Research Article

Roles of Human Sulfotransferases in Genotoxicity of Carcinogens Using Genetically Engineered *umu* Test StrainsYoshimitsu Oda,^{1*} Yu Zhang,¹ Sebastian Buchinger,² Georg Reifferscheid,² and Min Yang¹¹State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China²Division of Quantitative Hydrology, Federal Institute of Hydrology (BfG), Koblenz, Germany

Human sulfotransferase (SULT) 1A1, 1A2, and 1A3 cDNA genes were subcloned separately into the pTrc99A^{KM} vector. The generated plasmids were introduced into the *Salmonella typhimurium* O-acetyltransferase-deficient strain NM6000 (TA1538/1,8-DNP/pSK1002), resulting in the new strains NM7001, NM7002, and NM7003. We compared the sensitivities of these three strains with the parental strain NM7000 against 51 chemicals including aromatic amines, nitroarenes, alkylbenzenes, estrogens-like chemicals, and other compounds with and without S9 mix by making use of the *umu* test system that is based on the bacterial SOS induction. 2-Amino-6-methyl-dipyrido[1,2- α :3',2'-d']imidazole, 3-methoxy-4-aminoazobenzene, 3-nitrobenzanthrone, 5-nitroacenaphthene, and 3,9-dinitrofluoranthene caused high genotoxicity in the NM7001 strain. The genotoxic effects of

2-aminofluorene, 2-acetylaminofluorene, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, 2-nitrofluorene, 1-nitropyrene, and 2-nitropropane were stronger in the NM7002 strain compared with the NM7001 and NM7003 strains. Among the tested benzylic and allylic compounds, 1-hydroxymethylpyrene was detected in the NM7001 strain with the highest sensitivity. Estragole and 1'-hydroxysafrole exhibited strong genotoxicity in the NM7003 strain. The estrogen-like chemicals such as bisphenol A, genistein, *p,n*-nonylphenol, and 4-hydroxytamoxifen were not detected as genotoxins in any strain used. Collectively, the present results suggest that the generated test strains are valuable tools in order to elucidate the role of SULT enzymes in the bioactivation of chemicals to environmental carcinogens. Environ. Mol. Mutagen. 53:152–164, 2012. © 2011 Wiley Periodicals, Inc.

Key words: human sulfotransferases; carcinogens; genotoxicity; *umu* test

INTRODUCTION

Sulfonate conjugation has been shown to be an important pathway in the biotransformation of numerous xenobiotics and endobiotics such as drugs, chemical carcinogens, hormones, bile acids, neurotransmitters, peptides, and lipids [Coughtrie, 2002; Strott, 2002]. The universal sulfonate donor for these reactions is 3'-phosphoadenosine 5'-phosphosulfate (PAPS). The transfer of a sulfonate (SO₃⁻) to a hydroxyl or amino group is catalyzed by the super gene family of sulfotransferases (SULTs). In the case of most xenobiotics and small endogenous substrates, sulfonation has generally been considered as a detoxification pathway leading to more water-soluble products and thereby facilitating their excretion via kidneys or bile [Falany, 1997; Weinshilboum et al., 1997]. However, for xenobiotics such as *N*-hydroxy arylamines, *N*-hydroxy heterocyclic amines, and hydroxymethyl polycyclic aromatic hydrocarbons, sulfonation is a metabolic activation process leading to

highly reactive electrophiles that are both mutagenic and carcinogenic [Miller and Surh, 1994; Glatt, 2005].

The majority of in vitro cell systems which are currently used for the detection of genotoxicity suffer from the lack of metabolic activation of xenobiotics. These systems are therefore often combined with an exogenous

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metabolic activation system, usually rat liver S9 fractions supplemented with co-factors (S9 mix). This approach contributed significantly to the success of genotoxicity tests. However, the application of metabolic activation systems still has some drawbacks [Bridges and Hubbard, 1980]. When metabolic activation occurs extracellularly, the highly reactive and short-lived metabolic intermediates may not be stable enough to reach DNA or critical target proteins. In addition, the S9 fraction which is prepared from rat livers after induction by Aroclor 1254 or a combination of phenobarbital and β -naphthoflavone, consist of a mixture of various drug-metabolizing enzymes. Therefore, it can be difficult to clearly determine the contribution of an individual enzyme in the S9 mix to the metabolic activation of a compound.

In a recent study, we succeeded in the development of *umu* test strains which express human cytochrome P450s (CYPs) or *N*-acetyltransferases (NATs) intracellularly in *Salmonella typhimurium* (*S. typhimurium*). It was shown that these strains can be employed for mechanistic studies on genotoxicity caused by a variety of nitroarenes and arylamines [Oda et al., 1999, 2001].

The main aim of the present study was to investigate the roles of human SULT enzymes in the genotoxicity of chemical carcinogens. We describe the construction of three *umu* test strains that express human SULT1A1, SULT1A2, and SULT1A3 enzymes after introducing their cDNAs into the parent strain *S. typhimurium* NM6000. By using these new strains, we report that a variety of chemicals including aromatic amines, nitroarenes, and benzylic and allylic compounds, are metabolically activated by these SULT enzymes.

MATERIALS AND METHODS

Chemicals and Enzymes

Reference sources of chemicals were as follows: aniline, 2-amino-9*H*-pyrido[2,3-*b*]indole (A α C), 2-amino-6-methyl-dipyrido[1,2- α :3',2'-*d*]imidazole (Glu-P-1), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MA α C), 2-amino-3,5-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), 3-nitrofluoranthene, 4,4'-dinitrobiphenyl, 4-nitroquinoline 1-oxide, *p*-acetamidophenol, phenacetine, 4-hydroxytamoxifen, furfural, bisphenol A, *p*,*n*-nonylphenol, allylisothiocyanate were purchased from Wako Pure Chemical (Osaka); 2-acetylaminofluorene (2-AAF), β -naphthylamine, 2-nitrofluorene (2-NF), 5-nitroacenaphthene, nitrofuranone, 2-nitrotriphenylene, 1-nitronaphthalene, 1-nitropyrene, 1-nitrotriphenylene were obtained from Tokyo Kasei Kogyo Co., (Tokyo); 2-aminoanthracene and benzidine were purchased from Katayama Chemical (Osaka); PAPS, 6-aminochrysene, 2-nitropropane, 1-hydroxymethylpyrene (1-HMP), safrole, estragole, acrolein, 17 β -estradiol, genistein, hycanthone came from Sigma-Aldrich Com. (St. Louis); furylfuramide came from Ueno Pharmaceutical Co. (Osaka); 2-aminofluorene and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) were obtained from Aldrich Chemical Com. (Milwaukee); [³⁵S]PAPS (1.0 Ci/mmol) was purchased from New England Nuclear Research products; aminophenylnorharman (APNH) was a gift from Dr. Y. Totsuka of the National Cancer Center Research Institute (Tokyo); 4-aminobiphenyl was a gift from Dr. M. Plewa of University of

Illinois, Urbana, IL; 3-methoxy-4-aminoazobenzene (3-MeO-AAB) was a gift from Dr. M. Degawa of Shizuoka University, Shizuoka; 3-nitrobenzanthrone (3-NBA) was a gift from Dr. T. Watanabe of Kyoto Pharmaceutical University, (Kyoto); 3,7-dinitrofluoranthene and 3,9-dinitrofluoranthene were gifts from Dr. R. Nakagawa of Fukuoka Environmental Research Center, (Fukuoka); 1'-hydroxysafrole was a gift from Dr. Y. Okuno of Kinki University, (Osaka); 1,6-dinitropyrene was a gift from Dr. S. Sato of Kobe University, (Kobe); 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX) was a gift from Dr. N. Kinai of Shizuoka University, (Shizuoka). Rat liver S9 fractions and cofactors (S9 mix) were obtained from Oriental Yeast Co., Tokyo.

Bacterial Strains and Plasmids

The bacterial strains and plasmids used in this study are described in Table I. *Escherichia coli* (*E. coli*) DH5 α , *S. typhimurium* TA1538/1,8-DNP [Watanabe et al., 1990], and SJ10002 were used as host strains for transformation. Plasmid pTrc99A was used as a cloning vector, which was purchased from Amersham Biosciences.

Plasmid pTrc99A^{KM} (pNM70) was constructed by subcloning a DNA fragment (1.3 kb), carrying the kanamycin gene that was generated by *Pst*I digestion of pUC4K purchased from GE Healthcare, Japan.

Construction of Plasmids and Strains for the Expression of Human SULT Genes

The plasmids pNM71, pNM72, and pNM73 were prepared by subcloning the recombinant inserts pET28a (+)/SULT1A1, pET20b (+)/SULT1A2, and pET28a (+)/SULT1A3 (kindly provided by Dr. M.E. McManus, School of Biomedical Sciences, University of Queensland, Australia) into the vector plasmid pTrc99A^{KM} (pNM70). pET28a (+)/SULT1A1 and pET28a (+)/SULT1A3 encoding the human *SULT1A1* gene (GenBank accession number L10819) and *SULT1A3* gene (GenBank accession number L19956) were digested with *Nco*I and *Sal*I or *Xba*I and *Sal*I, respectively. pET20b (+)/SULT1A2 encoding the *SULT1A2* gene (GenBank accession number U28170) was digested with *Nco*I and *Sal*I. The resulting *Nco*I-*Sal*I and *Xba*I-*Sal*I DNA fragments were purified by agarose gel electrophoresis. These fragments were ligated into *Nco*I/*Sal*I or *Xba*I/*Sal*I regions of the plasmid vector pNM70. The *E. coli* DH5 α was transformed with each plasmid by making use of the CaCl₂ method [Maniatis et al., 1989]. The resulting transformants were initially selected for kanamycin resistance. Subsequently, the plasmid DNA isolated from the different transformants was digested completely with restriction enzymes *Nco*I and *Sal*I (in the case of SULT1A1 and SULT1A3) and *Xba*I and *Sal*I (in the case of SULT1A2) and subjected to agarose gel electrophoresis to verify the direction of respective *SULT* gene transcription. Plasmids which were suitable for the correct expression of SULT enzymes—as verified by the restriction analysis—were used for the further study. After modifying each plasmid with the *S. typhimurium* SJ10002 strain (restriction⁻, modification⁺), each plasmid was used to transform the *O*-acetyltransferase (*O*-AT)-deficient *S. typhimurium* strain NM6000 and ampicillin/kanamycin-resistant transformants were selected. The resulting strains were denominated as NM7001, NM7002, and NM7003, respectively. The control strain NM7000 was constructed by introducing the empty cloning vector pNM70 into *S. typhimurium* NM6000.

Enzyme Assay

SULT activities were determined in bacterial lysates (10,000g supernatant fraction) by a method described previously [Oda et al., 1993]. SULT1A1, SULT1A2, SULT1A3, and the vector activities were measured by the method of Foldes and Meek [1973] using *p*-nitrophenol or dopamine as substrates. The reaction mixture (500 μ l) for the characterization of SULT1A1, SULT1A2 activity, and the background activity

TABLE I. Bacterial Strains and Plasmids

Bacteria or plasmid	Description	Source
<i>Escherichia coli</i>		
DH5 α	F ⁻ , Φ 80 <i>dlacZ</i> M15, Δ (<i>lacZYA</i> <i>argF</i>)U169, <i>deoR</i> , <i>recA1</i> , <i>endA1</i> , <i>hsdR17</i> (<i>rk</i> ⁻ , <i>mk</i> ⁺) <i>phoA</i> , <i>supE44</i> , λ ⁻ , <i>thi-1</i> , <i>gyrA96</i> , <i>relA1</i>	P. F. Guengerich
<i>Salmonella typhimurium</i>		
SJ10002	<i>metA</i> , <i>metE</i> , <i>ilv</i>	K. Kutsukake
TA1538	<i>hisG46</i> , <i>gal</i> , Δ (<i>chl</i> , <i>uvrB</i> , <i>bio</i>), <i>rfa</i>	B. N. Ames
TA1538/1,8-DNP	As TA1538, but deficient in <i>O</i> -acetyltransferase	M. Watanabe
NM6000	As TA1538/1,8-DNP, but harbors pSK1002	Y. Oda
NM7000	As TA1538/1,8-DNP, but harbors pNM70 and pSK1002	This study
NM7001	As TA1538/1,8-DNP, but harbors pNM71 and pSK1002	This study
NM7002	As TA1538/1,8-DNP, but harbors pNM72 and pSK1002	This study
NM7003	As TA1538/1,8-DNP, but harbors pNM73 and pSK1002	This study
Plasmid		
pSK1002	<i>umuD</i> ⁺ - <i>umuC</i> ⁺ <i>lacZ</i> , Amp ^r	H. Shinagawa
pNM70	As pTrc99A, but KM ^r	This study
pNM71	As Trc99A, but has <i>SULT1A1</i> gene containing <i>NcoI</i> - <i>SalI</i> digested 6.3-kb fragment of pET28a/ <i>SULT1A1</i> subcloned at <i>NcoI</i> site in the kanamycin gene, KM ^r	This study
pNM72	As Trc99A, but has <i>SULT1A2</i> gene containing <i>NcoI</i> - <i>SalI</i> digested 6.3-kb fragment of pET20b/ <i>SULT1A2</i> subcloned at <i>NcoI</i> site in the kanamycin gene, KM ^r	This study
pNM73	As Trc99A, but has <i>SULT1A3</i> gene containing <i>XbaI</i> - <i>SalI</i> digested 6.7-kb fragment of pET28a/ <i>SULT1A3</i> subcloned at <i>NcoI</i> site in the kanamycin gene, KM ^r	This study

contained 20 mM potassium phosphate buffer (pH 6.4), 40 μ M [³⁵S] PAPS, 5 mM *p*-nitrophenol, and 1 mg/ml of the cytosolic fraction of the respective strain. The reaction mixture (500 μ l) for the characterization of SULT1A3 activity and the background activity consisted of 0.1 M glycine-NaOH buffer (pH 9.0), 40 μ M [³⁵S]PAPS, 5 mM dopamine, and 1 mg/ml cytosolic protein. The protein content was assayed by the Lowry method [Lowry et al., 1951].

umu Assay

The assay was performed with slight modifications according to a method published previously [Oda et al., 1985]. Briefly, the bacterial cells were grown overnight at 37°C in L-broth containing ampicillin (25 μ g/ml) and kanamycin (25 μ g/ml). The cultures were diluted 50-fold with TGA medium (1% Bactotryptone, 0.5% NaCl, 0.2% glucose, 20 μ g/ml ampicillin) supplemented with 1 mM isopropyl- α -D-thiogalactoside and incubated at 37°C for 3 hr until the bacterial density reached an absorbance of $\sim$ 0.3 at 600 nm. For the chemicals that did not require activation by S9 mix, the culture was subdivided into 1 ml aliquots in test tubes. The test chemicals (dissolved in 10 μ l dimethyl sulfoxide) were added to the tubes subsequently. For the chemicals that require activation by S9 mix, the cultures were subdivided into 850 μ l aliquots in test tubes, to which 150 μ l of S9 mix and 10 μ l of the test chemical were added. These mixtures were incubated at 37°C for 3 hr with vigorous shaking. After the incubation, the bacterial density and β -galactosidase activity were measured by the method of Miller [1972] with slight modification [Oda et al., 1985]. The measured values for the enzymatic activity were corrected by the subtraction of the background levels. A compound that induces the β -galactosidase activity to a level higher than twofold of the control value and shows a dose response curves is estimated as a genotoxic chemical.

RESULTS

Establishment of *S. Typhimurium* Strains Expressing the Human SULT1A1, SULT1A2, and SULT1A3

In order to express the three different SULT enzymes in *S. typhimurium*, we subcloned the respective genes into

the multicopy plasmid vector pNM70. The genes encoding the enzymes SULT1A1 and SULT1A3 were isolated from the plasmid vector pET28a (+) by an enzymatic digestion with *NcoI*/*SalI* (*SULT1A1*, 1.155 kb fragments) or *XbaI*/*SalI* (*SULT1A3*, 1.412 kb fragments). The gene encoding the enzyme SULT1A2 was isolated from the plasmid vector pET20b (+) by an enzyme digestion with *NcoI*/*SalI* (*SULT1A2*, 1.045 kb fragments). The fragments were subcloned into the multicopy plasmid vector pNM70, which was cut with the respective endonucleases. The resulting plasmids containing the kanamycin gene were denominated as pNM71, pNM72, and pNM73 (Fig. 1). These newly constructed plasmids were able to co-exist with plasmid pSK1002 containing the *umuC*⁺*lacZ* fusion gene which confers an ampicillin resistance.

The enzymatic activities in the bacterial extracts, which were determined by two substrates in four different strains, were shown in Table II. As expected, the SULT deficient strain NM7000 did not exhibit any detectable sulfating activity. Strain NM7001 showed lower sulfating activities than NM7002 with *p*-nitrophenol used as substrate. In case of dopamine as substrate, the strain NM7003 showed strong sulfating activities.

Effects of Aromatic Amines on *umuC* Induction in the *Salmonella* Test Strains

The induction of *umuC* gene expression by 15 aromatic amines was examined using the three constructed test strains which express the three different human SULTs in the presence of S9 mix. The parent strain NM7000, which contains the empty expression vector was used as reference. The sensitivity of the four bacterial strains to

Fig. 1. Plasmids constructed for the expression of human *SULT1A1*, *SULT1A2*, and *SULT1A3* genes. (A) Plasmid pNM71 for the expression of human *SULT1A1* gene; (B) plasmid pNM72 for the expression of human *SULT1A2* gene; (C) plasmid pNM73 for the expression of human

SULT1A3 gene. The cleavage sites of some restriction enzymes are shown with italic letters. KM, kanamycin gene; ori, the origin of replication from plasmid pBR322. The size of plasmids pNM71, pNM72, and pNM73 are 6.77, 6.76, and 6.40 kbs, respectively.

TABLE II. Sulfating Activity Toward *p*-Nitrophenol and Dopamine in *S. typhimurium* Test Strains

Strain	Sulfating activity (nmol/mg protein/min)
NM7001 (<i>SULT1A1</i>)	1.42 ^a
NM7002 (<i>SULT1A2</i>)	5.86 ^a
NM7003 (<i>SULT1A3</i>)	24.2 ^b
NM7000 (vector)	ND ^{a,b}

ND represents the activity below the detection limit (0.01 nmol/mg protein/min).

^a*p*-Nitrophenol.

^bDopamine.

aromatic amines was assessed by the lowest observed effect concentration of a given compound, i.e. the minimal concentration which induces the β -galactosidase activity at least twofold compared with the background levels. As shown in Tables III and IV, the *SULT1A1*-expressing strain NM7001 exhibited higher sensitivities toward *umuC* gene expression of Glu-P-1 and MeA α C than NM7002 and NM7003. The *SULT1A2*-expressing strain NM7002 exhibited higher sensitivities to *umuC* gene induction by 2-AF, 2-AAF, and PhIP than NM7001. NM7001 and NM7002 strains showed same sensitivities toward 2-aminoanthracene, 4-aminobiphenyl, APNH, and 3-MeO-AAB, whereas NM7003 and NM7000 strains exhibited relatively low sensitivities for these chemicals. NM7001, NM7002, and NM7003 strains were found to be similar sensitivities toward A α C and β -naphthylamine. In cases of 6-aminochrysene, MeIQ, Trp-P-1, and Trp-P-2, all strains used showed similar sensitivities. Aniline and benzidine did not induce *umuC* gene expression in any strains.

Effects of Nitroarene Compounds on *umuC* Induction in the Salmonella Test Strains

Similarly, as shown in Tables III and IV, the sensitivities of the four strains for 16 nitroarene compounds were examined. The *SULT1A1*-expressing strain NM7001 showed a strong *umuC* induction after exposure to 5-nitroacenaphthene, 3-NBA, 3-nitrofluoranthene, and 3,9-dinitrofluoranthene.

The strain NM7002, expressing *SULT1A2*, exhibited higher sensitivities toward 2-NF, 1-nitropyrene, and 2-nitropropane. Both NM7001 and NM7002 showed the same sensitivity for 4,4'-dinitrobiphenyl. In all strains *umuC* gene expression were found to be induced by furylfuramide, nitrofurazone, 3-nitrofluoranthene, 1-nitronaphthalene, 4-nitroquinoline 1-oxide, 2-nitrotriphenylene, 3,7-dinitrofluoranthene, and 1,6-dinitropyrene, but not by 1-nitrotriphenylene (Table III).

Effects of Various Benzylic and Allylic Compounds on *umuC* Induction in the Salmonella Test Strains

Because benzylic and allylic compounds have been reported to be converted to DNA- and protein-adducting species by sulfonation [Miller and Surh, 1994], we examined whether these compounds show any genotoxic responses via sulfation catalyzed by human SULTs using the generated SULT-expressing strains. As shown in Tables III and IV, the *umuC* induction of 1-HMP was strongest in the strain NM7001. Interestingly, estragole and 1'-hydroxylsafrole showed positive responses only in strain NM7003, but not in the strains NM7001 and NM7002. Both NM7001 and NM7003 showed the same sensitivity for hycanthone. The compounds allylisothiocyanate, furfural, 4-hydroxytamoxifen, and safrole showed no effects in any strains.

Effects of Estrogens-Like Compounds and Other Compounds on *umuC* Induction in the Salmonella Test Strains

Several members of the SULT family catalyze the sulfation of estrogen and estrogenic compounds. Particularly, *SULT1E1* is responsible for the high affinity sulfation of β -estradiol, estrone, and 17 α -ethinylestradiol [Falany et al., 1995; Schrag et al., 2004], whereas these physiological estrogens have low affinities to some members of the phenol SULT1 family [Falany et al., 1994]. We investigated whether the six estrogens-like compounds listed in Table III induce *umuC* gene expression using the SULT-expressing strains and the parent strain. As shown in

TABLE III. Genotoxicity Activity of a Variety of Chemicals in *S. typhimurium* Test Strains NM7000, NM7001, NM7002, and NM7003

Chemical	S9 ^a	Concentration (μ M)	β -Galactosidase activity (units) ^b			
			NM7000	NM7001	NM7002	NM7003
2-Aminoanthracene	+	0.78	ND	<0	<0	ND
		2.6	ND	44	38	ND
		7.8	7	302	293	10
		26	63	459	489	145
		78	160	450	455	457
2-AF	+	5.5	6	11	36	32
		16.4	14	27	45	71
		55	40	72	69	171
		164	79	144	118	554
2-AAF	+	0.05	0	0	2	1
		1.34	0	9	23	3
		4.5	2	42	61	4
		13.4	7	42	55	11
4-Aminobiphenyl	+	0.06	0	1	1	<0
		0.18	<0	4	6	<0
		0.6	0	123	139	<0
		1.8	5	223	270	16
6-Aminochrysene	+	0.41	1	<0	5	1
		1.23	5	8	11	9
		4.1	54	115	106	139
		12.3	137	294	237	338
APNH	+	0.3	0	102	235	1
		1	5	437	540	7
		3	47	720	677	126
		10	115	803	684	349
Aniline	+	5400	2	1	0	<0
		10,700	3	5	0	14
		21,500	<0	<0	<0	<0
A α C	+	1.65	<0	6	3	ND
		5.5	4	84	19	31
		16.5	12	254	70	84
		55	40	262	115	115
Benzidine	+	9.4	0	<0	3	<0
		31.2	0	<0	5	<0
		93.6	0	<0	3	<0
		312	10	<0	<0	<0
Glu-P-1	+	1.5	7	33	18	11
		5	20	90	51	63
		15	56	177	145	140
		50	90	189	219	354
MeA α C	+	0.51	0	1	<0	ND
		1.53	0	2	0	<0
		5.1	2	218	20	11
		15.3	10	252	47	15
MeIQ	+	0.47	9	10	9	ND
		1.41	16	27	18	46
		4.7	30	64	30	66
		14.1	56	92	50	109
3-MeO-AAB	+	1	0	45	21	<0
		3	2	128	112	3
		10	13	159	230	37
β -Naphtylamine	+	4.3	2	12	7	4
		12.8	8	22	22	23
		42.5	10	27	30	36
		128	7	22	19	26
PhIP	+	0.13	ND	14	20	<0
		0.44	ND	32	51	<0
		1.32	17	98	117	21
		4.4	30	201	203	132
		13.2	62	353	217	256
		44	93	390	177	380

TABLE III. Continued

Chemical	S9 ^a	Concentration (μ M)	β -Galactosidase activity (units) ^b			
			NM7000	NM7001	NM7002	NM7003
Trp-P-1	+	0.47	8	20	7	115
		1.41	27	36	30	171
		4.7	130	259	176	248
Trp-P-2	+	0.51	20	42	29	ND
		1.52	188	259	154	294
		5.1	246	345	211	351
Furylfuramide	—	15.2	208	288	164	248
		0.004	13	8	11	26
		0.012	33	24	31	67
5-Nitroacenaphthene	—	0.04	86	54	78	150
		0.12	189	135	167	207
		0.0015	11	28	16	5
3-NBA	—	0.005	26	65	39	21
		0.015	77	132	92	83
		0.05	158	142	159	198
2-NF	—	0.0003	<0	20	6	6
		0.001	1	50	12	4
		0.003	5	176	48	20
Nitrofurazone	—	0.01	69	297	117	52
		1.5	12	26	34	10
		5	39	62	84	19
3-Nitrofluoranthene	—	15	45	157	157	34
		50	59	194	195	46
		0.5	8	6	7	21
1-Nitronaphthalene	—	1.5	29	18	19	60
		5	54	45	47	133
		15	128	122	91	274
1-Nitropyrene	—	0.012	7	24	21	5
		0.04	25	50	49	7
		0.12	59	108	98	64
2-Nitropropane	—	0.4	116	157	160	136
		36	13	25	11	12
		72	28	41	21	30
4-Nitroquinoline 1-oxide	—	144	46	53	36	73
		289	65	41	35	86
		0.12	34	56	62	29
1-Nitrotriphenylene	—	0.4	93	138	136	75
		1.2	126	222	192	99
		4	164	214	207	100
2-Nitrotriphenylene	—	1000	5	32	100	21
		2000	6	49	165	36
		4000	4	92	242	68
4,4'-Dinitrobiphenyl	—	10,000	<0	4	262	71
		0.16	32	19	27	36
		0.53	104	53	76	138
1-Nitrotriphenylene	—	1.58	219	101	155	290
		5.3	486	282	373	363
		50	0	1	3	5
2-Nitrotriphenylene	—	100	<0	0	1	5
		200	<0	<0	<0	1
		0.03	12	29	28	27
3,7-Dinitrofluoranthene	—	0.1	43	67	63	72
		0.3	90	120	139	156
		1	84	137	149	140
4,4'-Dinitrobiphenyl	—	12.3	12	116	98	14
		41	14	120	100	13
		123	12	124	97	6
3,7-Dinitrofluoranthene	—	410	0	85	76	<0
		0.001	5	14	4	3
		0.0034	10	38	15	7
3,7-Dinitrofluoranthene	—	0.01	24	99	38	13
		0.034	65	211	83	42

TABLE III. Continued

Chemical	S9 ^a	Concentration (μ M)	β -Galactosidase activity (units) ^b			
			NM7000	NM7001	NM7002	NM7003
3,9-Dinitrofluoranthene	—	0.001	7	44	16	7
		0.0034	15	81	30	14
		0.01	42	227	92	51
		0.034	78	248	116	84
1,6-Dinitropyrene	—	0.003	62	71	82	88
		0.01	193	182	173	215
		0.034	428	330	334	447
		0.1	649	417	599	693
Allylisothiocyanate	+	2.5	0	3	<0	<0
		5	1	3	0	<0
		10	3	6	4	<0
Estragole	+	22.5	2	23	9	56
		67.6	2	26	13	79
		225	2	21	12	107
		676	<0	<0	<0	52
Hycanthone	—	3	5	18	16	21
		10	12	43	33	29
		30	44	108	98	190
		100	130	292	316	543
1'-Hydroxysafrole	—	20.5	2	13	7	39
		41	3	18	10	57
		82.5	5	25	16	89
		165	7	33	23	107
1-HMP	—	0.3	ND	34	ND	ND
		1	2	68	15	9
		3	0	72	30	25
		10	4	69	40	43
		30	10	74	57	58
Safrole	+	61.6	0	1	2	9
		184.8	2	7	6	13
Bisphenol A	+	30	<0	2	<0	2
		100	<0	1	0	3
		300	<0	<0	<0	<0
17 β -Estradiol	—	10	1	<0	1	0
		30	1	0	1	5
		100	<0	<0	<0	<0
Genistein	+	30	0	<0	<0	<0
		300	0	<0	<0	<0
		1000	<0	<0	<0	<0
4-Hydroxyestradiol	—	12.5	2	<0	4	0
		25	1	<0	4	3
		50	8	13	25	30
		100	40	59	110	182
4-Hydroxytamoxifen	—	2.5	2	<0	<0	<0
		5	<0	<0	<0	<0
		10	<0	<0	<0	<0
<i>p,n</i> -Nonylphenol	+	1	2	0	0	0
		3	1	<0	1	0
		10	1	<0	<0	0
		30	<0	<0	<0	<0
Acrolein	—	4.5	12	6	2	5
		8.9	19	8	24	30
		17.8	25	30	59	51
		35.6	40	38	87	62
<i>p</i> -Acetamidophenol	+	19.8	2	<0	<0	<0
		66.1	2	3	<0	<0
		198	1	1	<0	<0
Furfural	—	10	10	3	53	48
		30	7	5	57	47
		100	12	9	43	32

TABLE III. Continued

Chemical	S9 ^a	Concentration (μ M)	β -Galactosidase activity (units) ^b			
			NM7000	NM7001	NM7002	NM7003
MNNG	—	6.8	103	69	70	144
		20.4	280	155	162	316
		68	459	294	257	448
		204	32	120	35	17
MX	—	0.52	1	2	0	<0
		1.56	28	33	48	55
		5.2	82	104	131	153
		15.6	322	416	509	565
Phenacetine	+	1.7	<0	<0	<0	<0
		5.1	<0	<0	<0	<0
		17	<0	<0	<0	<0

The bold numbers for all results that indicated the genotoxic effect were shown. The basal β -galactosidase levels of *S. typhimurium* strains NM7000, NM7001, NM7002, and NM7003 in the absence of S9 mix were 32 ± 14 , 43 ± 24 , 48 ± 37 , and 57 ± 31 (SD, $n = 28$), respectively. The basal β -galactosidase levels of respective strains in the presence of S9 mix were 17 ± 5 , 37 ± 11 , 34 ± 13 , and 59 ± 32 (SD, $n = 24$). Data are the means of two or three independent experiments.

^aIn the presence of (+) or absence (−) of S9.

^bThe value of β -galactosidase activity for each chemical was calculated by subtracting from the basal activity in four test strains.

ND, not determined.

Tables III and IV, all strains used showed almost same sensitivity toward 4-hydroxyestradiol. In the cases of bisphenol A, 17 β -estradiol, genistein, 4-hydroxytamoxifen, and *p,n*-nonylphenol, no induction of the reporter gene was observed in any strains. From other five compounds that were tested, acrolein showed *umuC* induction (about twofold) in the strain NM7002 and in the parental strain, but <1.7-fold in the strains NM7001 and NM7003. In contrast, all strains showed a similar extent of sensitivity for MNNG and MX. The compounds *p*-acetamidophenol, furfural, and phenacetine exhibited negative responses in all strains used.

DISCUSSION

Establishment of *umu* Assay System

In this study, we developed a new *umu* assay system to investigate the functions of three different human SULTs, namely SULT1A1, SULT1A2, and SULT1A3, in the bioactivation of aromatic amines, nitroarene compounds, benzylic and allylic alcohols, and estrogens-like compounds to genotoxins. These strains NM7001, NM7002, and NM7003 were constructed by introducing human SULT1A1, SULT1A2, and SULT1A3 cDNAs into the strain NM6000 (TA1538/1,8-DNP/pSK1002) which is a derivative of the strain TA1538 which lacks the endogenous *Salmonella* O-AT and harboring the *umu''lacZ* genes. The generated strains were used to evaluate the genotoxicity of the above-mentioned chemicals of several different compound classes. Aromatic amines are known to be activated by *N*-oxidation that is catalyzed by CYPs or prostaglandin H synthase, followed by acetylation or

sulfation [Debaun et al., 1968; King and Phillips, 1968; Josephy, 1986; Grant, 1993]. Nitroarene compounds are activated by nitroreduction catalyzed by nitroreductases, followed by acetylation or sulfation [Rosenkranz and Mermelstein, 1983; Oda, et al., 1993, 1999; Glatt, 2000]. Our results of the current study using the described recombinant bacteria indicated that these strains can detect the potential genotoxicity of compounds such as aromatic amines and nitroarenes as well as benzylic and allylic alcohols with high sensitivity. Thus, it is concluded that these compounds are activated by metabolic sulfation and that the SULTs expressed in the bacterial cells were enzymatically active and can efficiently catalyze the sulfation of chemicals in the cells. Test systems for the assessment of genotoxicity are much less laborious if bacteria are used. Bacterial assays which heterologously express different enzymes that are involved in the metabolic activation of xenobiotics are therefore useful to examine the genotoxic effects of a large number of test compounds and are thus applicable for high-throughput screening.

Involvement of SULT Isoforms in the Genotoxicity of Chemical Carcinogens

Among eight aromatic amines, MeA α C and Glu-P-1 exhibited strong genotoxicity in the strain NM7001 in the presence of liver S-9 mix compared with the strains NM7002, NM7003, and NM7000. The results of MeA α C are consistent with Glatt et al. [2004] who reported that this aromatic amine showed strongly enhanced mutagenicity in a *S. typhimurium* strain expressing human SULT1A1 in the presence of rat liver postmitochondrial fraction compared with a control strain. Similarly, in case

TABLE IV. Comparison of Sensitivity of Four Strains of *S. typhimurium* With 39 Chemicals Based on Data Shown in Table III

Chemicals	S9 ^a	Minimal concentration (μM) ^b			
		NM7000	NM7001	NM7002	NM7003
Aromatic amines					
2-Aminoanthracene	+	11.9	1.71	1.89	15.3
2-AF	+	23.5	25.6	5.5	35.5
2-AAF	+	—	4.5	2.1	—
4-Aminobiphenyl	+	—	0.31	0.3	—
6-Aminochrysene	+	2.06	3.9	2.06	2.47
APNH	+	2.3	0.19	0.1	2.5
AαC	+	16.5	3.27	5.7	5.4
Glu-P-1	+	3.1	1.25	3.9	9.4
MeAαC	+	—	1.77	5	—
MeIQ	+	1.4	2.1	2.8	1.9
3-MeO-AAB	+	10	0.8	1.3	—
β-Naphthylamine	+	42.5	13	12.8	21.2
PhIP	+	0.9	0.55	0.27	2.2
Trp-P-1	+	0.85	0.83	1.5	0.62
Trp-P-2	+	0.46	0.3	0.4	0.9
Nitroarenes					
Furylfuramide	—	0.012	0.012	0.0097	0.0079
5-Nitroacenaphthene	—	5.4	2	4.1	9.1
3-NBA	—	5	0.5	1.85	7.4
2-NF	—	3.3	2.75	1.1	41
Nitrofurazone	—	1.4	4.0	3.3	1.4
3-Nitrofluoranthene	—	0.044	0.022	0.027	0.11
1-Nitronaphthalene	—	63.5	47.4	87.5	98.2
1-Nitropyrene	—	0.099	0.089	0.04	0.2
2-Nitropropane	-	—	—	1800	—
4-Nitroquinoline 1-oxide	—	0.15	0.21	0.15	0.14
2-Nitrotriphenylene	—	0.05	0.037	0.038	0.075
4,4'-Dinitrobiphenyl	—	—	4.1	4.1	—
3,7-Dinitrofluoranthene	—	0.011	0.0038	0.0074	0.027
3,9-Dinitrofluoranthene	-	0.0065	0.0006	0.0027	0.0074
1,6-Dinitropyrene	—	0.0017	0.0012	0.001	0.0017
Benzylic and allylic alcohols					
Estragole	+	—	—	—	22.5
Hycanthone	—	27	16.5	25.5	17.4
1'-Hydroxysafrole	—	—	—	—	30
m1-HMP	—	—	0.21	2.2	3.3
Estrogen like compounds					
4-Hydroxyestradiol	—	55	63	51.5	53
Other compounds					
Acrolein	—	30.3	—	38	—
MNNG	—	1.7	2.72	2.04	1.36
MX	—	2	2.72	3.93	3.66

The concentration of chemicals indicating inhibitory responses compared to the control strain was shown with italics. A short dash (—) indicates the chemicals showing negative responses in the genotoxic activities in test strains.

^aIn the presence of (+) or absence (—) of S9.

^bConcentration of chemicals that increase *umuC* gene expression by twofold over background levels extrapolated from doses resulting in increasing responses in Table III.

of Glu-P-1, Chu et al. [1995] reported that *N*-hydroxy-Glu-P-1 was selectively sulfonated by a human liver thermostable phenol SULT, which is termed SULT1A1 or SULT1A2 [Glatt and Meinel, 2004]. These results suggest that human SULT1A1 is involved in the bioactivation of

MeAαC and Glu-P-1 to genotoxic metabolites. On the contrary, 2-AF, 2-AAF, and PhIP exhibited stronger genotoxicity in the strain NM7002 compared with the strains NM7001, NM7003, and NM7000. These chemicals are bioactivated to potent genotoxins by human SULT1A2 expressed in *S. typhimurium*. In case of 2-AAF and PhIP, the results are in agreement with reports by Glatt which showed that the *N*-hydroxy-2-acetylaminofluorene (*N*-OH-2-AAF) is activated most efficiently by SULT1A2 expressed in *S. typhimurium* [Meinel et al., 2002; Glatt, 2005]. However, *N*-hydroxy-PhIP (*N*-OH-PhIP) is activated by SULT1A2 as well as by SULT1A1 [Glatt, 2005]. In addition, *N*-OH-2-AAF, *N*-hydroxy-2-AF, and *N*-OH-PhIP are also selectively sulfonated by the human hepatic thermostable phenol SULT [Chou et al., 1995]. Aromatic amines such as 2-aminoanthracene, 4-aminobiphenyl, APNH, and 3-methoxy-4-aminoazobenzene showed a similar genotoxic potential in the both strains NM7001 and NM7002, suggesting that these chemicals are bioactivated by SULT1A1 and SULT1A2. APNH, a carcinogenic heterocyclic amine, which is produced by coupling of norharman and aniline in the presence of S9 mix, induced liver and colon cancer in F344 rats [Kawamori et al., 2004]. Our study shows for the first time that human SULT1A1 and SULT1A2 are involved in the SULT-dependent bioactivation of APNH. In a previous article we already demonstrated that this compound can be bioactivated by the combined action of human CYP1A2 and human NAT2 enzymes [Oda et al., 2006]. In the case of 4-aminobiphenyl, the data are also consistent with findings of Chou et al. [1995], who showed that using an enzymatic assay consisting of a PAPS-regenerating system, 4-aminobiphenyl was sulfonated by the hepatic thermostable phenol SULT, but not the thermolabile phenol SULT. However, 6-aminochrysene, MeIQ, Trp-P-1, and Trp-P-2 showed an almost similar genotoxic effects in all strains used. This finding indicates that these chemicals are not activated by human SLT.

Nitroarene compounds identified in diesel exhaust and air particulate matter are potent mutagens and carcinogens [Rosenkranz and Mermelstein, 1985; Tokiwa and Ohnishi, 1986]. One major pathway of metabolic activation of these compounds is simple nitroreduction followed by *N*- and/or *O*-acetylation due to phase II enzymes. These enzymatic reactions result in the formation of highly reactive arylnitrenium ions, which interact with cellular DNA and proteins [Borlak et al., 2000; Yamazaki et al., 2000]. In a previous study, we showed that human NATs play an important role in the genotoxicity of nitroarenes using *S. typhimurium* strains expressing human NATs [Oda et al., 1999].

Of the 15 nitroarenes that were tested in the present study, 5-nitroacenaphthene, 3-NBA, and 3,9-dinitrofluoranthene showed the highest genotoxic potential in the strain NM7001 expressing human SULT1A1. Arlt et al.

[2002] reported that human NAT1 and NAT2 as well as human SULT1A1 are strongly involved in the metabolic activation of 3-NBA. They investigated the formation of DNA adducts using Chinese hamster lung fibroblast V79MZ cell lines that express human NATs or SULT1A1. This finding is consistent with our results that 3-NBA is bioactivated by human SULT1A1. Previously, we established an *umu* test strain series (termed OY strains) which is based on *S. typhimurium* expressing four different human CYP enzymes together with the NADPH-cytochrome P450 reductase (NPR) and the bacterial *O*-AT. Using these strains we demonstrated that in the presence of *O*-AT, 3-NBA can be bioactivated by human CYP1A1, CYP3A4, CYP1A2, and CYP1B1 together with NPR to a genotoxin, probably via nitroreduction [Oda et al., 2007]. The dinitro-compound 3,9-dinitrofluoranthene was observed to be bioactivated only by SULT1A1. In contrast, the structural isomer, 3,7-dinitrofluoranthene showed almost the same bioactivation by SULT1A1 and SULT1A2 (Table IV). A similar observation was reported by Glatt [2000]. It was shown that 2,4-dinitrotoluene and 2,4-dinitrobenzylalcohol were activated to genotoxins by human SULT1A1, which is expressed in strain *S. typhimurium* TA1538.

The strain NM7002 that expresses SULT1A2 was highly sensitive to 2-nitrofluorene, 1-nitropyrene, and 2-NP. In particular, 2-NP shows strong genotoxicity in *S. typhimurium* with and without exogenous metabolic activation [Speck et al., 1982; Göggelmann et al., 1988] and causes hepatocarcinoma in rats [Fiala et al., 1987]. The genotoxicity of this compound in rats has been attributed to the SULT-mediated formation of DNA-reactive arylnitrenium ions from propane 2-nitronate [Sodum et al., 1994]. Our data indicate that *O*-sulfonation by human SULT1A2 rather than SULT1A1 is a major metabolic pathway in activation of 2-NP. Using unscheduled DNA synthesis in Chinese hamster V79 cell lines that express allelic variants of the human phenol-sulfating phenol SULT or rat SULT enzymes it was reported that 2-NP showed higher mutagenic activities to cells that express the human SULT1A1*Arg (*1) alloenzyme compared to the cells expressing the human SULT1A1*His [Glatt, et al., 2000].

The compound 4,4'-dinitrobiphenyl showed the same genotoxic potential in the strains NM7001 and NM7002. However, in the case of nitroarenes such as furylfuramide, 3-nitrofluoranthene, nitrofurazone, 1-nitronaphthalene, 4-nitroquinoline 1-oxide, 2-nitrotriphenylene, 3,7-dinitrofluoranthene, and 1,6-dinitropyrene, the genotoxicity was almost similar in all strains. This finding indicates that the metabolic activation of these chemicals is independent of the SULT enzymes that were investigated in the present study.

The compound 1-HMP is a benzylic alcohol derived from hydroxymethyl polycyclic aromatic hydrocarbons and is a major metabolite formed in vitro from the hepatocarcinogen 1-methylpyrene [Rice et al., 1987]. For

S. typhimurium TA1538 and V79 Chinese hamster cells, it was reported that heterologous expression of rat or human SULTs leads to strong genotoxic effects caused by metabolites of 1-HMP which itself is inactive or only weakly active in the parental cells [Glatt et al., 1995]. We observed that human SULT1A1 expressed in *S. typhimurium* is more efficient in the activation of 1-HMP than human SULT1A2 and SULT1A3. In accordance to this finding, Glatt [2005] also reported that 1-HMP is activated by many different human SULTs, although SULT1A1 shows the highest efficiency. The reported order is SULT1A1 > SULT1E1 > SULT2A1 > SULT1A3 > SULT1C2 > SULT1A2. Thus, we suggest that 1-HMP can be activated to genotoxic metabolites mainly by human SULT1A1. Hycanthone, a benzylic alcohol-containing drug molecule that has been withdrawn from market, exhibited a similar genotoxic activity in all *S. typhimurium* strains expressing human SULTs. However, our results are different from results of Glatt et al. [2001] who proposed that human SULT2A1 is substantial for the mutagenic activity of hycanthone. Further research about the metabolic activation of hycanthone in the strain of *S. typhimurium* expressing human SULT2A1 is required.

Among numerous benzylic alcohols, 1'-hydroxysafrole, 1'-hydroxyestragole, and metabolites of safrole and estragole can induce hepatic tumors in mice and rats [Miller et al., 1983; Wiseman et al., 1987]. These conclusions are supported by the detection of DNA adducts formation in mice which are exposed to 1'-hydroxysafrole or 1'-hydroxyestragole [Phillips et al., 1981; Wiseman et al., 1985]. In addition, the 1'-hydroxysafrole, which occurs naturally in various food flavors, was reported to be not mutagenic in the strain TA1538 but it was mutagenic in two different strains expressing rat hydroxysteroid SULT and human hydroxysteroid SULT [Glatt et al., 1995]. Further in vitro studies undertaken with V79 cells expressing the human monoamine phenol SULT indicated that this enzyme can catalyze the protein adduct formation of estragole [Wakazono et al., 1998]. However, so far no experiment is performed to elucidate the substrate specificity of the human SULT enzyme for the bioactivation of such benzylic alcohols. Surprisingly, we found that these chemicals are strongly activated in the strain NM7003 that expresses the human SULT1A3. All other strains that were generated for this study showed negligible effects after exposure to this compound class. The presented result is the first evidence that human SULT1A3 plays an important role in the metabolic activation of benzylic alcohols to genotoxic intermediates.

Detoxification of Genotoxic Carcinogens Catalyzed by SULTs

Interestingly, the present study shows as well that the genotoxic potential of several chemicals is reduced by

SULT enzymes. For example, the genotoxicity of Glu-P-1, PhIP, 2-NF, 3-nitrofluoranthene, 1-nitropyrene, and 3,7-dinitrofluoranthene was inhibited by SULT1A3. In the case of acrolein, the genotoxicity was inhibited by SULT1A1 and SULT1A3. These findings suggest that SULT1A1 or SULT1A3 enzymes are involved in the detoxification of several genotoxic compounds. The *umu* test system with overexpressed SULT enzymes may be useful for a further investigation of the SULT-function in the metabolic inactivation of carcinogens.

Relationship Between the Localization of SULTs and the Bioactivation of Genotoxic Compounds

It is very important to determine whether tissues expressing SULTs are required for the activation of chemicals. At least 10 SULT forms have been detected in human tissues: SULT1A1 was detected in brain [Whittemore et al., 1986], liver [Falany et al., 1990; Riches et al., 2009], kidney, lung [Riches et al., 2009], and the gastrointestinal tract [Teubner et al., 2007]. SULT1A2 is expressed at low levels in the liver [Dooley et al., 2000; Teubner et al., 2007]. SULT1A3 was found in brain [Whittemore et al., 1985], platelets [Heroux and Roth, 1988], and the gastrointestinal tract [Teubner et al., 2007], but it is not detectable in the liver [Teubner et al., 2007; Riches et al., 2009]. A large number of chemicals used in this study are hepatocarcinogens in rodents. We showed that the bioactivation of most of the aromatic amines investigated in the presence of rat S9 mix with the exception of 6-aminochrysene, MeIQ, Trp-P-1, and Trp-P-2 depended on SULT1A1 and/or SULT1A2 activity and to a lesser extent on SULT1A3 (Table IV). This finding correlates with the tissue distribution of SULTs and is supported by other studies [Glatt, 1997, 2000; Banoglu, 2000]. Likewise to the aromatic amines, nitroarene compounds except for furylfuramide, nitrofurazone, 1-nitronaphthalene, 3,7-dinitrofluoranthene, 4-nitroquinoline 1-oxide, 2-nitrotriphenylene, and 1,6-dinitropyrene were activated by SULT1A1 and/or SULT1A2 but not by SULT1A3 (Table IV). Benzylic and allylic alcohols are bioactivated by all three investigated SULTs. Interestingly, 1'-hydroxysafrole and estragole showed only SULT1A3-dependent genotoxicity without and with S9 mix. It is known from immunoblot analyses that the SULT1A3 is not detectable in liver [Teubner et al., 2007; Riches et al., 2009]. However, Wang et al. [2006] reported that mRNA of SULT1A3 is detectable by reverse transcription-polymerase chain reaction in the human liver during the postmenopause. In contrast, recently Meinel et al. [2008] demonstrated that SULT1C3 has a SULT activity and that the SULT1C3 expression in *S. typhimurium* TA100 weakly activated 1'-hydroxysafrole. But it was not possible to detect the respective enzymatic activity in liver samples by an immunoblot assay. Further

studies are necessary to determine the genotoxicity of these chemicals with bacterial strains that express the human SULT1C3.

In conclusion, we established the *S. typhimurium* strains NM7001, NM7002, and NM7003 expressing human SULT1A1, SULT1A2, and SULT1A3, respectively. Our test systems which express the various SULTs in *S. typhimurium* cells are highly sensitive for SULT-dependent carcinogens without external supply of the cofactor PAPS. The main advantage of the heterologous expression of enzymes that are involved in the metabolic activation of xenobiotics is that the usually short-lived and reactive metabolites are able to react with intracellular biomolecules like proteins or DNA as they are generated inside the cell. We showed that SULT1A1 and SULT1A2 are mainly responsible for the activation of aromatic amines and nitroarenes whereas SULT1A1 and SULT1A3 contributed to the activation of 1-HMP and benzylic alcohols. The generated bacterial strains will also serve as valuable tools in order to elucidate the role and contribution of sulfation to the metabolism of drugs and xenobiotics in humans.

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