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Mutagenicity of nitrodibenzopyranones in the Salmonella plate-incorporation and microsuspension assays

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Abstract

The mutagenicity of three isomers of nitro-6*H*-dibenzo[*b*,*d*]pyran-6-one (NDBP), 2-NDBP, 3-NDBP and 4-NDBP, was characterized in the plate-incorporation (PI) and microsuspension (MS) assays using *Salmonella typhimurium* tester strains in the presence or absence of S9 mix. In both assays, all of the NDBPs showed mutagenicity in every strain. In the absence of S9 mix, TA98 was the strain most sensitive to the mutagenicity of NDBPs. The activity of NDBPs was reduced in TA98NR and TA98/1,8-DNP_6 strains relative to TA98, suggesting that NDBPs cause frameshift mutation and that nitroreduction by 'classical' nitroreductase and acetylation are significant steps for their metabolic activation. Mutagenic potency of NDBPs in TA98 without S9 mix in the MS assay (2-NDBP 104 300 rev./μg, 3-NDBP 23 500 rev./μg, and 4-NDBP 15 300 rev./μg) was much higher than that in the PI assay (2-NDBP 38 rev./μg, 3-NDBP 162 rev./μg, and 4-NDBP 7 rev./μg). Although additional S9 mix increased the mutagenicity of NDBPs in the PI assay, the mutagenic potency of NDBPs in the MS assay using strains TA98 and TA100 was decreased by the addition of S9 mix. In the PI assay, frameshift and base-substitution activities of both isomers were enhanced by the addition of the pKM101 plasmid, suggesting the induction by these isomers of complex frameshifts (frameshifts with associated base substitutions) in strain TA98. In the PI assay, 2-NDBP generally exhibited more base-substitution than frameshift activity; however, the reverse was true for 3-NDBP. In the MS assay, both isomers exhibited more frameshift than base-substitution activity.

Keywords: Nitrodibenzopyranone; *Salmonella typhimurium*; Plate-incorporation assay; Microsuspension assay

1. Introduction

Recently, 2-nitro-6*H*-dibenzo[*b*,*d*]pyran-6-one (NDBP) and 4-NDBP were identified as OH radical-initiated reaction products of phenanthrene in the presence of NOx (Helmig et al., 1992a; Arey et al., 1992). These compounds represented a new class of mutagens, i.e., nitroarene lactones, (El-Bayoumy

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Table 1
Mutagenicity of NDBPs in the standard tester strains in the plate-incorporation assay

Strain	Dose (μg/plate)	2-NDBP		3-NDBP		4-NDBP	
		− S9	+S9	− S9	+S9	− S9	+S9
TA98	0	30 ± 8	37 ± 4	24 ± 5	36 ± 3	29 ± 7	40 ± 5
	0.05	26 ± 2	58 ± 3	–	–	–	–
	0.1	32 ± 2	74 ± 1	46 ± 6	66 ± 14	–	–
	0.25	33 ± 3	117 ± 8	52 ± 6	85 ± 10	–	–
	0.5	40 ± 7	183 ± 15	95 ± 12	140 ± 15	–	–
	1	66 ± 16	295 ± 36	156 ± 17	240 ± 25	34 ± 7	51 ± 5
	2.5	137 ± 3	536 ± 21	431 ± 59	491 ± 26	42 ± 7	78 ± 2
	5	255 ± 3	–	856 ± 85	–	53 ± 1	130 ± 21
	10	482 ± 41	–	1621 ± 60	–	90 ± 21	227 ± 38
	25	–	–	–	–	148 ± 1	401 ± 19
LED [a] (μg/plate)		1	0.1	0.25	0.25	10	5
Slope [b] (rev./μg)		38 ± 4.6	259 ± 33.5	162 ± 17.3	211 ± 31.8	7 ± 0.7	18 ± 3.1
TA100	0	99 ± 19	111 ± 19	93 ± 15	102 ± 13	–	–
	0.05	–	169 ± 12	–	–	–	–
	0.1	116 ± 16	207 ± 2	–	–	–	–
	0.25	133 ± 20	348 ± 30	–	–	–	–
	0.5	137 ± 28	464 ± 23	132 ± 20	163 ± 1	–	–
	1	141 ± 2	655 ± 87	143 ± 21	180 ± 16	–	–
	2.5	198 ± 11	–	191 ± 24	213 ± 25	–	–
	5	299 ± 35	–	255 ± 44	295 ± 44	–	–
	10	439 ± 39	–	384 ± 79	450 ± 74	–	–
LED (μg/plate)		2.5	0.25	2.5	2.5	–	–
Slope (rev./μg)		38 ± 10.5	715 ± 189.1	31 ± 3.7	36 ± 5.3	–	–
TA1535	0	19 ± 5	13 ± 5	19 ± 5	13 ± 5	–	–
	0.5	23 ± 2	25 ± 1	–	–	–	–
	1	21 ± 2	32 ± 2	–	–	–	–
	2.5	26 ± 10	56 ± 12	–	–	–	–
	5	38 ± 2	82 ± 14	–	15 ± 3	–	–
	10	65 ± 3	96 ± 6	16 ± 2	16 ± 1	–	–
	50	111 ± 3	101 ± 4	22 ± 5	26 ± 2	–	–
	100	–	–	31 ± 1	32 ± 3	–	–
	200	–	–	39 ± 1	–	–	–
LED (μg/plate)		5	1	200	50	–	–
Slope (rev./μg)		9 ± 3.8	18 ± 0.8	0.1 ± 0.04	0.2 ± 0.03	–	–
TA1537	0	13 ± 4	26 ± 6	12 ± 4	26 ± 6	–	–
	0.5	22 ± 2	41 ± 6	17 ± 4	44 ± 6	–	–
	1	21 ± 6	52 ± 12	36 ± 8	60 ± 1	–	–
	2.5	37 ± 1	62 ± 1	65 ± 13	100 ± 5	–	–
	5	53 ± 12	118 ± 12	128 ± 7	190 ± 12	–	–
	10	100 ± 15	209 ± 12	201 ± 29	308 ± 9	–	–
LED (μg/plate)		2.5	1	1	1	–	–
Slope (rev./μg)		19 ± 7.9	26 ± 9.5	24 ± 1.4	30 ± 3.1	–	–
TA1538	0	8 ± 2	19 ± 2	9 ± 3	19 ± 2	–	–
	0.25	10 ± 4	34 ± 2	13 ± 3	32 ± 4	–	–
	0.5	10 ± 1	39 ± 2	14 ± 4	42 ± 4	–	–
	1	12 ± 2	51 ± 14	18 ± 5	49 ± 2	–	–
	2.5	9 ± 1	89 ± 6	34 ± 2	79 ± 6	–	–
	5	13 ± 2	148 ± 6	43 ± 1	137 ± 11	–	–
	10	19 ± 1	–	109 ± 6	211 ± 14	–	–
	50	22 ± 1	–	–	–	–	–
LED (μg/plate)		10	0.5	1	0.5	–	–
Slope (rev./μg)		1 ± 1.0	34 ± 8.6	8 ± 0.7	20 ± 0.2	–	–

[a] Lowest effective dose (μg/plate) tested that induced more than 2-fold induction over the spontaneous revertants.
[b] Mutagenicity determined by the method of Bernstein et al. (1982) and expressed as a linear slope (revertants/μg) ± S.D.

and Hecht, 1986; Arey et al., 1992) and were subsequently measured in ambient air particles (Helmig et al., 1992b; Lewtas et al., 1994). 4-NDBP was considered to be a unique atmospheric transformation product, because 4-NDBP was detected in all of the assayed air particle extracts but not in any combustion particle extracts (Lewtas et al., 1994). 2-NDBP, which is a potent direct-acting mutagen in a microsuspension (MS) assay (Kado et al., 1983; Kado et al., 1986), accounts for the majority of the mutagenic activity observed from gas-phase OH radical-initiated reactions of phenanthrene (Arey et al., 1992).

Although 2- and 4-NDBP have been detected as mutagens in strain TA98 (Arey et al., 1992), 3-NDBP has not been investigated for mutagenicity. Thus, the objective of this study was to characterize the mutagenicity of all three compounds further by evaluating them in a set of different tester strains in the presence and absence of S9. In addition, the compounds were evaluated in the standard plate-incorporation (PI) assay (Maron and Ames, 1983) and in a MS assay first developed by Kado et al. (1983) and modified by DeMarini et al. (1989). A MS assay was used because it requires less sample mass than the PI assay, and the available mass of organics from some air samples may be extremely limited. Thus, knowing the mutagenicity of these single compounds in such an assay is essential when evaluating the contribution of these compounds to the mutagenicity of ambient air.

2. Materials and methods

2.1. Chemicals

2-NDBP and 3-NDBP were purchased from Aldrich Chemical (Milwaukee, WI). The purities of commercially-available 2- and 3-NDBP isomers were more than 99% as judged by reversed-phase HPLC with monitoring of the eluate at 254 nm. 4-NDBP was isolated from the nitration products of 6*H*-dibenzo-[*b,d*]pyran-6-one (DBP) with nitric acid in acetic anhydride. DBP was synthesized from diphenic acid (Aldrich), with hydrogen peroxide and sulfuric acid (Ota and Okazaki, 1970). 4-NDBP was purified by repetitive silica-gel open-column chromatography, eluting with *n*-hexane–ethyl acetate (4 : 1), and normal-phase HPLC (Alltech Silica, 10 mm × 250 mm) using dichloromethane as the mobile phase. 4-NDBP was isolated as white crystal, m.p. 237–238°C. The structure of 4-NDBP was confirmed by mass spectrometry and proton nuclear magnetic resonance (^{1}H-NMR). Mass spectrum (m/z) (relative intensity), VG 70-250 SEQ, direct insertion probe, E 70 eV: 241 (M^+, 99%), 211 (M^+-30, 95%), 139 (M^+-102, 100%). ^{1}H-NMR (ppm, 500 MHz, Brucker AMX-500, acetone-d_6): 8.68, d, 1H, H_1, $J_{1,2} = 8.1$ Hz; 8.50, d, 1H, H_{10}, $J_{9,10} = 8.1$ Hz; 8.37, d, 1H, H_7, $J_{7,8} = 7.9$ Hz; 8.11, d, 1H, H_3, $J_{2,3} = 8.0$ Hz; 8.04, dd, 1H, H_9, $J_{8,9} = 7.4$ Hz, $J_{9,10} = 8.1$ Hz; 7.80, dd, 1H, H_8, $J_{7,8} = 7.9$ Hz, $J_{8,9} = 7.4$ Hz; 7.61, dd, 1H, H_2, $J_{1,2} = 8.1$ Hz, $J_{2,3} = 8.0$ Hz. This NMR data is consistent with that reported by Helmig and Arey (1992), and the mass spectrum (in particular, the abundance of the M^+-30 fragment) is very instrument-dependent. Normal-phase and reversed-phase HPLC analysis with dichloromethane or a methanol–water gradient (60 to 100% methanol over 20 min), respectively, indicated purity greater than 99% as judged by monitoring of the eluent at 254 nm.

2.2. Mutagenicity assays

The PI assay was performed as described by Maron and Ames (1983), and the MS assay as described by DeMarini et al. (1989). In the PI assay, the following strains were used for 2-NDBP and 3-NDBP and, where noted, for 4-NDBP: TA98 (4-NDBP), TA100, TA1535, TA1537, TA1538, TA98NR and TA98/1,8-DNP_6. The MS assay was performed with strains TA98, TA98NR, TA98/1,8-DNP_6, and TA100 (except for 4-NDBP). The genetic markers characteristic of each strain were verified for each master plate. In MS assay, overnight cultures of strains ($\sim 7 \times 10^8$ cells/ml) were concentrated ten-fold by centrifugation and resuspended in 0.015 M sodium phosphate buffer, pH 7.4, instead of 0.15 M sodium phosphate buffer. 50 μl of cell suspension, 50 μl of S9 mix or 0.015 M sodium phosphate buffer, and 2 μl of sample solution were mixed in a 13 × 150-mm test tube and incubated at 37°C for 90 min without shaking. After incubation, 2 ml of molten top agar was added, and the mixture was poured onto a minimal agar plate. Colonies were

counted after 72 h of incubation at 37°C. The chemicals were dissolved in DMSO. 2-Aminoanthracene (0.5 μg/plate for the PI assay; 0.25 μg/plate for the MS assay) and 2-nitrofluorene (3 μg/plate for the PI assay; 0.15 μg/plate for the MS assay) were used as positive controls with and without S9, respectively. All experiments were performed in duplicate or triplicate at least twice. Mutagenic potency was determined by two methods. One method, the lowest effective dose tested (LED) was the lowest tested dose that induced more than 2-fold increase in revertants over background. The second method determined the steepest initial linear slope using the method of Bernstein et al. (1982). The slope (revertants/μg or ng) ± S.D. was determined using the genetox manager system computer software (Claxton et al., 1993).

Exogenous metabolic activation was provided by Sprague–Dawley rat liver S9 induced with Aroclor 1254 (Organon Teknika, Durham, NC). The S9 mix was prepared according to Maron and Ames (1983). The S9 concentration was adjusted to 7% (v/v) in S9 mix (2.5 mg protein per 1 ml of S9 mix).

3. Results and discussion

The structures of the tested NDBP isomers are shown in Fig. 1. Their mutagenicity was evaluated in the PI assay by two ways: the lowest effective dose (LED) and a slope value (revertants/μg). A compound was judged to be positive when it induced a two-fold increase over the spontaneous revertant yield. LED is the lowest dose at which the plate counts exceeded 2 times the spontaneous revertant count. The mutagenic potency of NDBPs was estimated by a slope value. All the NDBPs were mutagenic in all strains with and without S9 mix (Table 1). TA98 was the strain most sensitive to 2-NDBP and 3-NDBP, suggesting that NDBPs induced frameshift mutation. Activity in TA1535, which detects mutagens that cause base-pair substitutions, was weak. Since availability of 4-NDBP was limited, 4-NDBP was tested in TA98 only. The order of mutagenicity of NDBPs in TA98 without S9 mix was 3-NDBP > 2-NDBP > 4-NDBP. The mutagenic potency of 2-NDBP in each strain was remarkably enhanced by the addition of S9 mix in the PI assay.

In order to characterize the mutagenicity of NDBPs further, their activity was tested in strains TA98NR and TA98/1,8-DNP_6, which are derivatives from TA98 deficient in 'classical' nitroreductase and acetyltransferase, respectively (Rosenkranz and Mermelstain, 1983; McCoy et al., 1983) (Table 2). Mutagenic potencies of the NDBPs were markedly reduced in TA98NR compared to TA98, suggesting that NDBPs were activated primarily by the 'classical' nitroreductase in TA98. In TA98/1,8-DNP_6, 2-NDBP showed 47% of the activity observed in TA98 without S9 mix; although, the potency of 3- and 4-NDBP in TA98/1,8-DNP_6 was only 14% and 28% of those in TA98, respectively. These results implied that nitroso and/or hydroxylamino derivatives from NDBPs metabolized by nitroreductase in TA98 were mutagenic and that acetylation was more a significant step in the metabolic activation of 3- and 4-NDBP than in that of 2-NDBP.

Mutagenic potencies of NDBPs in the MS assay are expressed as both slope values and LED values in Table 3 and 4. TA98 was highly sensitive to the mutagenicity of NDBPs in the MS assay, as it was in the PI assay. In the MS assay, however, 2-NDBP was more mutagenic than 3- and 4-NDBP in strains TA98 and TA98/1,8-DNP_6 without S9 mix, and the mutagenicity of 3-NDBP in strains TA98NR and TA98/1,8-DNP_6 was comparable to that in strain

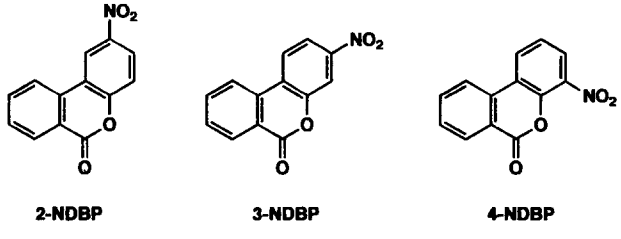

Fig. 1. Structure of 2-, 3- and 4-nitrobenzopyranone (NDBP).

TA98. On the other hand, addition of S9 mix decreased the mutagenic potency of NDBPs in strains TA98 and TA100 in the MS assay, though the activity of 2-NDBP in those strains was significantly increased by S9 mix in the PI assay. The decreased mutagenicity of the NDBPs in the presence of S9 in the MS assay compared to the PI assay could be due to extensive detoxication of putative active metabolites during the 90 min incubation in the MS assay. This difference in the effect of S9 mix between the PI assay and the MS assay was previously reported by Tamakawa et al. (1988), who showed that mutagenicity of airborne particulate extract in TA98 was also enhanced by S9 mix in the PI assay and the activity of extract was reduced by S9 mix in the MS assay at 0.043–6.88 mg protein/plate. The increased activity of the NDBPs in the MS assay is evident from the ratios of the potency in the MS assay to the potency in the PI assay, based on either slope or LED as also shown in Table 4.

Mutagenicity of 2- and 4-NDBP had previously been reported by Arey et al. (1992), using the MS

Table 2
Mutagenicity of NDBPs in strain TA98 and its derivatives in the plate-incorporation assay

Strain	Dose (μg/plate)	2-NDBP		3-NDBP		4-NDBP	
		− S9	+S9	− S9	+S9	− S9	+S9
TA98	0	30 ± 8	37 ± 4	24 ± 5	36 ± 3	29 ± 7	40 ± 5
	0.05	26 ± 2	58 ± 3	–	–	–	–
	0.1	32 ± 2	74 ± 1	46 ± 6	66 ± 14	–	–
	0.25	33 ± 3	117 ± 8	52 ± 6	85 ± 10	–	–
	0.5	40 ± 7	183 ± 15	95 ± 12	140 ± 15	–	–
	1	66 ± 16	295 ± 36	156 ± 17	240 ± 25	34 ± 7	51 ± 5
	2.5	137 ± 3	536 ± 21	431 ± 59	491 ± 26	42 ± 7	78 ± 2
	5	255 ± 3	–	856 ± 85	–	53 ± 1	130 ± 21
	10	482 ± 41	–	1621 ± 60	–	90 ± 21	227 ± 38
	25	–	–	–	–	148 ± 1	401 ± 19
LED [a] (μg/plate)		1	0.1	0.25	0.25	10	5
Slope [b] (rev./μg)		38 ± 4.6	259 ± 33.5	162 ± 17.3	211 ± 31.8	7 ± 0.7	18 ± 3.1
TA98NR	0	20 ± 5	36 ± 4	18 ± 5	36 ± 5	20 ± 5	36 ± 4
	0.1	–	43 ± 4	–	–	–	–
	0.25	–	56 ± 3	–	44 ± 5	–	–
	0.5	–	79 ± 12	24 ± 4	58 ± 9	19 ± 3	38 ± 1
	1	–	127 ± 26	28 ± 6	68 ± 10	22 ± 2	41 ± 1
	2.5	24 ± 4	245 ± 41	43 ± 15	107 ± 49	23 ± 4	47 ± 5
	5	26 ± 6	411 ± 84	59 ± 22	186 ± 83	21 ± 3	64 ± 1
	10	26 ± 4	–	123 ± 29	–	21 ± 2	114 ± 17
	25	27 ± 4	–	–	–	28 ± 3	206 ± 43
	100	43 ± 2	–	–	–	–	–
LED (μg/plate)		100	0.5	2.5	2.5	–	10
Slope (rev./μg)		0.2 ± 0.09	95 ± 14.0	9 ± 5.4	30 ± 16.5	0.5 ± 0.1	7 ± 1.1
TA98/1,8-DNP_6	0	16 ± 4	–	16 ± 4	–	17 ± 5	–
	0.5	24 ± 9	–	26 ± 5	–	–	–
	0.75	29 ± 6	–	36 ± 4	–	–	–
	1	35 ± 6	–	39 ± 4	–	18 ± 2	–
	2.5	68 ± 9	–	70 ± 21	–	22 ± 4	–
	5	132 ± 21	–	145 ± 30	–	27 ± 4	–
	10	255 ± 33	–	258 ± 59	–	37 ± 4	–
	25	–	–	–	–	49 ± 5	–
LED (μg/plate)		1	–	0.75	–	10	–
Slope (rev./μg)		18 ± 6.4	–	23 ± 5.5	–	2 ± 0.1	–

[a] Lowest effective dose (μg/plate) tested that induced more than 2-fold induction over the spontaneous revertants.
[b] Mutagenicity determined by the method of Bernstein et al. (1982) and expressed as a linear slope (revertants/μg) ± S.D.

assay of Kado et al. (1983) in strain TA98 without S9. In this assay, 2- and 4-NDBP gave 243 000 rev./μg (58 600 rev./nmole) and 1990 rev./μg (480 rev./nmole), respectively; in our MS assay, the potency of 2-NDBP was 1/2 lower and that of 4-NDBP was 7× higher (Table 4) than those re-

Table 3
Mutagenicity of NDBPs in the microsuspension assay

Strain	Dose (ng/plate)	2-NDBP		3-NDBP		4-NDBP	
		− S9	+S9	− S9	+S9	− S9	+S9
TA98	0	49 ± 7	44 ± 18	38 ± 5	47 ± 14	26 ± 2	25 ± 1
	1	109 ± 45	52 ± 10	57 ± 16	55 ± 1	–	–
	1.5	–	–	–	–	48 ± 1	28 ± 1
	5	609 ± 80	55 ± 14	176 ± 8	63 ± 1	–	–
	7.5	–	–	–	–	136 ± 5	27 ± 1
	10	1207 ± 28	57 ± 10	320 ± 15	62 ± 6	–	–
	30	–	–	–	–	464 ± 16	29 ± 4
	50	2746 ± 55	117 ± 5	1150 ± 288	112 ± 3	–	–
	75	–	–	–	–	995 ± 6	31 ± 2
	100	2506 ± 597	348 ± 65	1979 ± 44	286 ± 55	–	–
	150	–	–	–	–	–	42 ± 2
	300	–	–	–	–	–	51 ± 2
LED [a] (ng/plates)		1	50	5	50	7.5	300
Slope [b] (rev./ng)		104.3 ± 14.6	2.8 ± 0.39	23.5 ± 4.4	2.1 ± 0.29	15.3 ± 0.46	0.086 ± 0.009
TA100	0	112 ± 2	84 ± 13	112 ± 2	84 ± 13	–	–
	10	205 ± 45	94 ± 13	97 ± 5	78 ± 1	–	–
	50	305 ± 36	166 ± 18	102 ± 9	86 ± 8	–	–
	100	528 ± 148	290 ± 16	129 ± 3	91 ± 2	–	–
	500	–	–	270 ± 13	140 ± 1	–	–
	1000	–	–	533 ± 11	194 ± 1	–	–
LED (ng/plate)		50	100	500	1000	–	–
Slope (rev./ng)		4.3 ± 1.1	1.9 ± 0.042	0.37 ± 0.042	0.11 ± 0.007	–	–
TA98NR	0	26 ± 5	–	26 ± 5	–	18 ± 1	–
	1	29 ± 9	–	48 ± 9	–	–	–
	5	41 ± 4	–	109 ± 32	–	–	–
	7.5	–	–	–	–	27 ± 3	–
	10	57 ± 4	–	320 ± 2	–	–	–
	30	–	–	–	–	41 ± 3	–
	50	228 ± 24	–	1064 ± 112	–	–	–
	75	–	–	–	–	91 ± 6	–
	100	428 ± 46	–	–	–	–	–
LED (ng/plate)		10	–	5	–	30	–
Slope (rev./ng)		3.8 ± 0.54	–	22.8 ± 2.7	–	0.91 ± 0.049	
TA98/1,8-DNP_6	0	13 ± 3	–	16 ± 6	–	32 ± 11	
	0.5	31 ± 1	–	18 ± 1	–	–	–
	1	51 ± 1	–	22 ± 3	–	–	–
	1.5	–	–	–	–	47 ± 19	–
	5	228 ± 6	–	77 ± 36	–	–	–
	7.5	–	–	–	–	89 ± 18	–
	10	440 ± 37	–	232 ± 10	–	–	–
	30	–	–	–	–	304 ± 34	–
	50	2250 ± 8	–	1002 ± 63	–	–	–
	75	-	–	–	–	612 ± 48	–
LED (ng/plate)		0.5	–	5	–	7.5	–
Slope (rev./ng)		49.5 ± 3.7	–	18.4 ± 0.93	–	8.8 ± 0.74	–

[a] Lowest effective dose (ng/plate) tested that induced more than 2-fold induction over the spontaneous revertants.
[b] Mutagenicity determined by the method of Bernstein et al. (1982) and expressed as a linear slope (revertants/ng) ± S.D.

Table 4
Comparison of the mutagenic potencies of NDBPs in the plate-incorporation (PI) and microsuspension (MS) assays

Sample	Strain	S9	MS assay		PI assay		Increase in sensitivity	
		−/+	(rev./μg) Slope	(ng/plate) LED	(rev./μg) Slope	(ng/plate) LED	(MS/PI) Slope	(1 ÷ MS/PI) LED
2-NDBP	TA98	−	104300 ± 14600	1	38 ± 4.6	1000	2744	1000
		+	2800 ± 390	50	259 ± 33.5	100	11	2
	TA100	−	4300 ± 1100	50	38 ± 10.5	2500	113	50
		+	1900 ± 42	100	715 ± 189.1	250	2	2
	TA98NR	−	3800 ± 540	10	0.2 ± 0.09	100000	NC	NC
	TA98/1,8-DNP_6	−	49500 ± 3700	0.5	18 ± 6.4	1000	2750	2000
3-NDBP	TA98	−	23500 ± 4400	5	162 ± 17.3	250	146	50
		+	2100 ± 290	50	211 ± 31.8	250	10	5
	TA100	−	370 ± 42	500	31 ± 3.7	2500	12	5
		+	110 ± 7	1000	36 ± 5.3	2500	3	2
	TA98NR	−	22800 ± 2700	5	9 ± 5.4	2500	2533	500
	TA98/1,8-DNP_6	−	18400 ± 930	5	23 ± 5.5	750	800	150
4-NDBP	TA98	−	15300 ± 460	7.5	7 ± 0.7	10000	2185	1333
		+	86 ± 9	300	18 ± 3.1	5000	4	16
	TA100	−	NT	NT	NT	NT	NT	NT
		+	NT	NT	NT	NT	NT	NT
	TA98NR	−	910 ± 49	30	0.5 ± 0.1	NC	1820	NT
	TA98/1,8-NDP_6	−	8800 ± 740	7.5	2 ± 0.1	10000	4400	1333

NT, not tested; NC, not calculated; NB, not detected.

ported by Arey et al. (1992). The differences obtained using these two different MS assays may be due to the use of different buffers and/or the oxygen content in the reaction mixtures due to shaking during preincubation. Such differences in treatment conditions have previously been shown to affect the mutagenic activity of nitroarenes due to alteration of bacterial aeration (oxygenation) that affect production of 'classical nitroreductase' (Rosenkranz and Mermelstein, 1983). Nonetheless, both assays have proven sensitive and useful in evaluating the mutagenic activity of small masses of organics extracted from airborne particles (Kado et al., 1986; Matsushita et al., 1990), combustion emissions (Agurell and Stensman, 1992), and tobacco smoke (Ling et al., 1987; Lofroth et al., 1988). Like airborne particle extracts (Tamakawa et al., 1988; Agurell and Stensman, 1992), the NDBPs also were more mutagenic in the MS compared to the PI assay in TA98.

Recent molecular studies of revertants of the stan-

Table 5
Comparison of mutagenicity of NDBP isomers in the plate-incorporation assay

Mutation type (Strains)	Ratio of mutagenic potencies									
	+ S9						− S9			
	2-NDBP	3-NDBP	BaP [a]	Air	4-AB [c]	Glu-P-1 [d]	2-NDBP	3-NDBP	1-NP [e]	Air
Frameshift (FS) (TA98/TA1538)	8	11	3	1.6 [b]	2	1	38	20	5	1.5 [b]
Base-substitution (BS) (TA100/TA1535)	40	180	30	∝ [b]	∝	∝	4	310	∝	∝ [b]
BS/FS (TA100/TA98)	3	0.2	2	0.5 [f]–2 [a]	2	0.05	1	0.2	0.1	0.4 [f]–1.5 [b]

∝, denominator of ratio is zero.
[a] DeMarini et al. (1994); [b] Huisingh (1981); [c] Levine et al. (1994b); [d] Levine et al. (1994a); [e] Mermelstein et al. (1981); [f] Claxton et al. (1992).

dard Salmonella strains have begun to identify the types of mutations that can be recovered in these strains. For example, at the frameshift allele (*hisD3052*), the primary (or only) mutation induced in strain TA1538 is a hotspot 2-base deletion (DeMarini et al., 1993). For several chemicals that exhibit the same mutagenic potency in TA1538 and TA98, this same mutation was also the only one induced in TA98 (Levine et al., 1994a). However, for the agents that were more mutagenic in TA98 relative to TA1538, the enhancement of mutagenic potency mediated by the pKM101 plasmid involved the production of an additional class of mutations called complex frameshifts, which are frameshifts with an associated base substitution (Levine et al., 1994b). Table 5 shows the fold increase in mutagenic potencies of 2- and 3-NDBP among the different strains and comparisons to the results for other compounds for which molecular analysis of the revertants have been performed. Molecular analysis of revertants is available only for revertants generated in the PI assays, so only those data are used in the comparisons in Table 5.

At the frameshift allele, the plasmid enhanced the mutagenic potency of both NDBP isomers, suggesting that these isomers induce pKM101-mediated complex frameshifts in TA98 as seen with benzo[*a*]pyrene (BaP), urban air, and 4-aminobiphenyl (AB) (Levine et al., 1994b; DeMarini et al., 1994). At the base-substitution allele (*hisG46*), both isomers were similar to BaP in that the isomers did not require the plasmid to revert this allele, but the addition of the plasmid enhanced the mutagenic potency of the isomers by 400- to 300-fold. In contrast, 4-AB, 2-amino-6-methyldipyrido[1,2-a:3′,2′-*d*]imidazole (Glu-P-1), 1-nitropyrene (NP), and urban air required the plasmid to revert the base-substitution allele.

Similar to BaP and 4-AB, the 2-NDBP isomer exhibited base-substitution activity (TA100) that was greater than or equal to its frameshift activity (TA98), whereas the 3-NDBP isomer, similar to 1-NP and Glu-P-1, exhibited more frameshift than base-substitution activity. Thus, these two isomers in urban air would contribute differently to the base-substitution and frameshift activity of urban air as measured in the PI assay.

This study independently confirms the potent mutagenicity of the NDBP isomers, especially 2-NDBP, in the Salmonella MS mutation assay in the absence of metabolic activation as first reported by Arey et al. (1992). The mutagenic activity of these isomers was dramatically altered by assay conditions (e.g. PI assay as compared to MS assay), activation conditions and alterations in the nitroreductase and acetyltransferase present in these strains. Initial studies in our laboratory show that these NDBP isomers readily form DNA adducts in the presence of xanthine oxidase, consistent with the mutagenicity reported here.

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