ELSEVIER

Mutation Research 369 (1996) 209–220

MR
Genetic Toxicology

Role of *O*-acetyltransferase in activation of oxidised metabolites of the genotoxic environmental pollutant 1-nitropyrene

P.F. Rosser, P. Ramachandran, R. Sangaiah, R.N. Austin, A. Gold, L.M. Ball *

Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Received 20 June 1995; revised 4 March 1996; accepted 4 April 1996

Abstract

The genotoxic environmental contaminant 1-nitropyrene is metabolised in mammalian systems by pathways more complex than the straightforward nitroreduction which accounts for most of its biological activity in bacteria. In order to evaluate the role of *O*-acetyltransferase (OAT) activity in generation of genotoxic intermediates from 1-nitropyrene, the mutagenicity of the major primary oxidised metabolites of 1-nitropyrene was characterised in the Ames *Salmonella typhimurium* plate incorporation assay with strain TA98, and with variants of TA98 deficient (TA98/1,8-DNP_6) or enhanced (YG1024) in *O*-acetyltransferase. 1-Nitropyren-3-ol was more mutagenic in the absence than in the presence of S9, while 1-nitropyren-4-ol, 1-nitropyren-6-ol and 1-nitropyren-8-ol required S9 for maximum expression of mutagenicity. 1-Nitropyren-4-ol (176 rev/nmol without S9, 467 rev/nmol with S9 in TA98) and 1-nitropyren-6-ol (13 rev/nmol without S9, 266 rev/nmol with S9 in TA98) were overall the most potent nitropyrenol isomers assayed. 1-Acetamidopyren-8-ol and 1-acetamidopyrene 4,5-quinone were only minimally active. 1-Acetamidopyren-3-ol exhibited direct-acting mutagenicity. 1-Acetamidopyren-6-ol, previously shown to be a major contributor to mutagenicity in the urines of rats dosed with 1-nitropyrene (Ball et al., 1984b), was confirmed as a potent (359 rev/nmol) S9-dependent mutagen. Both the direct-acting and the S9-dependent mutagenicity of all the compounds studied was enhanced in the OAT-overproducing strain and much diminished (though not always entirely lost) in the OAT-deficient strain, showing that OAT amplifies expression of the genotoxicity of these compounds. 1-Acetamidopyren-6-ol required both S9 and OAT activity in order to exhibit any mutagenicity; this finding strongly implicates *N*-hydroxylation followed by *O*-esterification, as opposed to further S9-catalyzed ring oxidation, as a major route of activation for urinary metabolites of 1-nitropyrene.

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (nitroPAH) are widely distributed in the environment, as a result of combustion processes (Gibson, 1982; Gibson, 1983). Many compounds of this class are potent mutagens, and some are animal carcinogens (Rosenkranz and Mermelstein, 1985). The true extent of the hazard that these compounds represent to public health, however, remains to be established, because of uncertainty concerning their mechanisms of activation. 1-Nitropyrene (1-NP; Fig. 1), a widely-characterised model compound for environmental nitroPAH, owes its mutagenicity in microbial

* Corresponding author. Department of Environmental Sciences and Engineering, CB#7400, Rosenau Hall, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400, USA.

PII S0165-1218(96)00053-5

assay systems to an arylnitrenium ion generated through metabolism by nitroreductase enzymes. This pathway is illustrated in Fig. 2, Box A. The resulting *N*-(deoxyguanosin-8-yl)-1-aminopyrene adduct (Howard and Beland, 1982), though identified in bacterial incubations (Howard et al., 1983), accounts for only a small proportion of the total DNA binding seen in mammalian species in vivo (Jackson et al., 1984; Mitchell, 1988; Roy et al., 1989). Hence activation through routes other than direct nitroreduction must be inferred in mammalian systems.

The disposition of 1-NP in the intact organism involves a combination of reductive and ring-oxidation processes (Ball et al., 1984b; El-Bayoumy and Hecht, 1984). Oxidative metabolism is rapid and extensive, such that the overall pattern of DNA interaction and genotoxicity is likely to be due to the genotoxic properties of the oxidised metabolites rather than to active intermediates formed by direct nitroreduction of the parent compound. Adducts formed from the K-region epoxide of 1-NP (illustrated in Box B of Fig. 2) have been characterized with in vitro systems (Roy et al., 1991); 1-acetamidopyren-6-ol (Fig. 2) has been identified as the major contributor to mutagenicity excreted in urine by rats dosed with 1-NP (Ball et al., 1984b; Ball et al., 1991). Both of these metabolites thus constitute potential circulating proximate active species. We therefore proceeded to examine more closely the pathways involved in further activation of the principal metabolites of 1-NP, in the hope of gaining insight into the chemical species responsible for genotoxicity in mammalian systems.

The role of nitroreductases in activation of nitropyrenols was addressed in 1984 (Ball et al., 1984a), when we had available only very limited quantities of synthetic material isolated from a crude preparation by reverse-phase HPLC. In contrast to the parent 1-NP and to 1-nitropyren-3-ol, two of the three nitropyrenols studied (1-nitropyren-6-ol and 1-nitropyren-8-ol, Fig. 1) were as mutagenic in the nitroreductase-deficient strain TA98NR (also known as TA98ND) as in the fully nitroreductase-competent TA98. Hence we concluded that reduction by the 'classical' nitroreductase did not play a major *direct* role in the mutagenicity of these nitropyrenols, and other factors must contribute to the activity of these compounds.

O-Acetylation catalyzed by acetyl-CoA-dependent *N*-hydroxylamine *O*-acetyltransferase (OAT) is im-

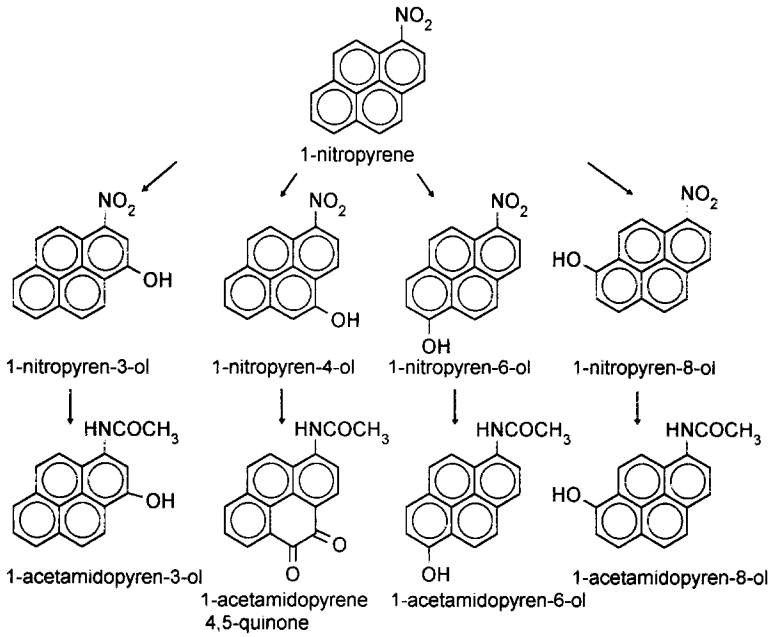

Fig. 1. Structures and numbering of metabolites of 1-nitropyrene.

portant in activation of many arylhydroxyl-amines; this pathway is illustrated in Box C of Fig. 2. The acetoxy moiety is a more potent leaving group than the un-esterified hydroxyl group, and thus the *N*-acetoxy derivative generates a reactive electrophilic species (arylnitrenium ion) with greater facility than does the *N*-hydroxylamine (Miller and Miller, 1981). Hence the mutagenicity of several arylamines and nitroarenes is enhanced in bacterial strains expressing elevated levels of OAT activity (Watanabe et al., 1990). This enzyme is not a requirement for expression of the mutagenicity of 1-NP itself, since the potency of this compound was not impaired in the OAT-deficient *Salmonella typhimurium* strain TA98/1,8-DNP$_6$ compared to the competent strain TA98 (Ball et al., 1984a). However, mutagenic activity in the urines of rats dosed with 1-NP, attributed to 1-NP metabolites, was reported to be considerably enhanced in the presence of OAT (Scheepers et al., 1991), suggesting that the OAT pathway makes a

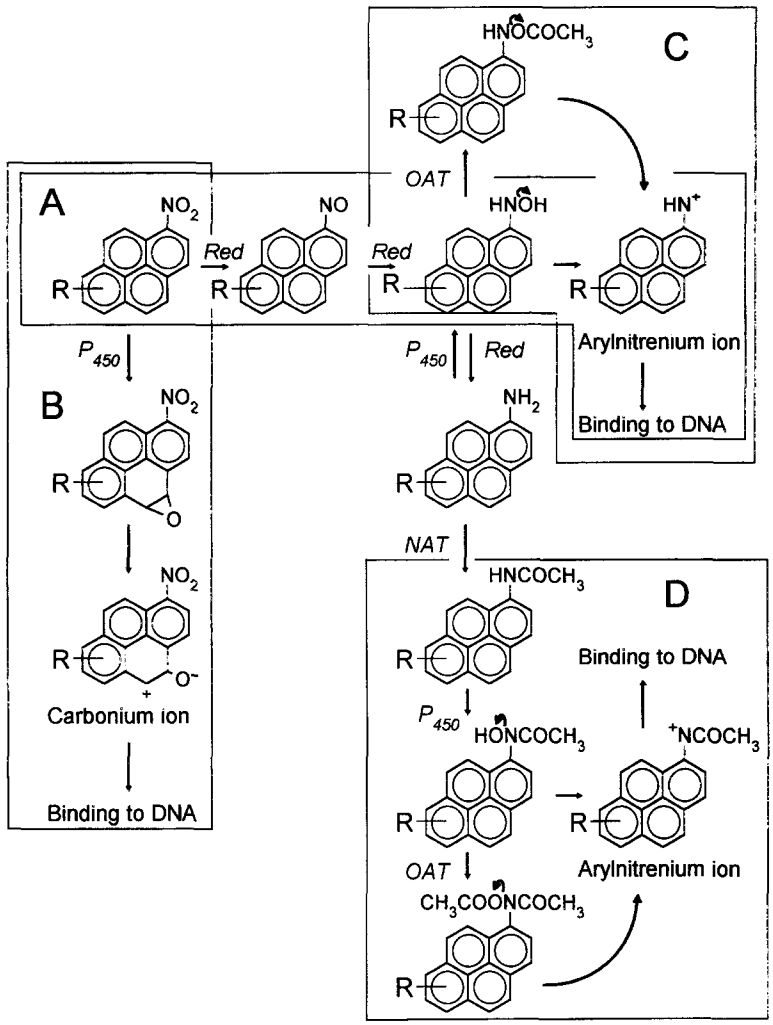

Fig. 2. Proposed pathways for activation of 1-nitropyrene and of its metabolites. The boxes denote major routes: A, activation by simple nitroreduction; B, activation by epoxidation; C, enhancement of the genotoxicity of a hydroxylamine intermediate by *O*-acetylation; D, further activation of an arylacetamide. Enzymes are indicated in italics: *Red*, nitroreductase; P_{450}, cytochrome P450; *NAT*, *N*-acetyltransferase; *OAT*, *O*-acetyltransferase. R = H in 1-nitropyrene or OH in phenolic metabolites.

greater contribution to the activity of oxidised 1-NP derivatives than to that of 1-NP itself.

To investigate this point further, we compared the bacterial mutagenicity of some of the major metabolites of 1-NP in *Salmonella typhimurium* strain TA98 which expresses OAT at constitutive levels, in TA98/1,8-DNP$_6$, a TA98 derivative in which OAT activity is abolished by a stop codon introduced by frameshift mutation in the coding region (Watanabe et al., 1992), and in YG1024, constructed from TA98 by inserting the OAT gene from TA1538 cloned into a multicopy tetracycline resistance plasmid, such that OAT is expressed at a level some 50-fold higher than in TA98 itself (Watanabe et al., 1990). For the present study synthesis of nitropyrenols on a larger scale, including the previously-untested isomer 1-nitropyren-4-ol, and also synthesis of the acetamidopyrenols (Fig. 1) previously available only as material isolated from rat urine or from enzyme-catalyzed oxidations (Ball et al., 1984a,b), enabled more rigorous characterization of these compounds, both structurally and biologically, than had been possible previously. We report here an evaluation of the importance of OAT activity in the bacterial mutagenicity of these compounds.

2. Materials and methods

2.1. Chemicals

1-Nitropyrene (1-NP) was synthesized and characterized as > 99.9% pure by the Midwest Research Institute, Kansas City, MO. HPLC-grade solvents were purchased from Burdick and Jackson, Muskegon, MI, and Fisher Scientific, Raleigh, NC. Other chemicals and solvents were purchased from commercial sources, Boehringer-Mannheim Inc., Indianapolis, IN, Sigma Chemical Co., St Louis, MO, Aldrich Chemical Co. and Fisher Scientific, at the highest degree of purity available, and used without further purification. Silica gel for open bed column chromatography was grade 60, from Aldrich Chemical Co.

Nitropyrenols. General preparation: 1-Acetylpyrene (Aldrich Chemical Co., Milwaukee, WI, 760 mg, 3.1 mmol, in CH_2Cl_2, 30 ml) was oxidised by stirring with *meta*chloroperoxybenzoic acid (mCPBA, 1.4 g crude weight, 8.14 mmol) and trifluoroacetic acid (356 mg) in CH_2Cl_2 (5 ml) for 3 days at room temperature. The reaction mixture was separated on a silica gel column eluted with benzene. 1-Acetoxypyrene was the second band to elute from the column, and fluoresced bright blue under long-wave UV light. Yield was 240 mg (30%). 1-Acetoxypyrene (182 mg) in acetic anhydride (4 ml) was nitrated by addition of 1.46 ml HNO_3 (70%, 0.5 ml, in 11 ml acetic anhydride). Yield was 211 mg (99%). The mixture of acetoxynitropyrenes was hydrolysed quantitatively by refluxing for 3 h with aq. NaOH (5%, w/v, 0.5 ml in MeOH, 5 ml). The products were chromatographed on a silica gel column (5 × 30 cm) eluted with CH_2Cl_2. The nitropyrenols eluted as three bands, red, brown-red and orange (in order), which were identified as 1,6-, 1,8- and 1,3-nitropyrenol, respectively, as described below. The nitropyrenols were shown to be over 99% pure by normal-phase and by reverse-phase HPLC. Structures are shown in Fig. 2.

1-Nitropyren-3-ol. The structure of this nitropyrenol isomer was confirmed by NMR spectroscopy, in which the presence of an upfield triplet corresponding to H_7 indicating no substitutions at C6, C7 and C8, and a singlet attributable to H2 are diagnostic, NMR. (500 MHz, acetone-d_6) δ ppm: 8.67 (d, 1 proton, H_{10}, $J_{10,9} = 9.5$ Hz); 8.55 (d, 1 proton, H_4, $J_{4,5} = 9.1$ Hz); 8.40 (d, 1 proton, H_6, $J_{6,7} = 7.6$ Hz); 8.37 (d, 1 proton, H_8, $J_{8,7} = 7.2$ Hz); 8.36 (d, 1 proton, H_5, $J_{5,4} = 9.1$ Hz); 8.29 (s, 1 proton, H_2); 8.26 (d, 1 proton, H_9, $J_{9,10} = 9.5$ Hz); 8.16 (t, 1 proton, H_7, $J_{7,6} = J_{7,8} = 7.6$ Hz).

1-nitropyren-4-ol. 1-NP (22 mg) in acetone (7 ml) was stirred in the dark with dimethyldioxirane (DMDO; 0.1 M in acetone, prepared as described in the literature (Murray and Jeyaraman, 1985), 10 ml) at room temperature. Product formation was monitored by TLC in methylene chloride. After 1 month, the solvent was removed by evaporation under a stream of N_2 gas, and the product was separated on a silica gel column eluted with 5% hexanes in methylene chloride. The first band to elute was unreacted 1-NP. A slower-moving, orange band was identified as 1-nitropyren-4-ol by the presence of an upfield triplet corresponding to H_7 indicating no substitutions at C6, C7 and C8, of an upfield singlet consistent with a proton *ortho* to a hydroxyl group and by

consideration of the downfield shift observed in the resonance attributed to H_3, *peri* to the hydroxyl group. NMR, 500 MHz, $CDCl_3$, δ ppm: 8.86 (d, 1H, H_{10}, $J_{10,9} = 9.4$ Hz); 8.69 (d, 1H, H_2, $J_{2,3} = 8.7$ Hz); 8.61 (d, 1H, H_3, $J_{3,2} = 8.7$ Hz); 8.31 (d, 1H, H_9, $J_{9,10} = 9.4$ Hz); 8.19 (d, 1H, H_6, $J_{6,7} = 7.6$ Hz); 8.16 (d, 1H, H_8, $J_{8,7} = 7.6$ Hz); 8.06 (t, 1H, H_7, $J_{7,8} = J_{7,6} = 7.6$ Hz); 7.53 (s, 1H, H_5).

1-Nitropyren-6-ol. The NMR spectrum was consistent with structure, and the UV spectrum identical to that of material isolated previously (Ball et al., 1984a).

1-Nitropyren-8-ol. The NMR spectrum was consistent with structure, confirmed by extensive homonuclear decoupling. The UV spectrum was identical to that of material isolated previously (Ball et al., 1984a).

Acetamidopyrenols: General procedure. Each nitropyrenol isomer (2 mg) was stirred with Zn (2 mg) and NH_4Cl (4 mg) in EtOH at room temperature until the orange solution turned colorless and exhibited fluorescence under long-wave UV light. The solution was filtered into acetic anhydride (2 drops). The solvent was evaporated under argon, then the solid was dried under vacuum. The position of substitution was fixed by the route of preparation, from a defined nitropyrenol isomer. Structures are shown in Fig. 2.

1-Acetamidopyren-3-ol. Unlike the other acetamidopyrenols, this isomer was orange-red. The NMR spectrum was consistent with structure, and the UV spectrum identical to that of material isolated previously from S9-catalyzed oxidation of 1-acetamidopyrene (Ball et al., 1984b).

1-Acetamidopyrene-4,5-quinone. 1-Acetamidopyrene (25 mg) was stirred in acetone with a 4-fold molar excess of DMDO (0.1 M in acetone) at room temperature for 24 h. Solvent was removed by evaporation under reduced pressure. A major orange product was recovered by chromatography on a silica gel column (3 × 25 cm) eluted with $CHCl_3$, which was further purified on 0.5 mm silica plates (20 × 20 cm) developed in $CHCl_3$. NMR analysis indicated that this product (yield, 2 to 10%) was 1-acetamidopyrene-4,5-quinone. NMR (400 MHz, $CDCl_3$) δ ppm: 9.1 (d, 1 proton, H_3, $J_{3,2} = 9.09$ Hz); 8.50 (dd, 1 proton, H_6, $J_{6,7} = 7.4$ Hz, $J_{6,8} = 1.3$ Hz); 8.18 (dd, 1 proton, H_8, $J_{8,7} = 7.4$ Hz, $J_{8,6} = 1.2$ Hz); 8.12 (d, 1 proton, H_2, $J_{2,3} = 9.1$ Hz); 7.77 (s, 2 protons, H_9 and H_{10}); 7.74 (t, 1 proton, H_7, $J_{7,6} = J_{7,8} = 7.4$ Hz); 2.37 (s, 3 protons, -COCH_3).

1-Acetamidopyren-6-ol. The NMR spectrum was consistent with structure, and the UV spectrum identical to that of material isolated previously from S9-catalyzed oxidation of 1-acetamidopyrene (Ball et al., 1984b).

1-Acetamidopyren-8-ol. The NMR spectrum was consistent with structure, and the UV spectrum identical to that of material isolated previously from S9-catalyzed oxidation of 1-acetamidopyrene (Ball et al., 1984b).

2.2. Instrumentation

Proton nuclear magnetic resonance (NMR) analysis was carried out at 500 MHz with a model AMX-500 spectrometer (Brucker Instrument Co., Billerica, MA). Chemical shifts are reported in ppm relative to TMS. For normal phase HPLC, an Alltech Sil 10 × 250 mm column was used with methylene chloride as eluent (model 2350 HPLC pump, ISCO, Lincoln, NE). For reverse-phase HPLC, a Zorbax ODS 9.4 × 250 mm column was eluted with a gradient from 50% MeOH in water to 100% MeOH generated with a model 2360 gradient programmer and model 2300 pump (ISCO, Lincoln, NE). In both modes, eluates were monitored at 254 nm. UV spectra were acquired with Spectronic 1201 (Milton Roy, Riviera Beach, FL) or model LC-85B (Perkin-Elmer, Norwalk, CT) spectrophotometers.

2.3. Mutagenicity assays

The *Salmonella typhimurium* plate incorporation assay was carried out as described (Ames et al., 1975), with the following minor modifications: minimal histidine was added to the base agar rather than to the soft agar overlay, and the number of histidine-prototrophic revertants was counted (Model 800 Colony Counter, Artek Inc., Farmingdale, NY) after 72 rather than 48 h (Claxton et al., 1982). *Salmonella typhimurium* strain TA98 (*hisD3052*, *rfa*, Δ*uvrB*, pKM101) and its variants were a generous gift from the stock of the Genetic Toxicology Branch, HERL (now the Environmental Carcinogenesis Division, NHEERL), U.S. Environmental Protection

Table 1
Mutagenicity of nitropyrenols towards *Salmonella typhimurium* variants

Dose	Activity in *Salmonella typhimurium* strain [a]					
(μg/plate)	TA98		TA98/1,8-DNP_6		YG1024	
	− S9	+S9	− S9	+S9	− S9	+S9
1-Nitropyren-3-ol						
0.00	33 ± 4	38 ± 4	24 ± 10	24 ± 4	50 ± 26	104 ± 36
0.05	–	–	–	–	99 ± 40	110 ± 30
0.10	45 ± 5	59 ± 7	32 ± 13	26 ± 7	268 ± 76	132 ± 35
0.25	–	–	–	–	–	144 ± 39
0.50	116 ± 20	66 ± 14	82 ± 13	27 ± 5	579 ± 85	228 ± 49
1.0	221 ± 41	111 ± 29	159 ± 30	30 ± 8	844 ± 113	410 ± 50
2.5	341 ± 81	288 ± 42	396 ± 82	40 ± 9	433 ± 52	–
5.0	221 ± 103	560 ± 51	218 ± 178	81 ± 17	–	–
Positive controls [b]	407 ± 34	683 ± 93	109 ± 19	482 ± 24	3126 ± 203	1357 ± 510
Specific activity [c]	50 ± 11	28 ± 3	40 ± 7	3 ± 1	204 ± 26	80 ± 6
r^2 [d]	≥ 0.96 (1.0)	≥ 0.99 (5.0)	≥ 0.99 (2.5)	≥ 0.91 (5.0)	≥ 0.89 (1.0)	≥ 0.97 (1.0)
1-Nitropyren-4-ol						
0.00	28 ± 2	34 ± 5	27 ± 6	32 ± 8	66 ± 30	101 ± 47
0.005	–	–	–	–	120 ± 72	–
0.01	–	–	–	–	159 ± 86	230 ± 78
0.05	–	126 ± 12	–	–	387 ± 73	439 ± 83
0.10	84 ± 5	222 ± 29	66 ± 26	39 ± 17	669 ± 98	699 ± 119
0.25	219 ± 16	392 ± 51	131 ± 11	–	–	1340 ± 112
0.50	380 ± 48	907 ± 73	239 ± 15	53 ± 22	1764 ± 186	2365 ± 547
1.00	692 ± 123	1814 ± 223	460 ± 30	80 ± 28	–	–
2.50	992 ± 208	–	911 ± 42	141 ± 33	–	–
5.00	–	–	–	315 ± 70	–	–
Positive controls	386 ± 30	486 ± 136	114 ± 16	451 ± 34	2545 ± 305	1307 ± 269
Specific activity	176 ± 35	467 ± 28	93 ± 1	15 ± 3	1552 ± 114	1452 ± 252
r^2	≥ 0.99 (1.0)	≥ 0.99 (1.0)	≥ 0.99 (2.5)	≥ 0.96 (5.0)	≥ 0.98 (0.1)	≥ 0.99 (0.5)
1-Nitropyren-6-ol						
0.00	30 ± 4	40 ± 5	25 ± 11	27 ± 12	39 ± 10	69 ± 14
0.05	–	–	–	–	50 ± 4	138 ± 36
0.10	33 ± 6	114 ± 17	27 ± 9	33 ± 17	54 ± 7	208 ± 51
0.25	–	–	–	–	–	478 ± 117
0.50	62 ± 12	507 ± 120	23 ± 12	38 ± 20	89 ± 21	1100 ± 263
1.0	84 ± 16	1341 ± 215	32 ± 11	41 ± 16	136 ± 46	2360 ± 378
2.5	172 ± 18	2514 ± 270	33 ± 11	61 ± 25	260 ± 101	–
5.0	266 ± 23	3386 ± 80	42 ± 16	125 ± 42	–	–
Positive controls	442 ± 40	740 ± 120	102 ± 12	403 ± 123	2696 ± 362	2039 ± 832
Specific activity	13 ± 1	266 ± 26	1 ± 1	9 ± 6	23 ± 12	610 ± 105
r^2	≥ 0.97 (5.0)	≥ 0.97 (2.5)	≥ 0.40 (5.0)	≥ 0.97 (5.0)	≥ 0.99 (2.5)	≥ 0.99 (1.0)
1-Nitropyren-8-ol						
0.00	34 ± 5	37 ± 5	16 ± 5	17 ± 7	45 ± 24	68 ± 42
0.05	–	–	–	–	48 ± 25	–
0.10	42 ± 19	75 ± 28	18 ± 4	25 ± 11	57 ± 38	127 ± 39
0.25	–	–	–	–	–	214 ± 48
0.50	68 ± 34	130 ± 10	19 ± 4	44 ± 16	64 ± 41	439 ± 112
1.0	72 ± 28	213 ± 21	22 ± 8	71 ± 28	68 ± 39	885 ± 194

Table 1 (continued)

Dose	Activity in *Salmonella typhimurium* strain [a]					
(μg/plate)	TA98		TA98/1,8-DNP$_6$		YG1024	
	− S9	+S9	− S9	+S9	− S9	+S9
1-Nitropyren-8-ol						
2.5	75 ± 27	385 ± 62	23 ± 7	165 ± 64	88 ± 33	1723 ± 462
5.0	80 ± 27	559 ± 66	27 ± 10	229 ± 95	–	–
Positive controls	418 ± 27	716 ± 88	106 ± 19	540 ± 45	2801 ± 107	2643 ± 376
Specific activity	2 ± 1	40 ± 5	1 ± 0.5	15 ± 7	4 ± 1	203 ± 28
r^2	≥ 0.38 (5.0)	≥ 0.98 (2.5)	≥ 0.71 (5.0)	≥ 0.98 (2.5)	≥ 0.69 (2.5)	≥ 0.99 (2.5)

[a] Activity is expressed as His$^+$ revertants/plate (mean ± SD) determined with the *Salmonella* plate incorporation assay (Ames et al., 1975) in duplicate on three separate occasions.
[b] Positive controls without S9 were 2-nitrofluorene, 3 μg/plate; positive controls with S9 were 2-anthramine, 0.5 μg/plate for strains TA98 and YG1024, and 3 μg/plate for TA98/1,8-DNP$_6$.
[c] Specific mutagenicity, expressed as His$^+$ revertants/nmole, was calculated by least squares linear regression from the linear portion of the dose-response curve.
[d] Correlation coefficients r^2, with limits of linearity (μg per plate) shown in parentheses.

Agency, Research Triangle Park, NC. TA98/1,8-DNP$_6$ was originally obtained from Dr. H.S. Rosenkranz, Case Western Reserve University, Cleveland, OH, USA, and YG1024 from Dr. M. Watanabe, National Institute of Hygienic Sciences, Tokyo, Japan. Bacteria were maintained on master-plates and tested for genetic markers as described (UV and crystal violet sensitivity, ampicillin resistance, His and biotin requirement, Claxton et al., 1982). For growth of YG1024, tetracycline was included both in overnight broth (20 μg/ml) and in the master-plates (Watanabe et al., 1990). 0.1 ml of overnight bacterial broth was used per assay plate. S9 fraction (9000 × *g* supernatant) prepared from the livers of Aroclor 1254-treated male Sprague-Dawley rats was purchased from Mol-Tox, Annapolis, MD and stored at −80°C until used. For exogenous metabolic activation, the S9 was supplemented with an NADPH-generating co-factor mix as described (Ames et al., 1975). 20 μl of S9 protein (0.8–0.9 mg) was added per plate. Each compound was assayed with duplicate plates on at least three separate occasions, at doses ranging from 0.005 to 5 μg per plate, both with and without exogenous

Table 2
Summary of ratios of mutagenic potencies in *O*-acetyltransferase-competent and -deficient strains [a]

Compound	Without S9			With S9		
	P6/98	YG/98	YG/P6	P6/98	YG/98	YG/P6
1-Nitropyrene [b]	1.0	7	7	0.08	7	88
1-Nitropyren-3-ol	0.8	4	5	0.1	3	27
1-Nitropyren-4-ol	0.5	9	17	0.03	3	97
1-Nitropyren-6-ol	0.08	2	23	0.03	2	68
1-Nitropyren-8-ol	0.5	2	4	0.4	5	14
1-Acetamidopyren-3-ol	0.5	0.9	1.7	0.2	5	30
1-Acetamidopyrene 4,5-quinone	0	1	∝ [c]	0.7	3	5
1-Acetamidopyren-6-ol	0	1	∝	0.003	3	925
1-Acetamidopyren-8-ol	0	2	∝	0	0.5	∝

[a] Potency ratios were derived from the specific activities (in rev/nmole, determined as described previously) in the strains specified. 98 = TA98, P6 = TA98/1,8-DNP$_6$, YG = YG1024.
[b] Data from Ball et al., 1994 (the specific activity of 1-NP was 168 ± 22 rev/nmole in TA98 without S9, 113 ± 17 rev/nmole with S9).
[c] The denominator was 0.

Table 3
Mutagenicity of acetamidopyrenols towards *Salmonella typhimurium* TA98 variants

Dose	Activity in *Salmonella typhimurium* strain [a]					
(μg/plate)	TA98		TA98/1,8-DNP$_6$		YG1024	
	− S9	+S9	− S9	+S9	− S9	+S9
1-Acetamidopyren-3-ol						
0.00	30 ± 3	41 ± 4	18 ± 4	23 ± 9	37 ± 11	64 ± 7
0.0005	–	–	–	–	38 ± 6	112 ± 31
0.010	–	–	–	–	51 ± 13	56 ± 1
0.05	45 ± 11	50 ± 10	28 ± 7	24 ± 6	114 ± 56	72 ± 13
0.10	75 ± 52	47 ± 15	38 ± 16	27 ± 7	158 ± 75	90 ± 20
0.25	–	–	–	–	–	120 ± 43
0.5	242 ± 140	69 ± 26	141 ± 110	28 ± 7	361 ± 110	204 ± 81
1.0	345 ± 38	100 ± 47	162 ± 58	31 ± 10	363 ± 67	443 ± 202
2.5	296 ± 201	209 ± 102	201 ± 116	48 ± 21	194 ± 160	761 ± 384
5.0	146 ± 121	256 ± 172	–	–	167 ± 4	840 ± 389
Positive controls [b]	438 ± 27	738 ± 72	108 ± 5	584 ± 38	2315 ± 598	2051 ± 177
Specific activity [c]	128 ± 75	18 ± 8	68 ± 68	3 ± 3	114 ± 87	89 ± 43
r [d]	≥ 0.96 (1.0)	≥ 0.98 (2.5)	≥ 0.99 (2.5)	≥ 0.60 (2.5)	≥ 0.91 (1.0)	≥ 0.93 (2.5)
1-Acetamidopyrene-4,5-quinone						
0.0	22 ± 8	27 ± 5	14 ± 4	18 ± 2	72 ± 18	103 ± 27
0.1	23 ± 10	27 ± 8	17 ± 4	21 ± 3	76 ± 27	103 ± 10
0.5	22 ± 5	35 ± 8	17 ± 2	24 ± 7	80 ± 24	105 ± 24
1.0	25 ± 4	39 ± 7	20 ± 5	28 ± 8	78 ± 27	134 ± 21
2.5	33 ± 8	55 ± 28	19 ± 4	38 ± 13	82 ± 19	187 ± 33
5.0	41 ± 16	49 ± 5	23 ± 2	49 ± 17	96 ± 18	214 ± 40
Positive controls	339 ± 70	403 ± 150	63 ± 17	396 ± 76	2767 ± 405	2220 ± 162
Specific activity	1 ± 1	3 ± 3	0	2 ± 1	1 ± 1	10 ± 2
r^2	≥ 0.72 (5.0)	≥ 0.76 (5.0)	≥ 0.36 (5.0)	≥ 0.92 (5.0)	≥ 0.49 (5.0)	≥ 0.93 (2.5)
1-Acetamidopyren-6-ol						
0.00	33 ± 3	39 ± 5	26 ± 4	22 ± 5	58 ± 25	82 ± 34
0.05	–	174 ± 53	–	–	57 ± 22	257 ± 34
0.10	39 ± 11	404 ± 268	29 ± 9	28 ± 6	62 ± 27	424 ± 36
0.25	–	–	–	–	–	926 ± 91
0.50	46 ± 13	915 ± 338	27 ± 12	27 ± 6	68 ± 16	1254 ± 215
1.0	43 ± 15	1188 ± 256	27 ± 10	30 ± 8	69 ± 25	1606 ± 272
2.5	43 ± 24	1449 ± 205	30 ± 10	32 ± 4	64 ± 20	–
5.0	48 ± 26	–	30 ± 12	35 ± 7	–	–
Positive controls	419 ± 42	709 ± 101	102 ± 16	423 ± 90	2977 ± 287	2285 ± 551
Specific activity	1 ± 0.5	359 ± 14	0 ± 0	1 ± 1	1 ± 1	925 ± 51
r^2	≥ 0.82 (5.0)	≥ 0.87 (0.5)	≥ 0.11 (5.0)	≥ 0.51 (5.0)	≥ 0.21 (2.5)	≥ 0.99 (0.25)
1-Acetamidopyren-8-ol						
0.00	35 ± 5	39 ± 5	23 ± 7	27 ± 8	32 ± 11	70 ± 35
0.01	–	–	–	–	37 ± 2	–
0.05	–	–	–	–	42 ± 16	66 ± 17
0.10	38 ± 3	46 ± 11	26 ± 6	27 ± 5	47 ± 20	68 ± 15
0.25	–	–	–	–	–	58 ± 1
0.5	44 ± 6	53 ± 12	27 ± 9	26 ± 8	49 ± 19	70 ± 16
1.0	45 ± 6	56 ± 14	31 ± 9	32 ± 7	47 ± 23	74 ± 17
2.5	47 ± 5	55 ± 11	27 ± 8	28 ± 4	56 ± 19	72 ± 20
5.0	60 ± 14	56 ± 13	27 ± 8	28 ± 6	–	–

Table 3 (continued)

Dose (μg/plate)	Activity in *Salmonella typhimurium* strain [a]					
	TA98		TA98/1,8-DNP$_6$		YG1024	
	− S9	+S9	− S9	+S9	− S9	+S9
1-Acetamidopyren-8-ol						
Positive controls	402 ± 33	744 ± 106	96 ± 15	516 ± 101	2710 ± 106	2187 ± 361
Specific activity	1 ± 1	1 ± 0.5	0 ± 0	0 ± 0	2 ± 1	0.5 ± 0.5
r^2	≥ 0.60 (5.0)	≥ 0.30 (5.0)	≥ 0.01 (5.0)	≥ 0.01 (5.0)	≥ 0.13 (2.5)	≤ 0.33 (2.5)

[a] Activity is expressed as His$^+$ revertants/plate (mean ± SD) determined with the *Salmonella* plate incorporation assay (Ames et al., 1975) in duplicate on three separate occasions.

[b] Positive controls without S9 were 2-nitrofluorene, 3 μg/plate, and with S9, 2-anthramine, 0.5 μg/plate for strains TA98 and YG1024, and 3 μg/plate for TA98/1,8-DNP$_6$.

[c] Specific mutagenicity, expressed as His$^+$ revertants/nmole, was calculated by least squares linear regression from the linear portion of the dose-response curve.

[d] Correlation coefficients r^2, with limits of linearity (μg per plate) shown in parentheses.

metabolic activation (S9), at doses selected for each compound and bacterial strain to span the range from slightly above spontaneous up to the dose level at which toxicity (as manifested by loss of linearity in revertant counts) was evident. Compounds were freshly dissolved in DMSO immediately before each assay. Positive controls were included in each experiment. Specific mutagenicity was calculated by least-squares linear regression from the linear portion of the dose-response curve.

3. Results

The nitropyrenols all exhibited some mutagenicity in the absence of S9 (Table 1), which can reasonably be attributed to nitroreduction (Box A in Fig. 2). Involvement of *O*-acetylation in enhancing formation of the arylnitrenium ion from the reductive pathway (Box C in Fig. 2) is indicated by decreased activity in TA98/1,8-DNP$_6$ compared to TA98, and enhanced potency in YG1024. This is expressed by calculating the ratios of activity in the acetyltransferase deficient or enhanced strains compared to activity in TA98, and more strikingly, the ratio of potency in YG1024 to the potency in TA98/1,8-DNP$_6$ (Table 2); potency ratios calculated for 1-NP from data obtained in concurrent experiments but published previously (Ball et al., 1994) are included for comparison.

Addition of S9 enhanced the potency of all the nitropyrenols in strain TA98, with the exception of 1-nitropyren-3-ol. The latter compound presents a pattern of mutagenicity consistent with activation occurring primarily by direct nitroreduction, and impaired by the presence of S9. The relatively small decrease observed in the direct activity of 1-nitropyren-3-ol in TA98/1,8-DNP$_6$, and similarly small increase in YG1024 (Table 2) point to a minimal role for esterification of the hydroxylamine; this reaction may be hindered by steric or electronic effects due to the *meta* substitution.

1-Nitropyren-4-ol proved to be more potent than the nitropyrenols tested previously (Ball et al., 1984a), and exhibited substantial activity in the absence of S9. For this isomer also, activity can be attributed to nitroreduction, but with a larger amplification from esterification than was the case for 1-nitropyren-3-ol.

In contrast, the two remaining nitropyrenols exhibited strong S9-dependence. 1-Nitropyren-6-ol was proportionally the most sensitive to loss of activity in the acetylase-deficient TA98/1,8-DNP$_6$ variant, and correspondingly also strongly enhanced in YG1024, in either absence or presence of S9 (Table 2). 1-Nitropyren-8-ol was overall the least potent of the isomers tested, and required both S9 and *O*-acetyltransferase for any expression of mutagenicity.

Among the acetamide derivatives (Table 3 1-acetamidopyren-3-ol exhibited both direct-acting mutagenicity and toxicity, while 1-acetamidopyren-8-ol was hardly active under any of the test conditions, and can therefore be regarded as a true detoxication product. 1-Acetamidopyrene-4,5-quinone and 1-

acetamidopyren-6-ol were active in the presence of S9, and approximately 3-fold enhancement of activity was seen in strain YG1024. In the absence of *O*-acetyltransferase, these products were inactive, with or without S9. This pattern of activation is consistent with S9-catalyzed *N*-oxidation and/or deacetylation, followed by bacterial *O*-acetylation (though some level of *O*-acetyltransferase activity in the S9 cannot be excluded). The esterification step may provide sufficient enhancement in biological activity to compensate for losses of extracellularly-generated hydroxylamine by binding to S9 or membrane proteins or decomposition that decrease the absolute amount of hydroxylamine available for diffusion into the bacterial cell.

4. Discussion

The present study confirms previous observations on the mutagenicity of mammalian metabolites of 1-NP (Ball et al., 1984a), and points to a role for S9 in further metabolic activation of these compounds. The values for mutagenic potency recorded in this study are broadly similar to those reported previously (Ball et al., 1984a,b), with the exception that enhancement of the activity of 1-nitropyren-6-ol and -8-ol in the presence of S9 was more distinct here; the S9 used in the present study was purchased commercially, while material prepared in-house was used in the earlier study. One crucial finding of the present study concerns the importance of *O*-esterification in activation of oxidised metabolites of 1-NP; the major urinary metabolite 1-acetamidopyren-6-ol is virtually inert in the absence of bacterial *O*-acetylation.

1-Nitropyren-4-ol stands out as being the most active nitropyrenol isomer in TA98, and 1-nitropyren-8-ol the least active. The activity of 1-nitropyren-3-ol is predominantly direct-acting, and is decreased in the presence of S9, whereas the activity of the other nitropyrenol isomers tested is enhanced by S9. These observations have implications for the genotoxicity of these compounds in the intact organism, in that the 1-nitropyren-4-ol and 1-nitropyren-6-ol isomers in particular cannot be considered to be detoxication products. The dependence of mutagenicity on the presence of both oxidation and esterification is more typical of an arylamine than of a nitroarene compound; explanation of the activation pattern seen with the nitropyrenols must either postulate nitroreduction to the amine (most likely bacterial in origin) followed by extracellular, S9-catalyzed *N*-oxidation then another intra-bacterial biotransformation step, *O*-acetylation, or invoke action of the S9 on the hydrocarbon nucleus, such as a K-region epoxidation, as shown in Box B of Fig. 2. In the former scenario, repeated transfer into and out of the cell would result in low overall yields of ultimate active species because of losses at each step and extracellular dilution, hence underestimation of the potency in this assay system compared to a situation where both the activation processes and the molecular target occur inside the same cell. In the latter case, the role of *O*-acetyltransferase in the activation process is not immediately obvious.

Distinct differences in activity between isomers point to a role for electronic effects. The electron-donating properties of hydroxyl substituents at positions conjugated with C1 (such as C4, C6, C8) would be expected to resonance-stabilise the positive charge of the arylnitrenium ion, thus enhancing its electrophilic reactivity (Cole et al., 1980), and also to increase the susceptibility of the arylamine or arylamide to *N*-oxidation (Hanna et al., 1979), whereas hydroxylation at other positions would have a net deactivating effect. The influence, if any, of electron-donating or -withdrawing substituents on susceptibility to bacterial nitroreduction has not been fully explored. As nitroreduction is a crucial step in generation of active electrophiles from nitroarenes in the assay system used for the present study, decreased affinity for the nitroreductase enzyme could modulate the expectation of higher intrinsic potency for the nitropyrenols studied here compared to 1-NP itself (168 rev/nmole in TA98, Ball et al., 1994).

The activity of the major urinary metabolite of 1-NP, 1-acetamidopyren-6-ol, in the *Salmonella typhimurium* plate incorporation assay clearly depends on *N*-oxidation followed by *O*-acetylation, since this compound is virtually inactive in systems devoid of either of these two pathways; the other acetamidopyrenols exhibit little activity in the presence of S9, confirming that the 1,6 isomer is the principal isomer responsible for mutagenic activity in the urines of rats dosed with 1-NP. The cytochrome P450 iso-

forms responsible for the *N*-oxidation step may include the 2B and 1A subfamilies (Edwards et al., 1994; Yamazaki and Shimada, 1992), since these are known to participate in activation of other genotoxic arylamines. Studies on the further disposition of 1-acetamidopyren-6-ol will be needed to evaluate whether the action of the S9 fraction is confined to cytochrome P450-catalyzed *N*-oxidation (giving rise to an *N*-acylarylnitrenium ion as the active intermediate, hence *N*-acetylated DNA adducts) or whether deacetylation also occurs, to yield an arylnitrenium ion, and arylamine-rather than arylamide-derived DNA adducts.

Substantial differences exist between bacterial and mammalian enzymes with respect to both nitroreduction and acetylation reactions, which complicate use of these results to predict genotoxicity in mammalian systems. Several mammalian enzymes are capable of catalysing nitroreduction, including DT-diaphorase, aldehyde oxidase, xanthine oxidase, and cytochrome P450 itself (Saito et al., 1984); all require anaerobic conditions and are sensitive to oxygen, unlike the 'classical' bacterial oxygen-insensitive nitroreductase (nitroreductase II, Peterson et al., 1979), and their substrate preferences may not parallel those of the bacterial enzyme. The *N,O*-transesterification involved in activation of acetamides such as 2-acetamidofluorene is not found in *S. typhimurium* bacteria (Saito et al., 1985), hence genotoxicity of acetamides may in fact be underestimated in bacterial assays. Hamster liver (Saito et al., 1986; Trinidad et al., 1990), mouse liver (Mattano et al., 1989) and human recombinant *N*-acetyltransferase NAT1 expressed in *E. coli* (Hein et al., 1993) can all catalyse *N,O*-transesterification as well as *N*- and *O*-acetyl transfers; the polymorphic NAT2 enzyme, which accounts for the majority of human acetylase activity, exhibits both of the latter two activities (Hein et al., 1993). In contrast, *N*-acetyltransferase activity is apparently linked to but not necessarily identical to the enzyme catalysing *O*-acetyltransferase in bacteria (Orr et al., 1985). 2-Aminofluorene is activated by NAT1 rather than NAT2 (Minchin et al., 1992), whereas the converse holds for heterocyclic amines (Wild et al., 1995); by analogy NAT1 would be the enzyme responsible for acetylation of the aminopyrene derivatives. In the intact organ, esterification by *O*-sulfation or *O*-glucuronidation may also play a role in metabolic activation which cannot at present be adequately evaluated in a bacterial assay system. However, the importance of esterification in generating active species from 1-NP metabolites demonstrated by the present study emphasizes the role played by the metabolic specificity of individual tissues in determining susceptibility to genotoxic effects arising from 1-NP.

Acknowledgements

Portions of this paper are based on research submitted by PFR and PR in partial fulfillment of the requirements for the degree of MSPH from the University of North Carolina at Chapel Hill. This research was supported by PHS RO1-ES05732.

References

Ames, B.N., J. McCann and E. Yamasaki (1975) Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test, Mutation Res., 31, 347–364.

Ball, L.M., M.J. Kohan, L.D. Claxton and J. Lewtas (1984a) Mutagenicity of derivatives and metabolites of 1-nitropyrene: Activation by rat liver S9 and bacterial enzymes, Mutation Res., 138, 113–125.

Ball, L.M., M.J. Kohan, J.P. Inmon, L.D. Claxton and J. Lewtas (1984b) Metabolism of 1-nitro[^{14}C]pyrene in vivo in the rat and mutagenicity of urinary metabolites, Carcinogenesis, 5, 1557–1564.

Ball, L.M., J.J. Rafter, J.-Å. Gustafsson, B.-E. Gustafsson, M.J. Kohan and J. Lewtas (1991) Formation of mutagenic urinary metabolites from 1-nitropyrene in germ-free and conventional rats: role of the gut flora, Carcinogenesis, 12, 1–5.

Ball, L.M., P. Rosser-Duncan and M.N. Boucher (1994) Oxidative and bacterial pathways in metabolic activation of nitrated polycyclic aromatic hydrocarbons, Polycyc. Arom. Compounds, 7, 35–42.

Claxton, L.D., M. Kohan, A.C. Austin and C. Evans. 1982. The Genetic Bioassay Branch protocol for bacterial mutagenesis including safety and quality assurance procedures. U.S. Environmental Protection Agency, Research Triangle Park, NC. NTIS, EPA-600/2-82-008.

Cole, C.-A., H.-L. Pan and T.L. Fletcher (1980) New 9-substituted 3-*N,O*-diacetylhydroxylaminofluorenes: enhanced electrophilicity and mutagenicity. Derivatives of fluorene 38. Cancer Lett., 9, 61–66.

Edwards, R.J., B.P. Murray, S. Murray, T. Schulz, D. Nebert, T.W. Gant, S.S. Thorgeirsson, A.R. Boobis and D.S. Davies (1994) Contribution of CYP1A1 and CYP1A2 to the activa-

tion of heterocyclic amines in monkeys and humans, Carcinogenesis, 15, 829–836.

El-Bayoumy, K. and S.S. Hecht (1984) Metabolism of 1-nitro[U-4,5,9,10-^{14}C]pyrene in the F344 rat, Cancer Res., 44, 4317–4322.

Gibson, T.L. (1982) Nitro derivatives of polynuclear aromatic hydrocarbons in airborne and source particulate matter, Atmos. Environ., 16, 2037–2040.

Gibson, T.L. (1983) Sources of direct-acting nitroarene mutagens in airborne particulate matter, Mutation Res., 122, 115–121.

Hanna, P.E., R.E. Gammas, R.D. Sehon and M.K. Lee (1979) Modulation of the metabolic *N*-hydroxylation of arylamides by substituent variation, in: Fall Abstracts, Am. Chem. Soc., Medicinal Chemistry Section, No. 70.

Hein, D.W., M.A. Doll, T.D. Rustan, K. Gray, Y. Feng, R.J. Ferguson and D.M. Grant (1993) Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases, Carcinogenesis, 14, 1633–1638.

Howard, P.C. and F. Beland (1982) Xanthine oxidase catalyzed binding of 1-nitropyrene to DNA, Biochem. Biophys. Res. Commun., 104, 727–732.

Howard, P.C., R.H. Heflich, F.E. Evans and F.A. Beland (1983) Formation of DNA adducts in vitro and in *Salmonella typhimurium* upon metabolic reduction of the environmental mutagen 1-nitropyrene, Cancer Res., 43, 2052–2058.

Jackson, M.A., L.C. King, L.M. Ball, S. Ghayourmanesh, A.M. Jeffrey and J. Lewtas (1984) Nitropyrene: DNA binding and adduct formation in respiratory tissues, Environ. Health Perspect., 62, 203–207.

Mattano, S.S., S. Land, C.M. King and W.W. Weber (1989) Purification and biochemical characterization of hepatic arylamine *N*-acetyltransferase from rapid and slow acetylator mice: identity with arylhydroxamic acid, *N,O*-acetyltransferase and *N*-hydroxylamine *O*-acetyltransferase, Mol. Pharmacol., 35, 599–609.

Miller, E.C. and J.A. Miller (1981) Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules, Cancer, 47, 2327–2345.

Minchin, R.F., P.T. Reeves, C.H. Teitel, M.E. McManus, B. Mojarabbi, K.F. Ilett and F.F. Kadlubar (1992) *N*- and *O*-acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in COS-1 cells, Biochem. Biophys. Res. Commun., 185, 839–944.

Mitchell, C.E. (1988) Formation of DNA adducts in mouse tissues after intratracheal instillation of 1-nitropyrene, Carcinogenesis, 9, 857–860.

Murray, R.W. and R. Jeyaraman (1985) Dioxiranes: Synthesis and reactions of methyldioxiranes, J. Org. Chem., 50, 2847–2853.

Orr, J.C., D.W. Bryant, D.R. McCalla and M.A. Quilliam (1985) Dinitropyrene-resistant *Salmonella typhimurium* are deficient in an acetyl-CoA acetyltransferase, Chem.-Biol. Interact., 54, 281–288.

Peterson, F.J., R.P. Mason, J. Hovsepian and J.L. Holzman (1979) Oxygen-sensitive and -insensitive nitroreduction by *Escherichia coli* and rat hepatic microsomes, J. Biol. Chem., 254, 4009–4014.

Rosenkranz, H.S. and R. Mermelstein (1985) The genotoxicity, metabolism and carcinogenicity of nitrated polycyclic aromatic hydrocarbons, J. Environ. Sci. Health, C3, 221–272.

Roy, A.K., K. El-Bayoumy and S.S. Hecht (1989) ^{32}P-postlabeling analysis of 1-nitropyrene-DNA adducts in female Sprague-Dawley rats, Carcinogenesis, 10, 195–198.

Roy, A.K., P. Upadhyaya, F.E. Evans and K. El-Bayoumy (1991) Structural characterization of the major adducts formed by reaction of 4,5-epoxy-4,5-dihydro-1-nitropyrene with DNA, Carcinogenesis, 12, 577–581.

Saito, K., T. Kamataki and R. Kato (1984) Participation of cytochrome P-450 in reductive metabolism of 1-nitropyrene by rat liver microsomes, Cancer Res., 44, 3169–3173.

Saito, K., A. Shinohara, T. Kamataki and R. Kato (1985) Metabolic activation of mutagenic *N*-hydroxyarylamines by *O*-acetyltransferase in *Salmonella typhimurium* TA98, Arch. Biochem. Biophys., 239, 286–295.

Saito, K., A. Shinohara, T. Kamataki and R. Kato (1986) *N*-Hydroxyarylamine *O*-acetyltransferase in hamster liver: identity with arylhydroxamic acid *N,O*-acetyltransferase and arylamine *N*-acetyltransferase, J. Biochem. (Tokyo), 99, 1689–1697.

Scheepers, P.T.J., J.L.G. Theuws and R.P. Bos (1991) Mutagenicity of urine from rats after 1-nitropyrene and 2-nitrofluorene administration using new sensitive *Salmonella typhimurium* strains YG1012 and YG1024, Mutation Res., 260, 393–399.

Trinidad, A., D.W. Hein, T.D. Rustan, R.J. Ferguson, L.S. Miller, K.D. Bucher, W.G. Kirlin, F. Ogolla and A.F. Andrews (1990) Purification of hepatic polymorphic arylamine *N*-acetyltransferase from homozygous rapid acetylator inbred hamster: identity with polymorphic *N*-hydroxyarylamine *O*-acetyltransferase, Cancer Res., 50, 7942–7949.

Watanabe, M., M. Ishidate Jr. and T. Nohmi (1990) Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: New derivatives of *Salmonella typhimurium* tester strains possessing elevated *O*-acetyltransferase levels, Mutation Res., 234, 337–348.

Watanabe, M., T. Sofuni and T. Nohmi (1992) Involvement of Cys^{69} residue in the catalytic mechnaism of *N*-hydroxyarylamine *O*-acetyltransferase of *Salmonella typhimurium*, J. Biol. Chem., 267, 8429–8436.

Wild, D., W. Feser, S. Michel, H.L. Lord and P.D. Josephy (1995) Metabolic activation of heterocyclic aromatic amines catalyzed by human arylamine *N*-acetyltransferase isozymes (NAT1 and NAT2) expressed in *Salmonella typhimurium*, Carcinogenesis, 16, 643-648.

Yamazaki, H. and T. Shimada (1992) Activation of 6-aminochrysene to genotoxic products by different forms of rat liver cytochrome P450 in an *O*-acetyltransferase-overexpressing *Salmonella typhimurium* strain (NM2009), Biochem. Pharmacol., 44, 913–920.