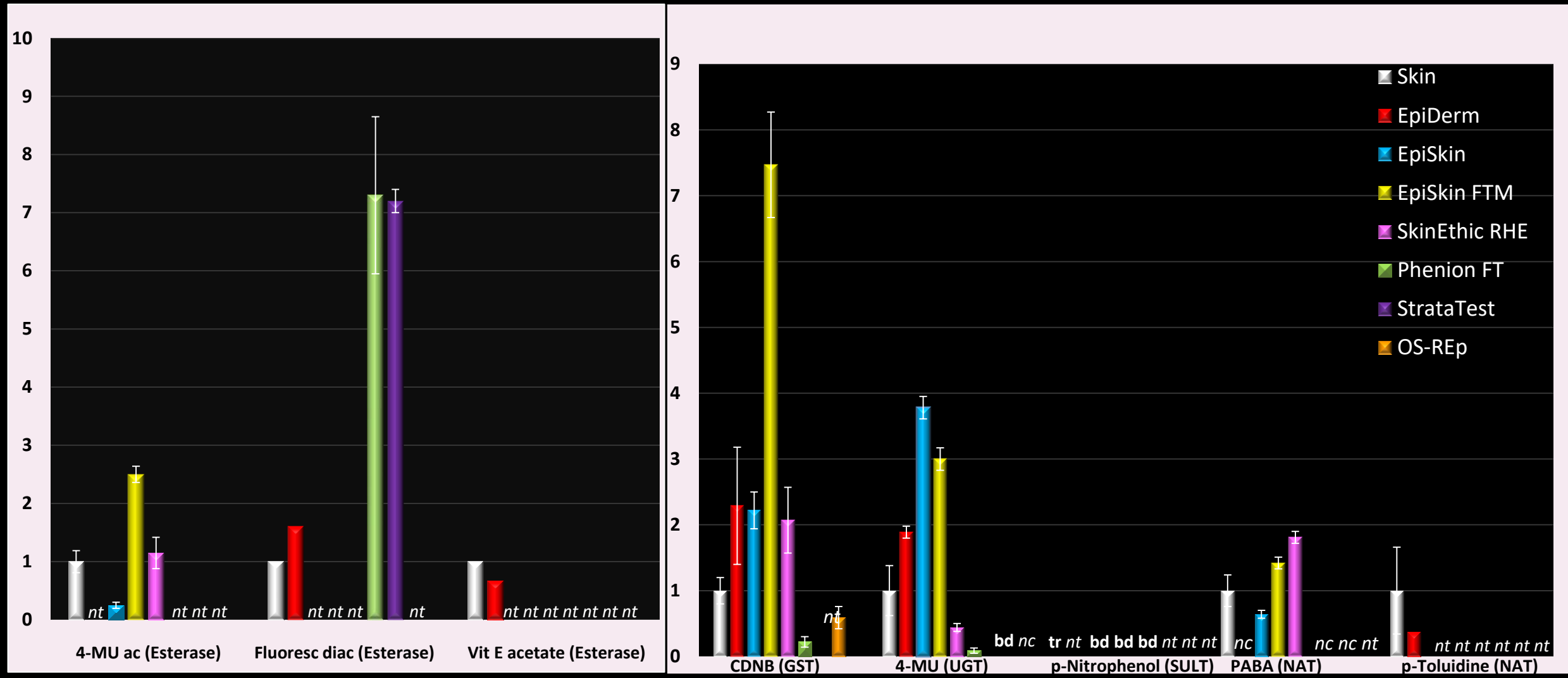
ADH, alcohol dehydrogenase;

NQR, NAD(P)H quinone reductase;

nc, not comparable;
nt, not tested;
bd, below detection

Oesch et al. Arch Toxicol 92, 2411-2456, 2018

Xenobiotic-metabolizing esterases and conjugases in human 3D models compared with human skin



Abbreviations: **4-MU ac,** 4-methylumbelliferone acetate; **Fluoresc diac;** fluorescein diacetate; **Vit E acetate,** vitamin E acetate; **CDNB,** 1-chloro-2,4-dinitrobenzene; **GST,** glutathione S-transferase; **UGT,** UDP-glucuronyltransferase; **SULT**, sulfotransferase; **PABA,** para-aminobenzoic acid; **NAT**, N-acetyl transferase; *nc,* not comparable; **tr,** trace; *nt*, not tested; **bd**, below detection

Oesch et al. Arch Toxicol 92, 2411-2456, 2018

RELATIVE SUITABILITY OF HUMAN 3D SKIN MODELS COMPARED WITH HUMAN SKIN

Very tentative because of paucity of data

Arbitrary: Compared with human skin: 1-2x: Excellent; >2-3x: Good; >3-10x: Marginally acceptable; >10x: Too distant

Enzyme	EpiDerm™	Episkin™	Episkin™FTM	SkinEthik™RHE	Phenion®FT	StrataTest®	OS-REp
Cytochrome P450	**exc, but vlt**	**good, but vlt**	**exc, but vlt**	**exc, but vlt**	too distant	too distant	*nt*
Cyclooxygenase	marg accept	*nt*	*nt*	*nt*	*nt*	*nt*	*nt*
ADH	too distant	*nt*	*nt*	*nt*	*nt*	too distant	*nt*
NQR	**good, but vlt**	*nt*	**exc, but vlt**	marg accept	*nt*	*nt*	*nt*
Esterase	**exc, but vlt**	marg accept	**good, but vlt**	**exc, but vlt**	marg accept	marg accept	*nt*
GST	**good, but vlt**	**good, but vlt**	marg accept	**exc, but vlt**	marg accept	*nt*	**exc, but vlt**
UGT	**exc, but vlt**	marg accept	**good, but vlt**	**good, but vlt**	marg accept	too distant	*nc*
Sulfotransferase	*nt*	*nc*	*nc*	*nc*	*nt*	*nt*	*nt*
N-Acetyltransferase	**good**	**exc**	**exc**	**exc**	*nc*	*nc*	*nt*

Abbreviations: **exc, excellent; vlt, very little tested**; marg accept, marginally acceptable; *nt*, not tested; *nc*, not comparable; ADH, alcohol dehydrogenase;

NQR, NADH/NADPH quinone reductase; GST, glutathione S-transferase; UDP-glucuronyltransferase

Oesch et al. Arch Toxicol 92, 2411-2456, 2018

The effect of sensitizers and non-sensitizers on CD86 expression and cytokine release from **VG-KDF-Skin**

	CD86 expression	IL-1a concentration (% of control)	IL-4 concentration (% of control)
Control	±	100 ± 8	100 ± 18
DNCB 1 mmol/l	+	133 ± 16*	140 ± 36
DNCB 2 mmol/l	**+ / ++**	**598 ± 28*** **	**262 ± 91***
HCA 1 mmol/l	±	108 ± 8	127 ± 34
HCA 2 mmol/l	**+**	**256 ± 39****	**150 ± 81**
DNFB 0.5 mmol/l	**+ / ++**	**181 ± 24****	**270 ± 107***

DNCB, 2,4-dinitrochlorobenzene; DNFB, 2,4-dinitro furuolobenzene; HCA, a-hexyl cinnamic aldehyde; **VG-KDF-Skin, three-dimensional human skin model composed of Keratinocytes, Dendritic cells and Fibroblasts using collagen VitriGel membrane**

Uchino et al. Toxicol in vitro 23, 333-337, 2009

A novel in vitro test "EpiSensA" that uses reconstructed human epidermis
(RhE "LabCyte EPI-MODEL", Japan tissue Engineering, Aichi, Japan)

	EpiSensA	DPRA	KeratinoSens	h-CLAT
A. Lipophilic chemicals				
N	29	27	26	26
Sensitivity (%)	93	44	67	46
Specificity (%)	100	100	0	10
Accuracy (%)	93	48	62	50
B. Hydrophilic chemicals				
N	43	43	42	41
Sensitivity (%)	96	81	70	81
Specificity (%)	75	81	93	87
Accuracy (%)	88	81	79	83
C. Pre/pro-haptens				
N	11	11	11	10
Sensitivity (%)	100	55	73	80
Specificity (%)	–	–	–	–
Accuracy (%)	100	55	73	80
D. Overall				
N	72	70	68	67
Sensitivity (%)	**94**	**63**	**69**	**64**
Specificity (%)	**78**	**83**	**82**	**88**
Accuracy (%)	**90**	**69**	**72**	**70**

Saito et al. Toxicol in vitro 40, 11-25, 2017 (compared with LLNA)

Protein and cyctochrome P450 content and activities of xenobiotic-metabolizing enzymes in treshly isolated rat liver parenchymal cells (PC), a rat hepatoma cell line (FAO), and different hybrid cell lines (HPCT).

		HPCT		
	FAO	Clone 1B1E3	Four further clones	PC
Protein (mg/10^6cells)	0·21 ± 0·09	0·69 ± 0·03	0·47 to 0·74	1·61 ± 0·36
Percentage	(13 ± 5)	(43 ± 2)	(29 to 46)	(100 ± 22)
P450 (nmol/10^6cells)	< 0·02	< 0·02	< 0·09	0·37 ± 0·06
Percentage	(< 5)	(< 5)	(< 5)	(100 ± 16)
Microsomal EH (U/10^6cells)[a]	0·04 ± 0·03	0·32 ± 0·01	0·26 to 0·31	2·80 ± 0·94
Percentage	(1 ± 1)	(11 ± 0)	(9 to 11)	(100 ± 34)
Cytosolic EH (U/10^6cells)[a]	< 0·02	2·5 ± 1·1	0·2 to 6·7	23·4 ± 12·6
Percentage	(< 0·1)	(11 ± 5)	(1 to 29)	(100 ± 54)
GST (U/10^6cells)[a]	9·6 ± 3·3	96·7 ± 18·8	60·8 to 80·0	744 ± 341
Percentage	(1 ± 0)	(13 ± 3)	(8 to 11)	(100 ± 46)
Sulphotransferase (U/10^6cells)[a]	< 0·01	0·04 ± 0·02	0·03 to 0·07	3·58 ± 1·30
Percentage	(< 0·3)	(1 ± 1)	(1 to 2)	(100 ± 36)
UDPGT (U/10^6cells)[a]	5·4 ± 2·3	25·9 ± 4·8	20·0 to 33·9	22·6 ± 7·8
Percentage	(24 ± 10)	(115 ± 21)	(88 to 150)	(100 ± 35)

Utesch et al. Xenobiotica 22, 1451-1457, 1992

Metabolic conversion of testosterone and benzo[*a*]pyrene in freshly isolated rat liver parenchymal cells (PC), a rat hepatoma cell line (FAO), and two hybrid cell lines (HPCT) (nmol/h per 10^6cells).

	FAO	HPCT	PC
Testosterone	<1	5 to 20[a]	386 ± 26
Percentage	$(<0{\cdot}3)$	(1 to 5)	(100 ± 7)
Benzo[*a*]pyrene	$1{\cdot}6 \pm 0{\cdot}6$	$5{\cdot}2 \pm 1{\cdot}9$[b]	$9{\cdot}0 \pm 1{\cdot}4$
Percentage	(17 ± 7)	(58 ± 21)	(100 ± 16)

Utesch et al. Xenobiotica 22, 1451-1457, 1992

TABLE 4. Monooxygenase Activities in V79 Cells and Lines Derived from Them through Transfection of cDNA Coding for Rat P-450IA1 (XEM1, XEM2, XEM3) or P-450IIB1 (SD1)[a]

Cells	Specific activity (pmol/min·mg protein)		
	7-Pentoxyresorufin dealkylase	7-Ethoxycoumarin dealkylase	Arylhydrocarbon hydroxylase
V79	n.d. (<0.1)	~0.2[c], ~0.2[c]	~0.2[c], ~0.2[c]
SD1	33, 37, 38	n.t.	~0.2[c], ~0.2[c]
XEM1	n.t.	15	9, 9, 10
XEM2	n.t.	85	51, 49
XEM3	n.t.	43	20, 21
Hepatocytes, untreated[b]	10, 10, 12	36	45
Hepatocytes, Aroclor 1254-treated[b]	130, 201,193	n.t.	n.t.

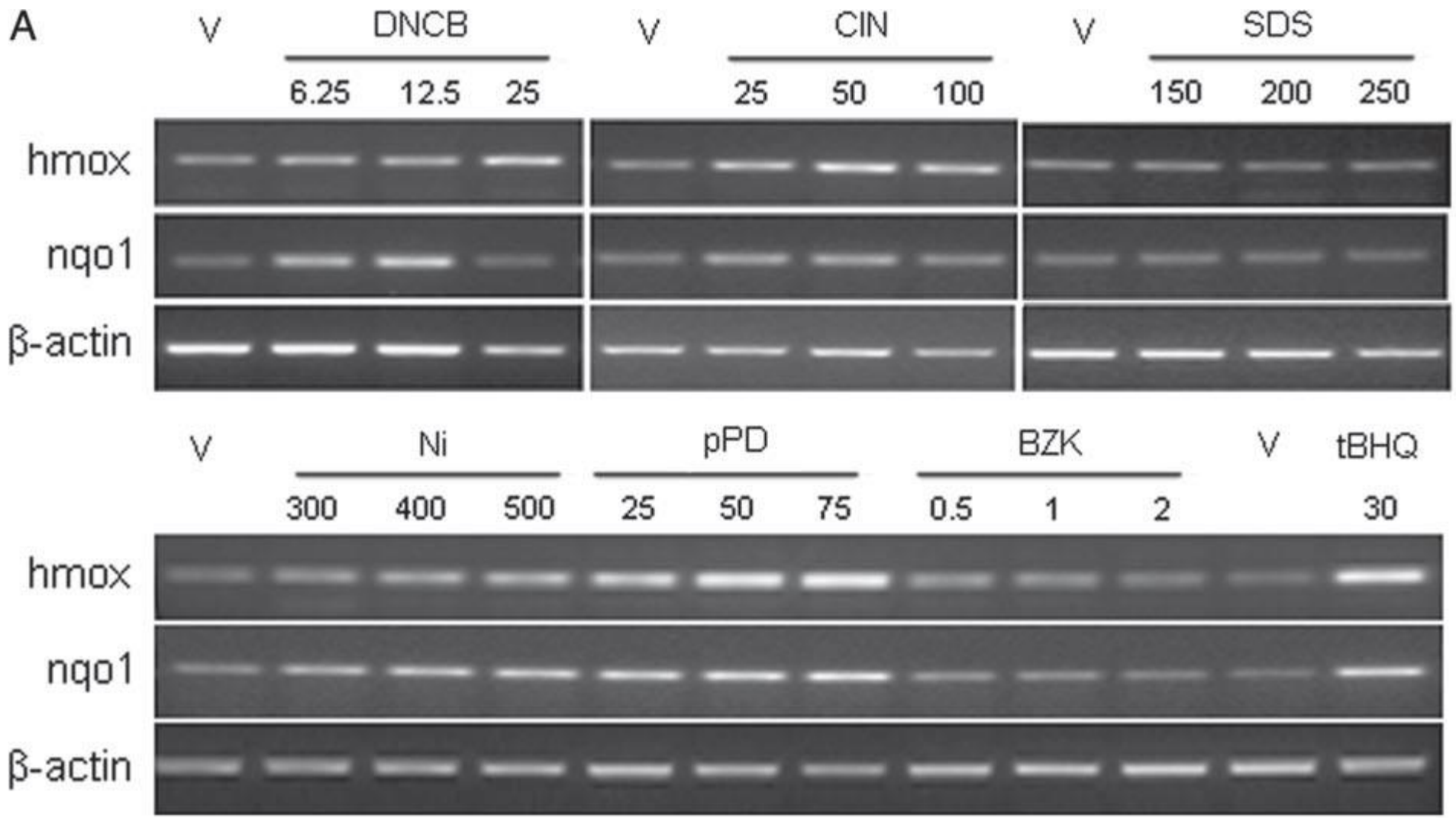
n.d., not detected (detection limit in parenthesis); n.t., not tested

Glatt et al. Mol Toxicol 1, 313-334, 1987

SUMMARY

- **Cells currently used in KE2 and KE3 sensitization tests posses several important xenobiotica-metabolizing enzymes, but lack some other important xenobiotica-metabolizing enzymes**
- **Despite of this shortcoming, the use of these cells surprisingly has led to apparently quite satisfactory accuracies compared with LLNA and human data**
- **Hence, apparently no absolute necessity for improvements, which, however, would be desirable for consumer's and companies protection for old and, especially, new compounds with new structures**
- **Possibilities for improvement:**
  - **S9 fortified with cofactors of all relevant enzymes**
  - **Use of co-cultures**
  - **Search for more ideal cell lines**
  - **Use of differentiation inducers**
  - **Use of 3D human skin models**
  - **Construction of hybrids**
  - **Transfection of the genes for the missing enzymes**

END OF PRESENTATION



Expression of hmox1 and nqo1 mRNAs in human CD34-DC in response to various concentrations of chemicals. mRNA expression visualized on a 2% agarose gel.
Ade et al. Toxicol Sci 107: 451-460, 2009

	Human data				LLNA data			
	Se [%]	Sp [%]	Acc [%]	n	Se [%]	Sp [%]	Acc [%]	n
2 out of 3' approach: DPRA, KeratinoSens, h-CLAT	**90**	**90**	**90**	**101**	**81**	**83**	**82**	**103**
DPRA	84	84	84	102	77	85	79	105
KeratinoSens™	82	84	82	102	74	73	74	103
h-CLAT	89	64	82	98	86	68	81	101
LuSens	78	79	79	60	73	70	71	62
(m)MUSST	74	88	78	85	71	83	75	87
LLNA	91	64	82	111	–	–	–	–

Se = sensitivity; Sp = specificity; Acc = accuracy; n = number of substances analyzed

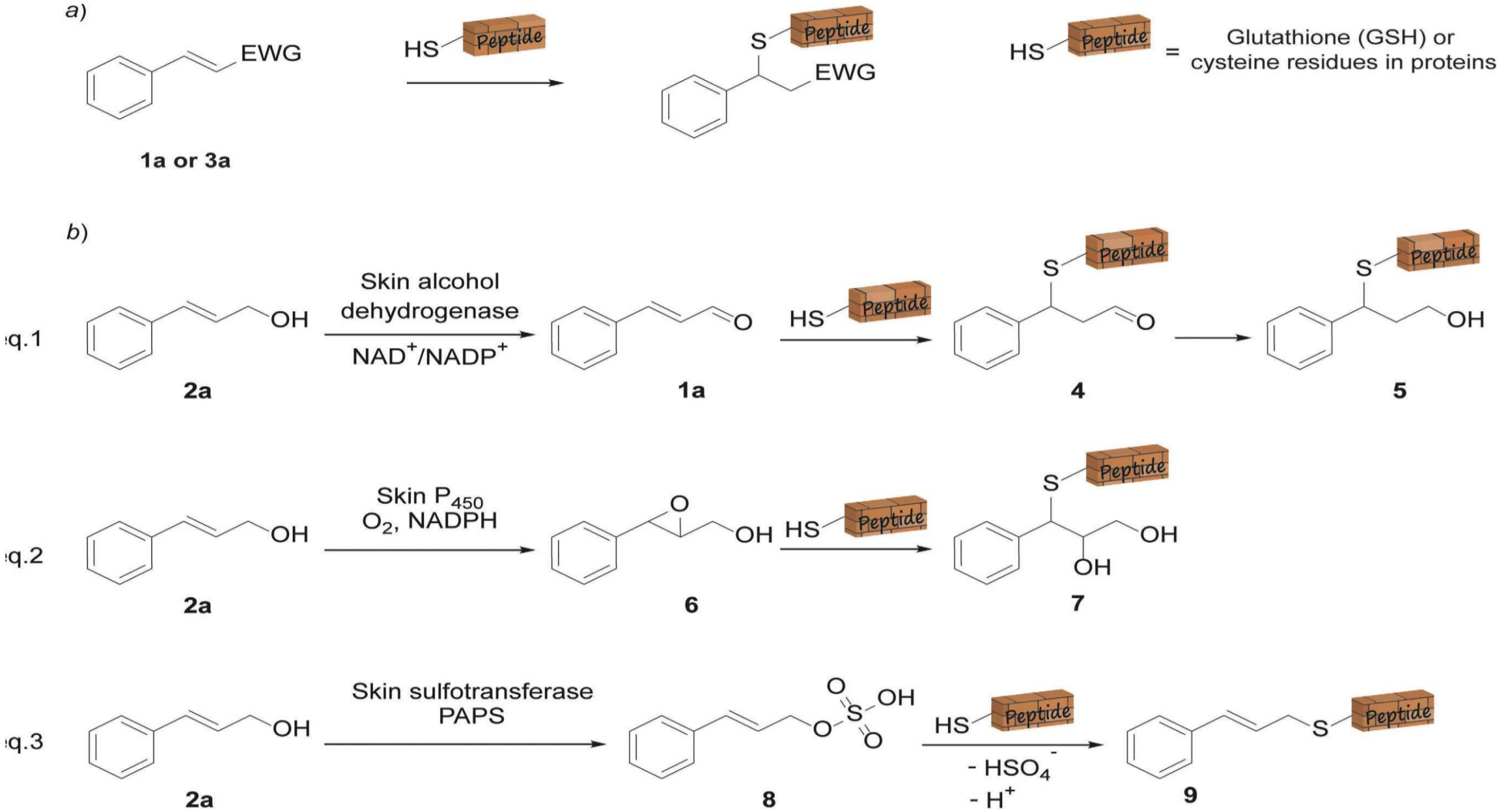
Urbisch et al. Regul Toxicol Pharmacol, 71:337-351, 2015

A. Conservative approach applied to ambiguous classifications:
Assign Human NC/1B as Positive

Performance vs. Human Data	"2 out of 3" DA	LLNA
Accuracy (%)	**69.7**	72.2
Sensitivity (%)	**71.7**	86.0
Specificity (%)	62.5	20.0
Balanced Accuracy (%)	67.1	53.0

B. Default to Negative approach applied to ambiguous classifications:
Assign Human NC/1B as Negative

Performance vs. Human Data	"2 out of 3" DA	LLNA
Accuracy (%)	**67.1**	55.6
Sensitivity (%)	**81.6**	89.2
Specificity (%)	52.6	20.0
Balanced Accuracy (%)	67.1	54.6



Charpantier et al. Chem Biodivers 15(4), e1800013, 2018

DETAILED SUMMARY

- KeratinoSens and LuSens cells used in KE2 as well as U327 and THP-1 cells used in KE3 sensitization tests are well equipped esterase activity and with N-acetyltransferase-1 activity, enzymes of high importance in the metabolism of sensitizers
- KeratinoSens and LuSens cells used in KE2 sensitization tests in addition are well equipped with aldehyde dehydrogenase activity, but not U327 and THP-1
- Alcohol dehydrogenase activity was quantifiable only in THP-1
- The important CYP1A, 2B and 3A-depndent EROD; PROD and BROD as well as FMO and UGT activities were not detected in any of these cells
- Nevertheless, the combination of the combination of KE1, KE2 and KE3 or a 2/3 WoE approach led to satisfactory accuracies when compared with LLNA or human data
- Hence, a further improvement of xenobiotic metabolism for KE2 and KE3 cells does not appear absolutely necessary, but is desirable for protection of consumers and of producing/selling companies
- Possibilities for improvement:
  S9 fortified with cofactors of all relevant enzymes
  Search for more ideal cell lines
  Use of differentiation inducers
  Use of 3D human skin models
  Use of co-cultures
  Construct hybrids
  Transfect the genes for the missing enzymes

SUMMARY

- If the metabolite responsible for desired or deleterious effects is known or is reasonably presumed, models relatively close to the human skin with respect to generating and removing the responsible metabolite may be chosen. Only few models fulfill the requirements of good closeness to human skin **and** studies with several substrates comparing the activity for the same substrates with human skin:
  - **For esterases: Pig: Excellent**
  - **For glutathione S-transferases: Rat: Good**
  - **For N-acetyltransferase: NHEC, HaCaT, Episkin, Episkin FTM, SkinEthikRHE: Excellent; EpiDerm: Good**

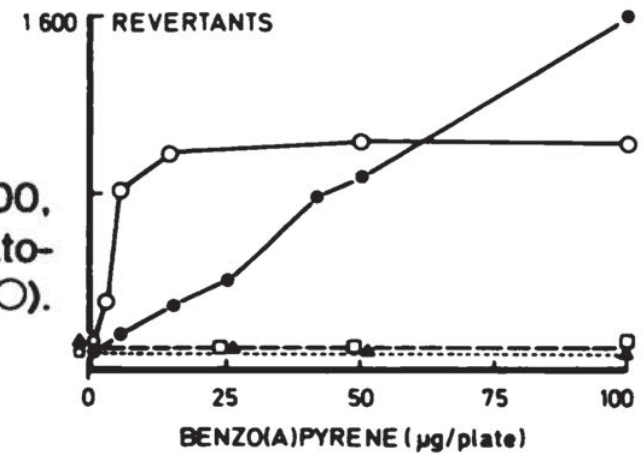
- If only one or two substrates tested is tentatively accepted, the following models may be included in the choice:
  - For CYPs: Guinea pig, pig; NCTC 2544; EpiDerm™, Episkin™, Episkin™FTM, SkinEthik™RHE
  - For ADH: Mouse, guinea pig
  - For NQR: NCTC 2544; EpiDerm™, Episkin™FTM
  - For esterase: U937, THP-1; EpiDerm™, Episkin™FTM, SkinEthik™RHE
  - For GST: Pig; EpiDerm™, Episkin™, SkinEthik™RHE, OS-REp
  - For UGT: EpiDerm™, Episkin™FTM, SkinEthik™RHE
  - For SULT: Rat

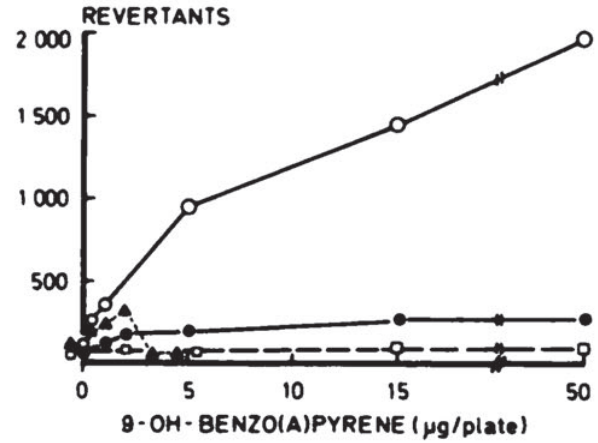
Fragrance substances that have been experimentally shown to act as prehaptens and/or prohaptens

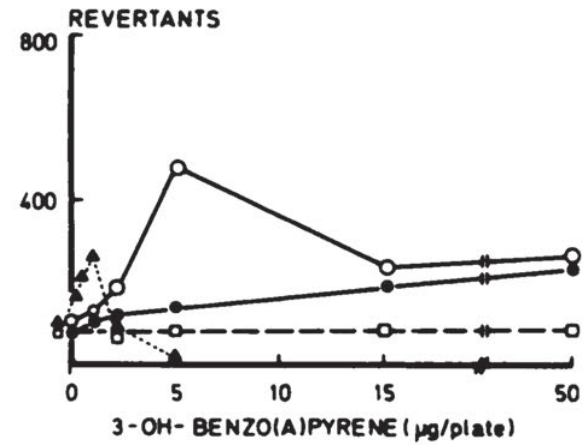
Fragrance Substance	Activation by air oxidation	Bioactivation (oxidation)
Cinnamyl alcohol	Yes	Yes
Eugenol	No	Yes
Geranial	Yes	No
Geraniol	Yes	Yes
Isoeugenol	No	Yes
Limonene	Yes	No
Linalool	Yes	No
Linalyl acetate	Yes	No
α-Terpinene	Yes	Yes

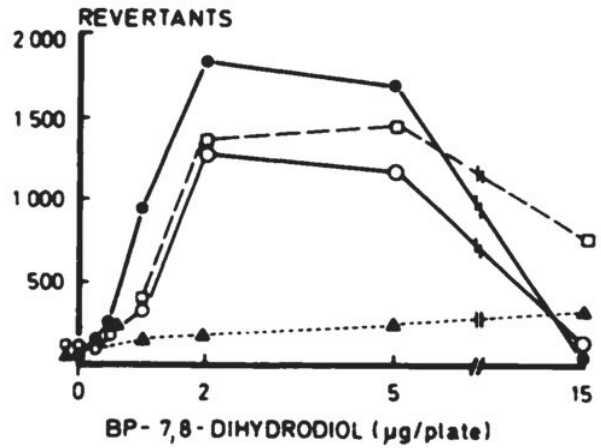
Karlberg et al. Contact Dermat 69, 323-334, 2013

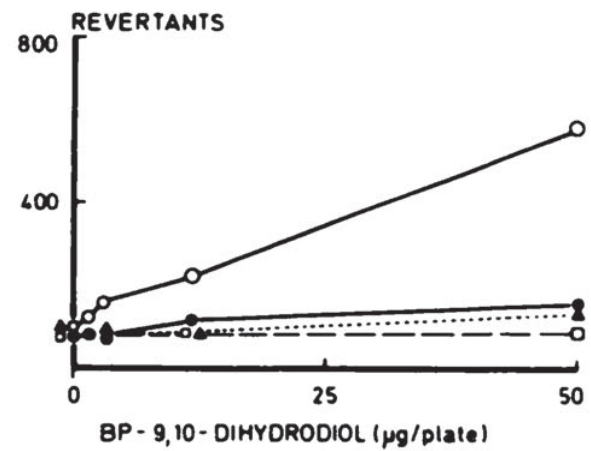
Chart 1. Mutagenicity of BP and BP metabolites in *S. typhimurium* TA 100, directly (▲) or in the presence of intact hepatocytes (●), homogenized hepatocytes (□), or homogenized hepatocytes and a NADPH-generating system (○).











Glatt et al. Cancer Res 41, 270-277, 1981

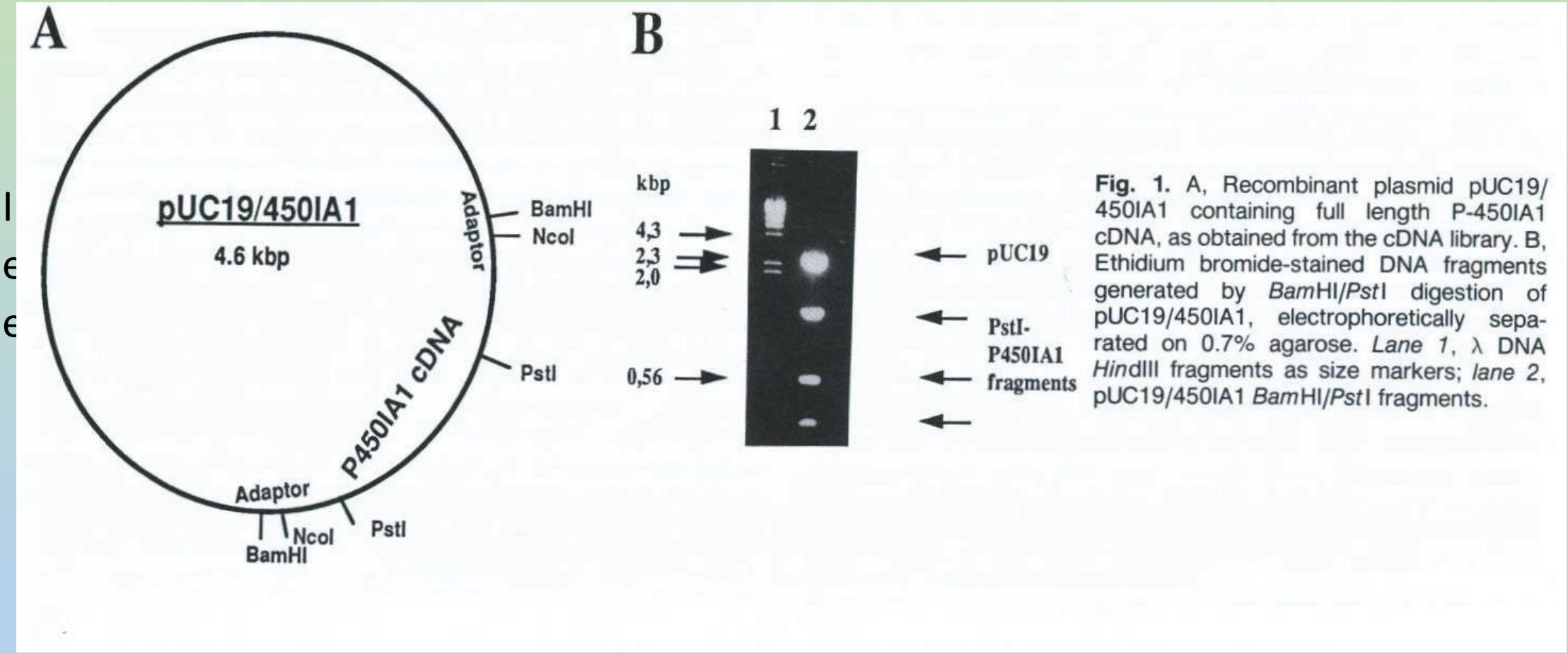
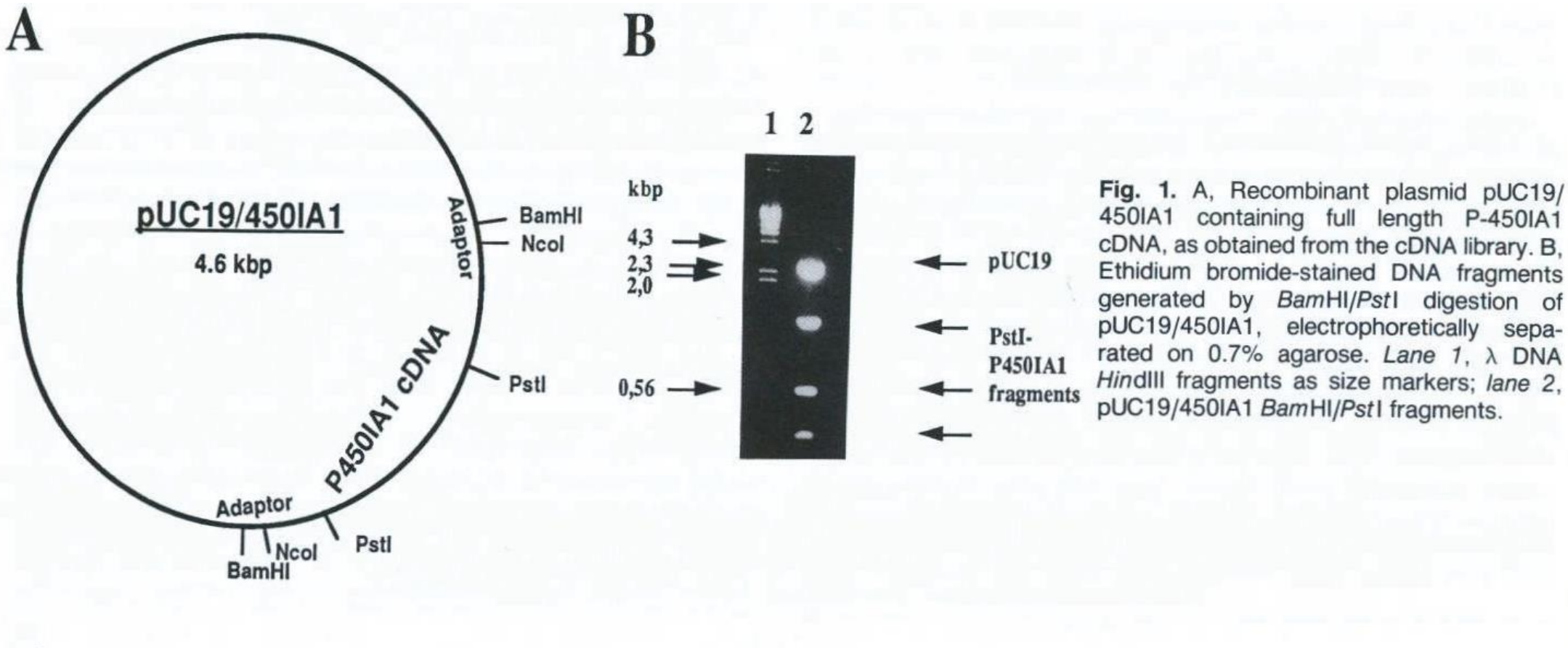


Fig. 1. A, Recombinant plasmid pUC19/450IA1 containing full length P-450IA1 cDNA, as obtained from the cDNA library. B, Ethidium bromide-stained DNA fragments generated by *Bam*HI/*Pst*I digestion of pUC19/450IA1, electrophoretically separated on 0.7% agarose. *Lane 1*, λ DNA *Hin*dIII fragments as size markers; *lane 2*, pUC19/450IA1 *Bam*HI/*Pst*I fragments.



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SATISH DOGRA, JOHANNES DOEHMER, HANSRUEDI GLATT, HORST MÖLDERS, PETER SIEGERT, THOMAS FRIEDBERG, ALBRECHT SEIDEL, and FRANZ OESCH

Mol Pharmacol 37, 608-613, 1990

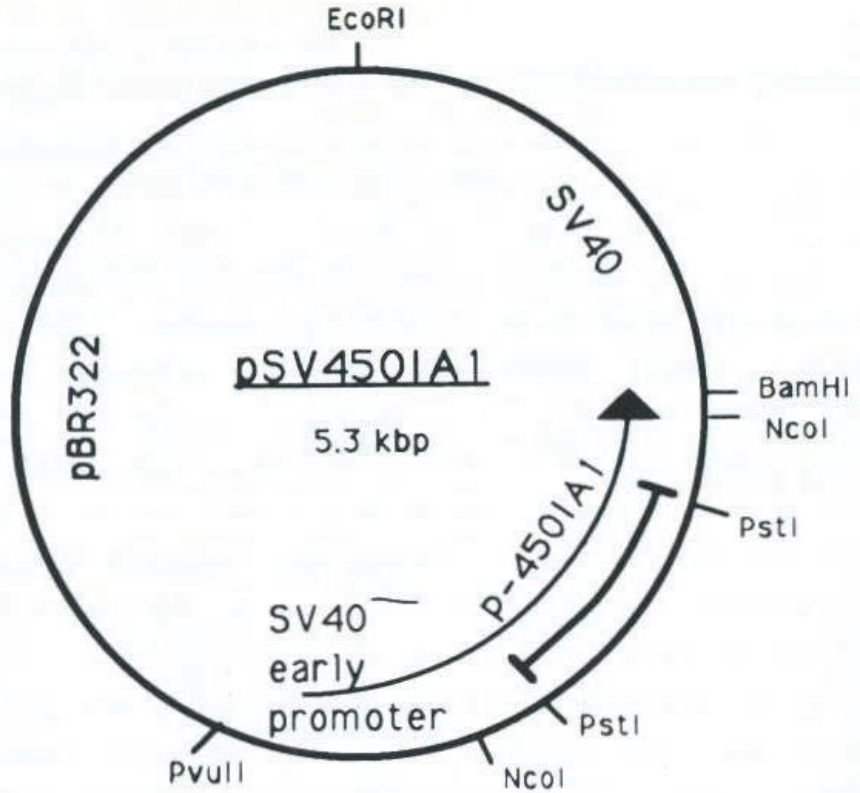


Fig. 2. Recombinant plasmid pSV4501A1 as used for gene transfer into V79 cells.

SATISH DOGRA, JOHANNES DOEHMER, HANSRUEDI GLATT, HORST MÖLDERS, PETER SIEGERT, THOMAS FRIEDBERG, ALBRECHT SEIDEL, and FRANZ OESCH

Mol Pharmacol 37, 608-613, 1990

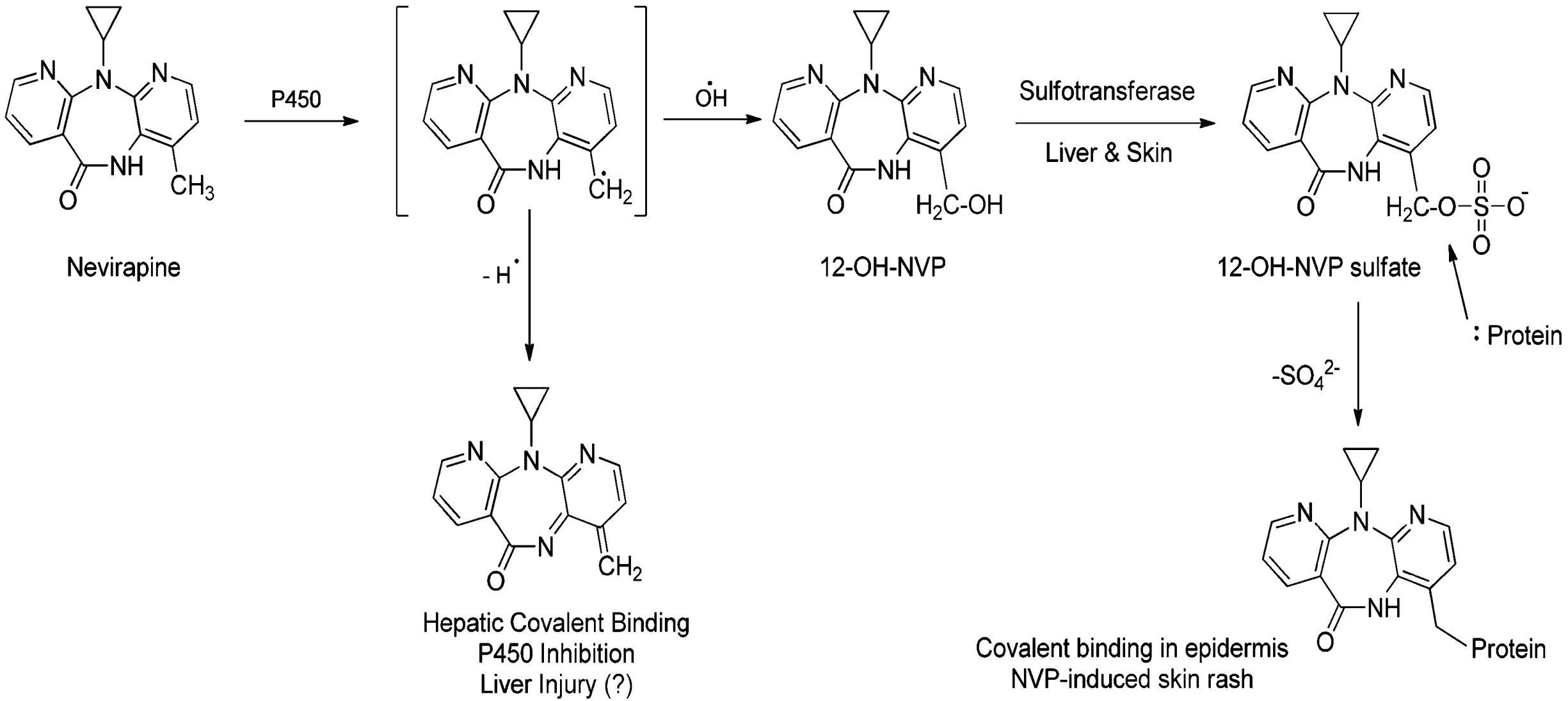
Enzymatic activity in V79, XEM1, XEM2, and XEM3 cells and hepatocytes of untreated Sprague-Dawley rats

Cells	Specific activity	
	7-Ethoxycoumarin	AHH
	$pmol \cdot mg^{-1} \cdot min^{-1}$	
V79	<0.2	<0.2
XEM1	15.2	9.5
XEM2	84.2	50.2
XEM3	43.4	21.3
Hepatocytes from untreated rats	39.3	45.0

SATISH DOGRA, JOHANNES DOEHMER, HANSRUEDI GLATT, HORST MÖLDERS, PETER SIEGERT, THOMAS FRIEDBERG, ALBRECHT SEIDEL, and FRANZ OESCH

Mol Pharmacol 37, 608-613, 1990

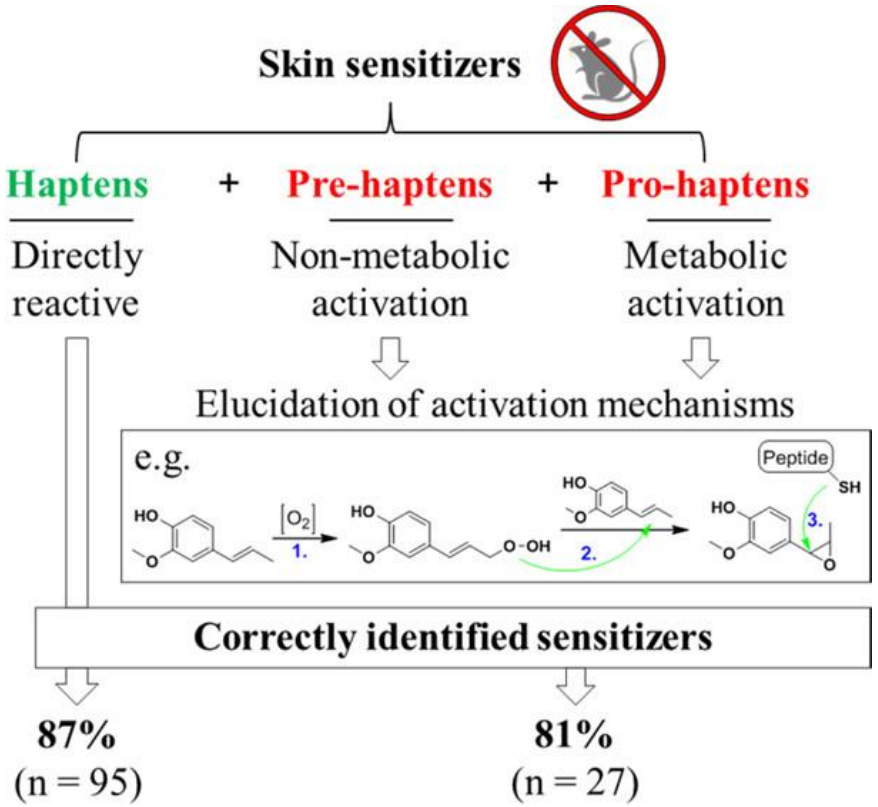
Sensitivity (%)	TP : (TP + FN) x 100
Specificity (%)	TN : (TN + FP) x 100
Positive predictive value (%)	TP : (TP + FP) x 100
Negative predictive value (%)	TN : (TN + FN) x 100
Accuracy (%)	(TP + TN) : (TP + FP + TN + FN) x 100


P450
OH
Sulfotransferase
Liver & Skin
Nevirapine
12-OH-NVP
12-OH-NVP sulfate
- H
: Protein
-SO4 2-
Hepatic Covalent Binding
P450 Inhibition
Liver Injury (?)
Covalent binding in epidermis
NVP-induced skin rash
Protein

CONSEQUENCES OF SKIN TREATMENT WITH NEVIRAPINE

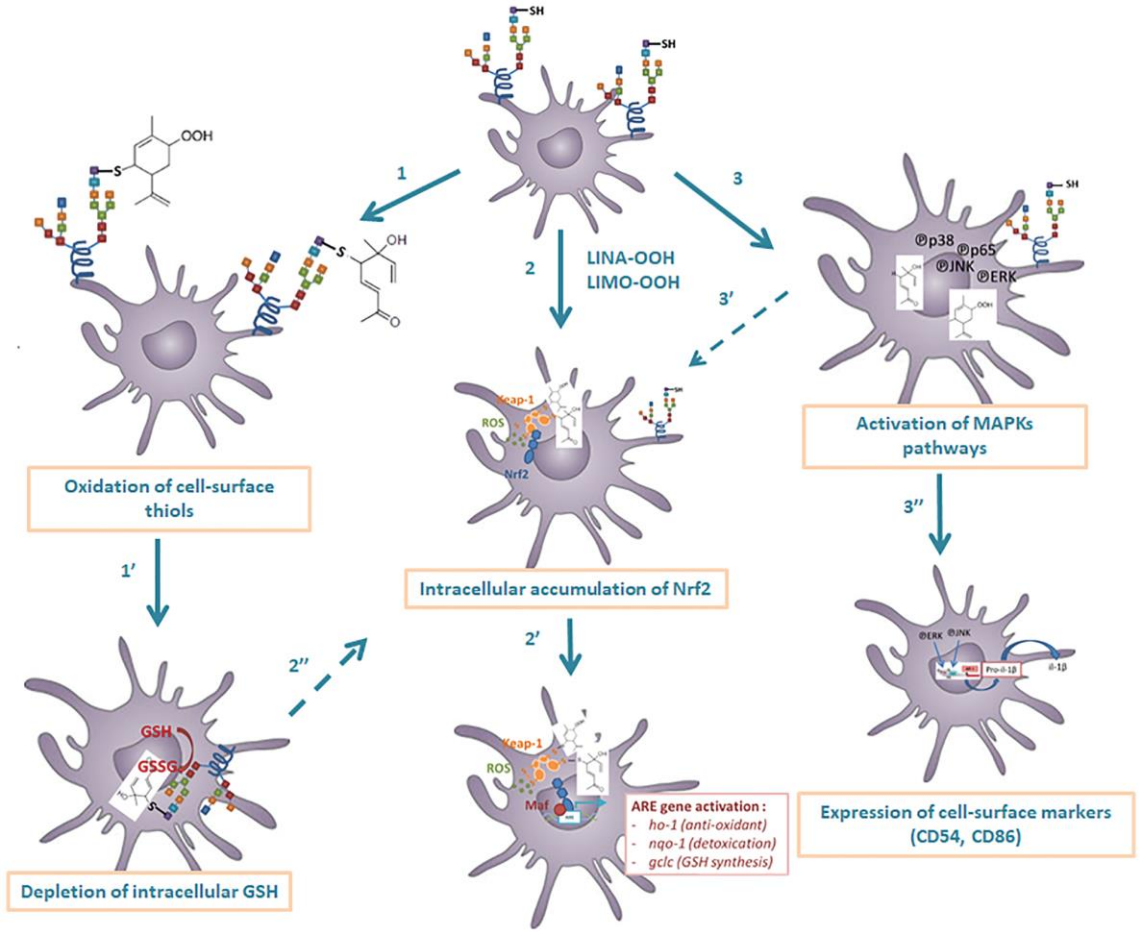
Human	Rat	Mouse
Formation of reactive sulfate	Formation of reactive sulfate	No formation of sulfate
Covalent binding to skin protein	Covalent binding to skin protein	No covalent binding to skin protein
Severe skin rash	Severe skin rash	No skin rash
All abolished by SULT inhibitor	All abolished by SULT inhibitor	No effect of SULT inhibitor

Data from Sharma et al. *Chem. Res. Toxicol.* 2013, 26: 410−421 and Sharma et al. *Chem. Res. Toxicol.* 2013, 26: 817−827



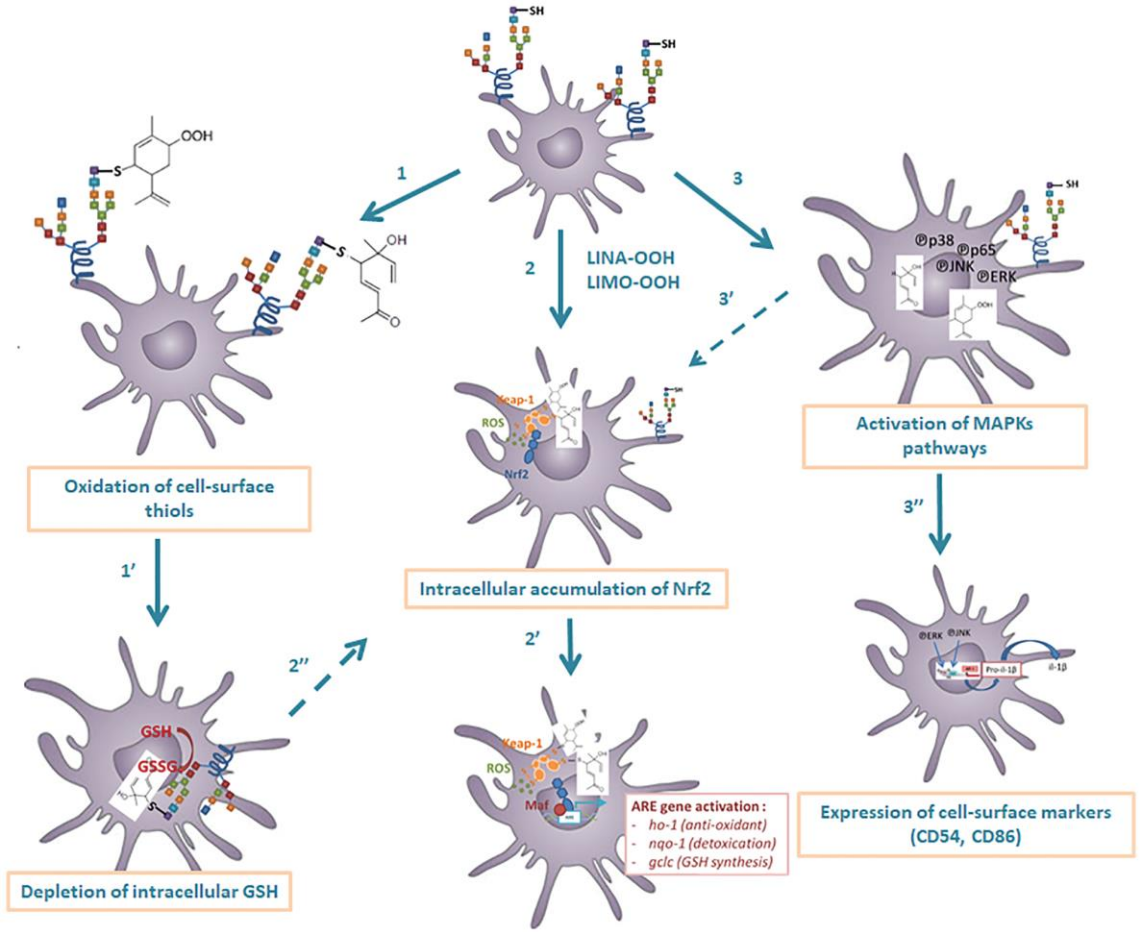
Published in: Daniel Urbisch; Matthias Becker; Naveed Honarvar; Susanne Noreen Kolle; Annette Mehling; Wera Teubner; Britta Wareing; Robert Landsiedel; *Chem. Res. Toxicol.*
DOI: 10.1021/acs.chemrestox.6b00055

Mechanism of action of terpenes and hydroperoxides on THP-1 cells.



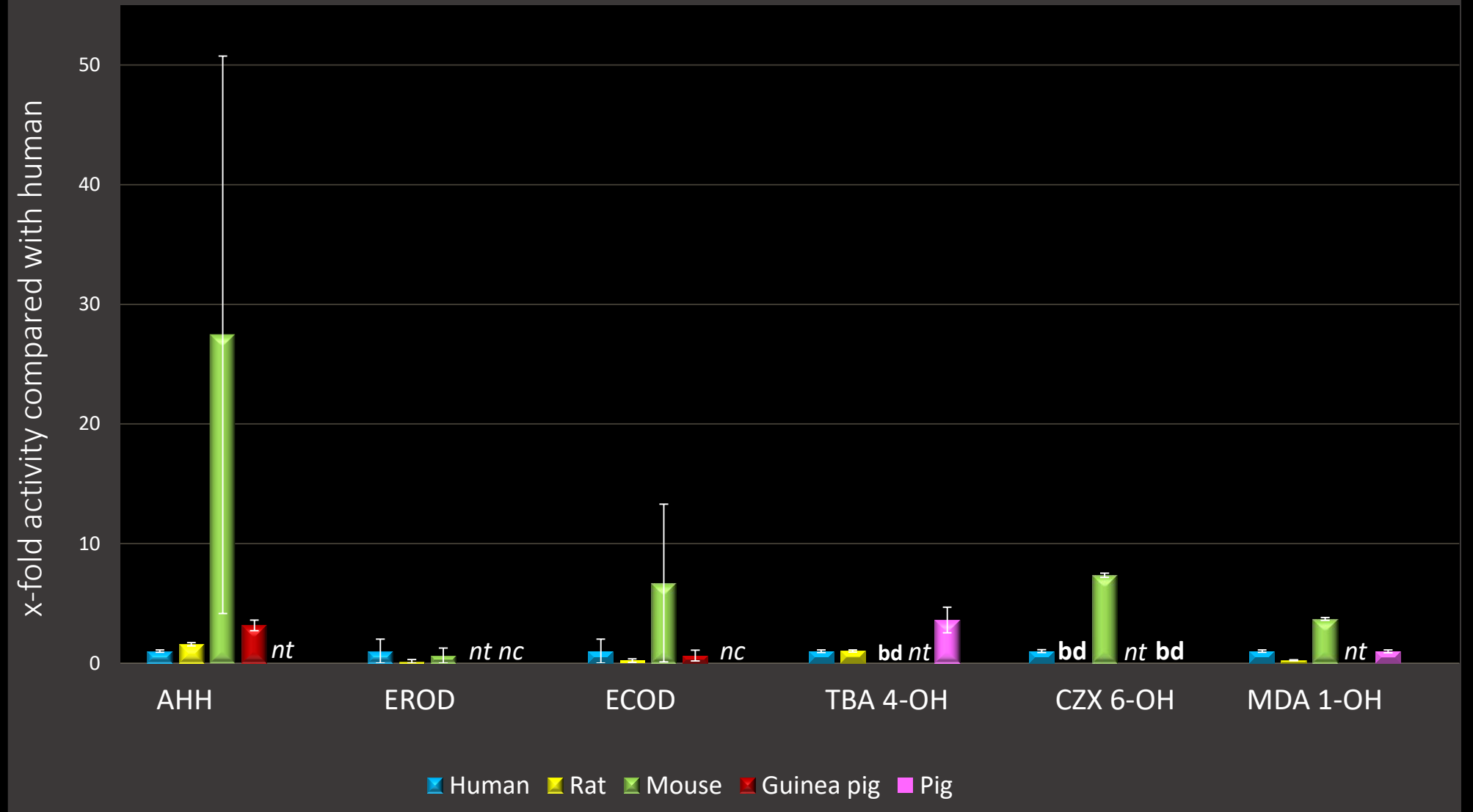
Raffalli et al. 2018. Toxicological Sciences, Volume 161, Issue 1, January Pages 139–148, https://doi.org/10.1093/toxsci/kfx207

Mechanism of action of terpenes and hydroperoxides on THP-1 cells.



Toxicological Sciences, Volume 161, Issue 1, January 2018, Pages 139–148, https://doi.org/10.1093/toxsci/kfx207

CYP activities in skin of various species compared with human



Abbreviations

AHH, Aryl Hydrocarbon Hydroxylase (CYP1);

EROD, 7-Ethoxyresorufin O-deethylase (CYP1);

ECOD, 7-Ethoxycoumarin O-deethylase (CYP1, 2B, 2D, 3A);

TBA 4-OH, Tolbutamide 4-hydroxylation (CYP2C9);

CZX 6-OH, Chlorzoxazone 6-hydroxylation (CYP2E1);

MDA 1-OH, Midazolam 1-hydroxylation (CYP3A);

nt, not tested;
nc, not comparable;
bd, below detection

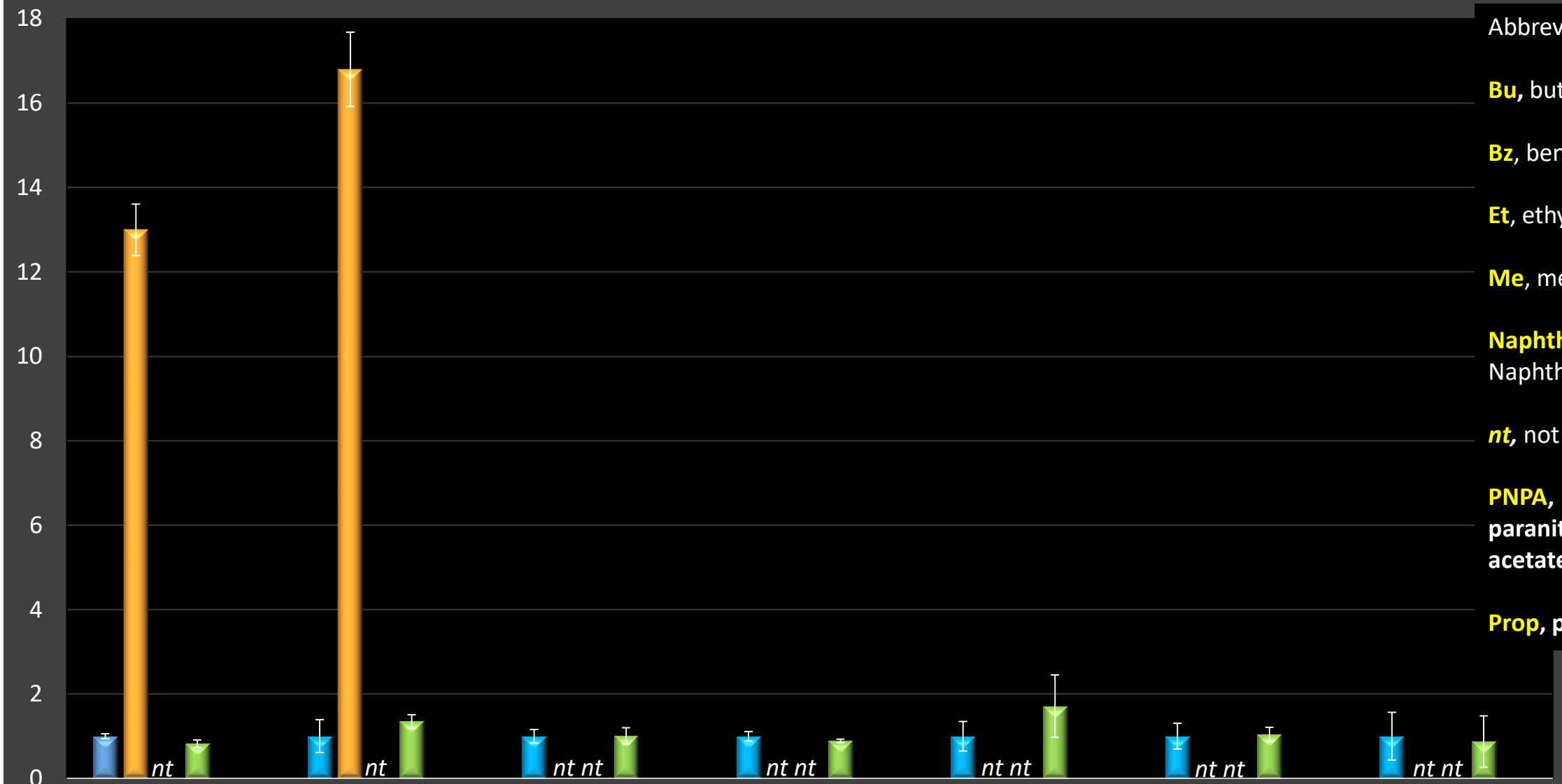
Non-CYP-mediated oxidoreductase activities[a] in skin of various mammalian species

Model substrate (for)	Human	Rat	Mouse	Guinea pig
Ethanol (ADH)	0.3-0.4	2.06	1.1-1.2	0.6
2,6-Dichlorophenolindophenol (NQR)	~375	*nt*	*nt*	23.4 - 159

[a] nmol product/mg cytosolic protein/min

Abbreviations: ADH, alcohol dehydrogenase; NQR, NADH/NADPH quinone reductase; *nt*, not tested

Esterase activities in skin of various species compared with human



Abbreviations

Bu, butyl;

Bz, benzyl;

Et, ethyl;

Me, methyl;

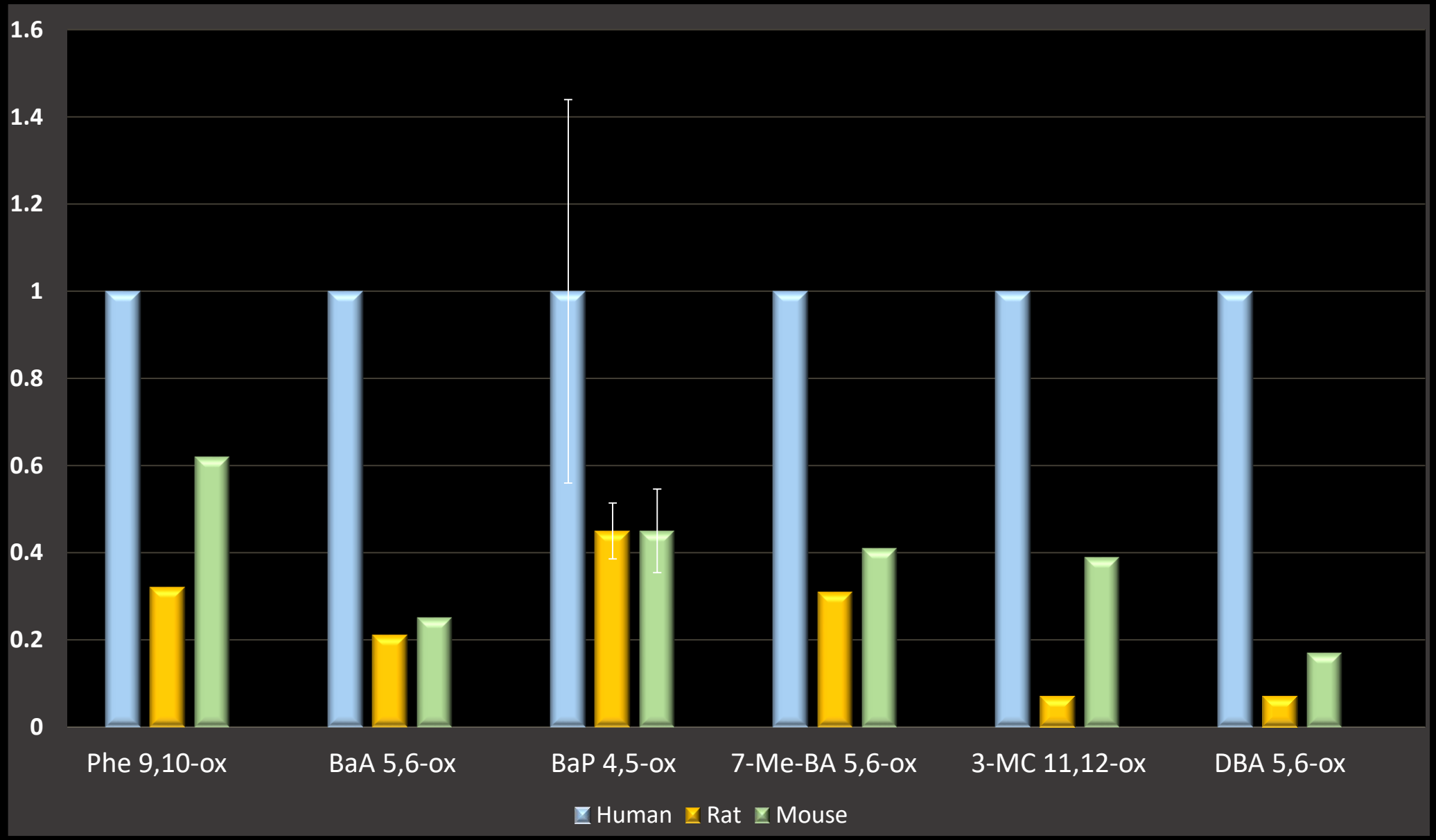
Naphth acetate, Naphthyl acetate;

***nt*,** not tested;

PNPA, paranitrophenyl acetate;

Prop, propyl

Microsomal epoxide hydrolase activities in skin of various species compared with human



Abbreviations

BaP 4,5-ox, Benzo(a)pyrene 4,5 oxide;

Phe 9,10-ox, Phenanthrene 9,10-oxide;

BaA 5,6-ox, Benz(a)anthracene 5,6-oxide;

7-Me-BA 5,6-ox, 7-Methylbenz(a)anthracene 5,6-oxide;

3-MC 11,12-ox, 3-Methylcholanthrene 11,12 oxide;

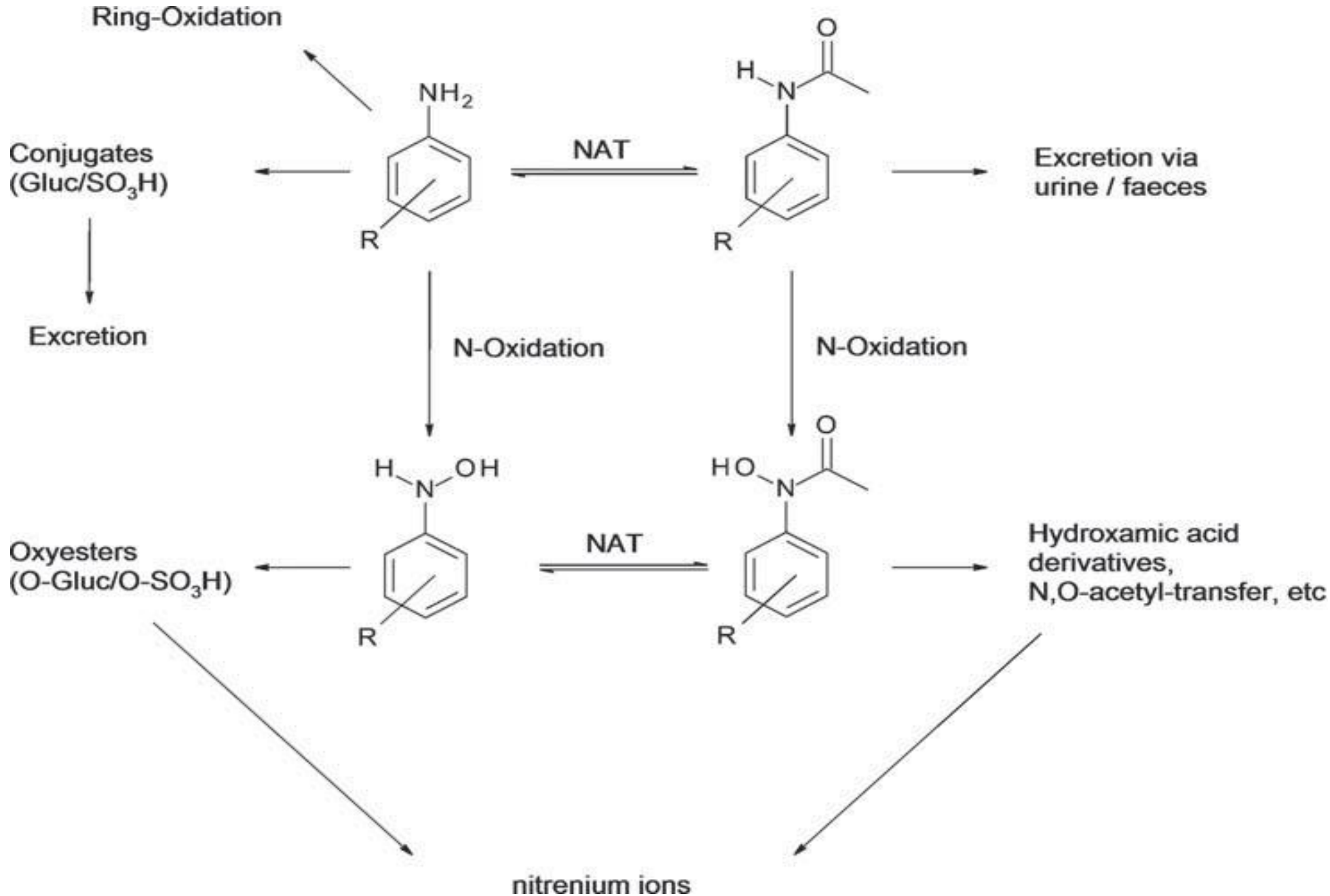
DBA 5,6-ox, Dibenz(a,h)anthracene 5,6-oxide

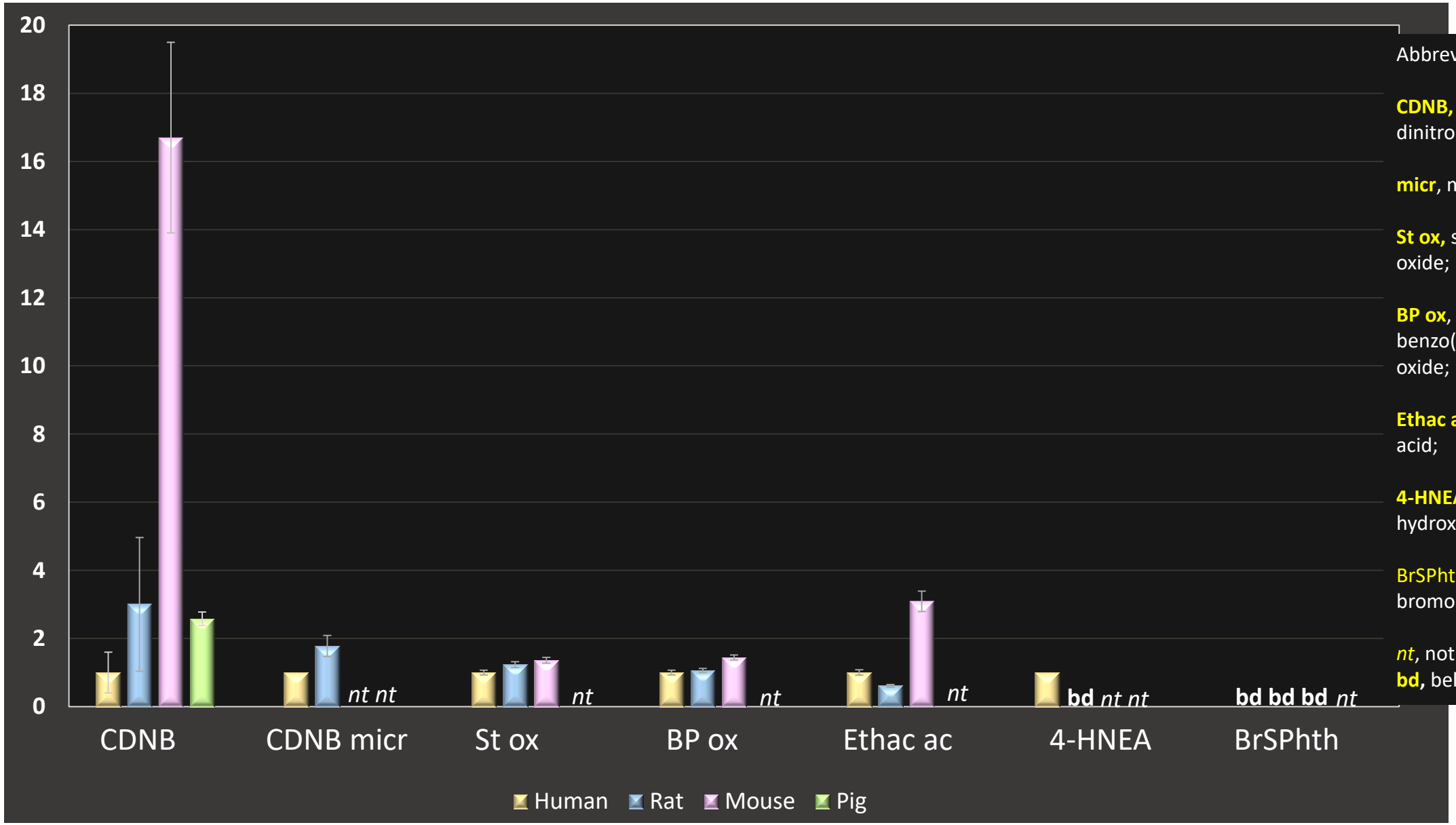
GENOTOXICITY TEST RESULTS

	Ames	MN/CA	Comet
2,5-Diaminotoluene	**pos**	**pos**	**pos**
2-Acetyl-2,5-diaminotoluene	**neg**	*nt*	**neg**
5-Acetyl-2,5,diaminotoluene	**neg**	*nt*	**neg**
2,5-Diacetyl-2,5-diaminotoluene	**neg**	*nt*	**neg**
Para-phenylenediamine	**pos**	**pos**	**pos**
Acetyl-para-phenylenediamine	**neg**	**neg**	**neg**
Diacetyl-para-phenylenediamine	**neg**	**neg**	**neg**
4-Amino-2-hydroxytoluene	**neg**	**pos**	**pos**
4-Acetylamino-2-hydroxytoluene	*nt*	**neg**	**neg**

Abbreviations: MN, micronucleus; CA, chromosome aberration; pos, positive; neg, negative; *nt*, not tested.

Data from Garrigue et al. *Mutat. Res*. 608: 58–71, 2006 and from Zeller and Pfuhler *Mutagenesis* 29: 37-48, 2014


Ring-Oxidation
NH2
Conjugates
(Gluc/SO3H)
NAT
Excretion via
urine / faeces
R
Excretion
N-Oxidation
N-Oxidation
H
N
OH
HO
O
Oxyesters
(O-Gluc/O-SO3H)
NAT
Hydroxamic acid
derivatives,
N,O-acetyl-transfer, etc
nitrenium ions



Abbreviations:

CDNB, 1-chloro-2,4-dinitrobenzene;

micr, microsomal;

St ox, styrene 7,8 oxide;

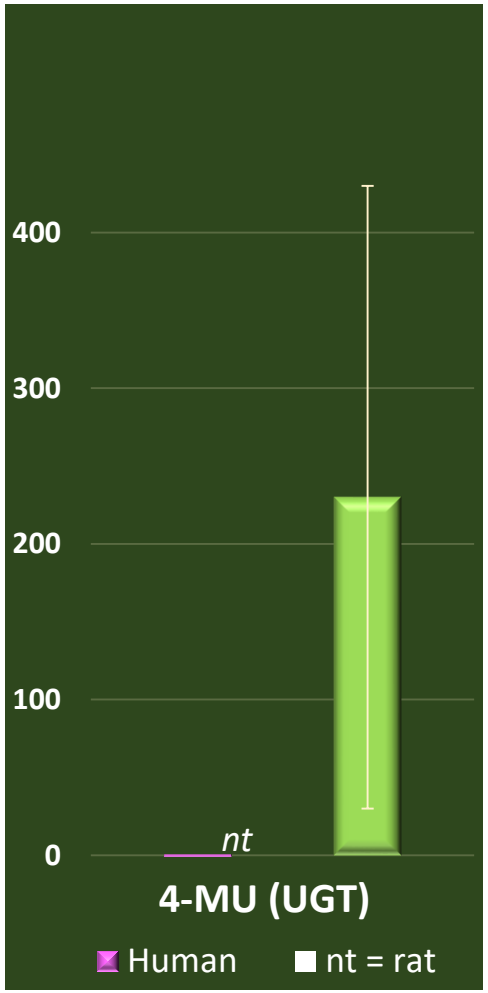
BP ox, benzo(a)pyrene 4,5-oxide;

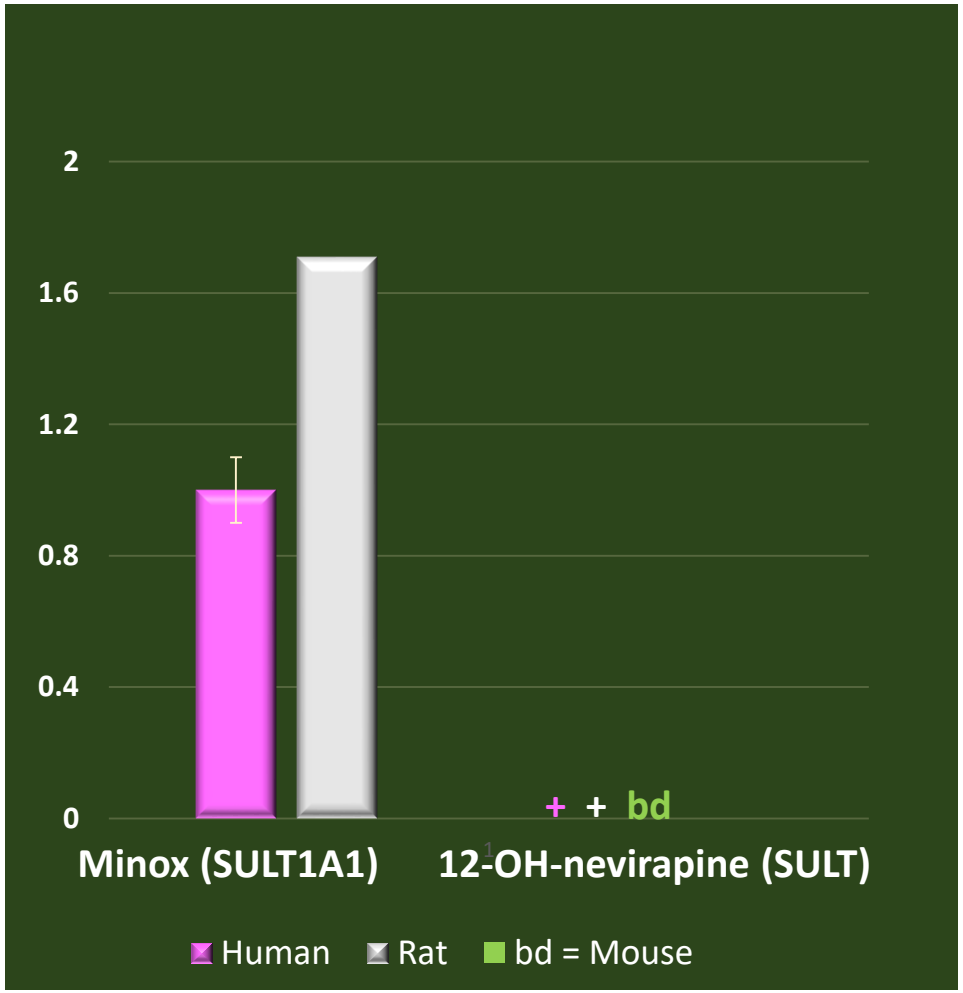
Ethac ac, ethacrynic acid;

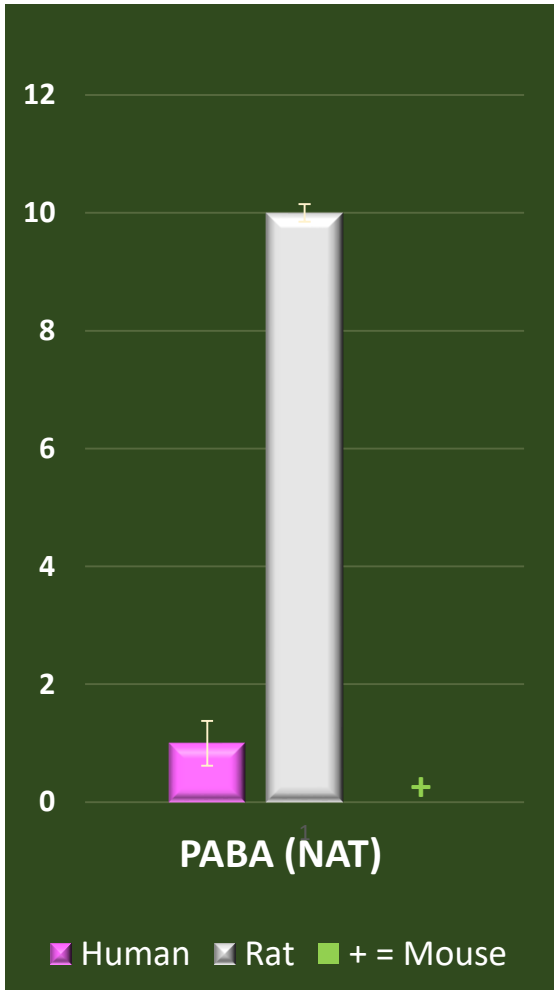
4-HNEA, 4-hydroxynoneal;

BrSPhth, bromosulphthalein;

nt, not tested;
bd, below detection







Abbreviations:

4-MU, 4-methyl umbelliferone;

Minox, minoxidil;

NAT, N-acetyltransferase;

PABA, para-amino benzoic acid;

SULT, sulfotransferase;

UDP, UDP-glucuronyl transferase;

+ = activity not quantified;

nt, not tested;

bd, below detection

RELATIVE SUITABILITY OF SKIN OF VARIOUS SPECIES AS MODEL FOR HUMAN SKIN

Very tentative because of paucity of data

Arbitrary: Compared with human skin: 1-2x: Excellent; >2-3x: Good; >3-10x: Marginally acceptable; >10x: Too distant

Enzyme	Rat	Mouse	Guinea Pig	Pig
CYP	marginally acceptable	too distant	**good, but vlt**	**good, but vlt**
Non-CYP OxRed: ADH	marginally acceptable	**good, but vlt**	**excellent, but vlt**	*nt*
NQR	*nt*	*nt*	marginally acceptable	*nt*
Esterase	too distant	*nt*	*nt*	**excellent**
mEH	too distant	marginally acceptable	*nt*	*nt*
GST	**good**	too distant	*nt*	**good, but vlt**
UGT	*nt*	too distant	*nt*	*nt*
Sulfotransferase	**excellent, but vlt**	*nt*	*nt*	*nt*
N-Acetyltransferase	marginally acceptable	*nc*	*nt*	*nt*

Abbreviations: **vlt, very little tested**; ADH, alcohol dehydrogenase; NQR, NADH/NADPH quinone reductase; *nt,* not tested; mEH, microsomal epoxide hydrolase; GST, glutathione S-transferase; UGT, UDP-glucuronyltrasferase; *nc*, not comparable