Bull. Environ. Contam. Toxicol. (1985) 34:715–721

Bulletin of Environmental Contamination and Toxicology

Studies on Comparative Decomposition Rate by Rat Liver Homogenate and on Micronucleus Test of Nitrated Polycyclic Aromatic Hydrocarbons

Takeshi Ohe

Kyoto City Institute of Public Health, 1-2, Higashi-Takadacho, Mibu, Nakagyoku, Kyoto, 604, Japan

Nitrated polycyclic aromatic hydrocarbons (nitrated PAHs) have been detected in various environmental samples and shown to be responsible for a substantial portion of the observed direct-acting mutagenicity in the Salmonella assay by many researchers (Jäger 1978; Morita et al. 1983; Nachtman et al. 1981; Pitts Jr. 1983; Ramdahl et al. 1982; Rosenkranz 1982; Salmeen et al. 1982; Schuetzle et al. 1982; Tokiwa et al. 1983). Since these potent mutagens constitute a possible hazard to humans (McCoy and Rosenkranz 1982; Rosenkranz and Mermelstein 1983), further informations on the pharmacological and toxicological properties are needed. This paper describes the results on the comparative decomposition rate of some nitrated PAHs by rat liver homogenate and the micronucleus test in mice after administering 1-nitropyrene, 2-nitrofluorene and 1-aminopyrene.

MATERIALS AND METHODS

1-nitropyrene (Tokyo Kasei Co. Ltd.,) was purified by silica gel column chromatography and was checked for purity by FID- and ECD-GC. 2-nitrofluorene, 9-nitroanthracene, 2-nitronaphthalene and 1-aminopyrene were purchased from Aldrich Chemical Industries Ltd.. Pyrene and benzo(a)pyrene were purchased from Gaschro Kogyo Inc.,.

Male six-week old ICR strain mice and male six-week old Wistar rats were obtained commercially. They were caged and allowed free access to water and lab chow under standard conditions of temperature and humidity.

Determination of comparative decomposition rate of nitrated PAHs by rat liver homogenate was carried out according to the method shown in Figure 1. Rat liver homogenate (9000 g homogenate, S9) was prepared from phenobarbital sodium (0.1 % in the drinking water)-, 3-methylcholanthrene (80 mg/kg body weight; i.p.)-and PCB (KC-500, 500 mg/kg body weight; i.p.)-treated male rats as described by Ames et al. (1973).

The micronucleus test in mice was performed as described by Schmid (1975). The mice were treated intraperitoneally with a single dose or five consecutive daily doses of each test chemical.

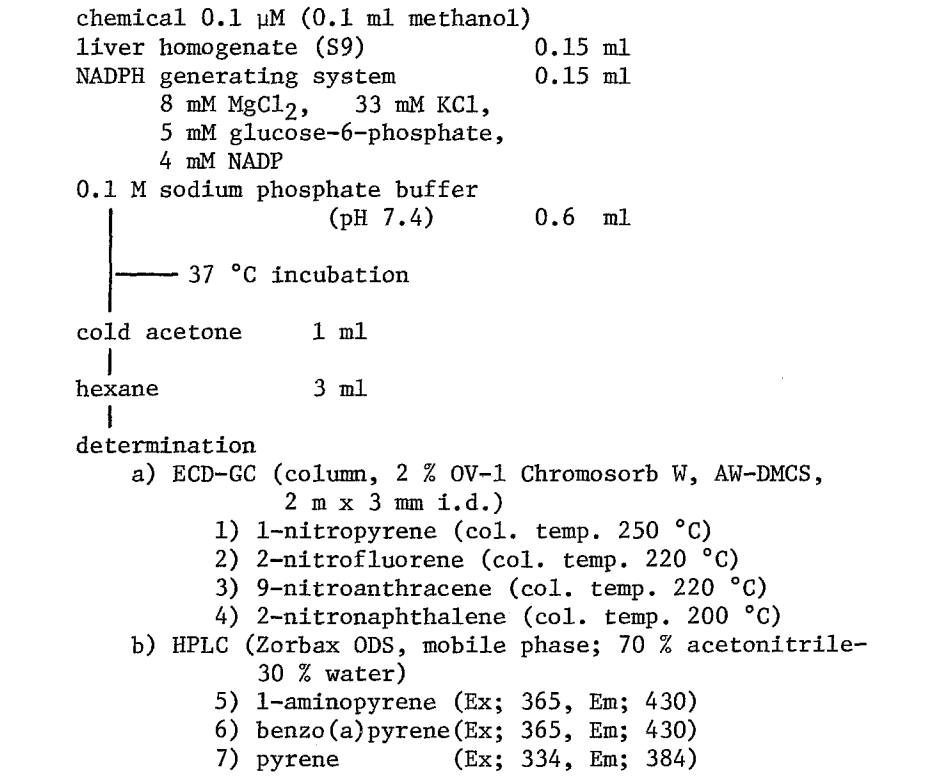
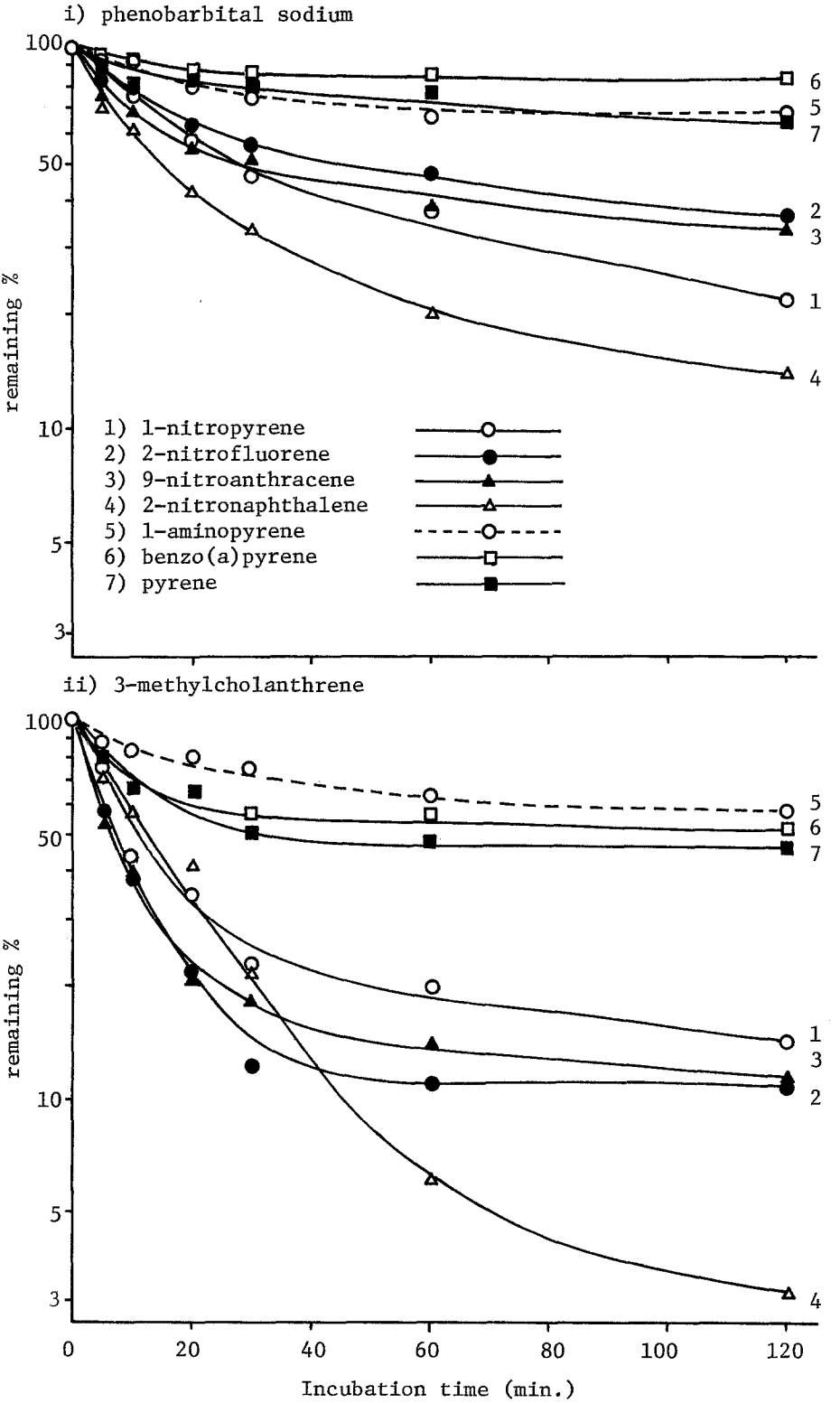


Figure 1. Determination procedure for comparative decomposition rate.

The doses are given in Table 1. Each chemical was dissolved in DMSO to the desired concentration. The solvent control groups received an equal amount of DMSO (0.25 ml/100 g body weight), while positive control group receieved mitomycin C at a dose level of 3 mg/kg body weight. Mice were killed 30 hr after the single injection or 6 hr after the fifth injection. Bone-marrow smears were stained with May-Gruenwald-Giemsa solution and the incidence of micronuclei in 2,000 erythrocytes (polychromatic and normochromatic erythrocytes) per each mouse were scored. Additional smears prepared from mice were stained with new methylene blue and the frequencies of reticulocytes in 1,000 erythrocytes were also examined (Yamamoto and Kikuchi 1981).

RESULTS AND DISCUSSION

Figure 2 shows the comparative decomposion rates of nitrated PAHs by rat liver homogenate treated by 3 different drug metabolizing inducers. The data clearly demonstrates that four kinds of nitrated PAHs were more rapidly decomposed than unsubstituted PAHs such as benzo(a)pyrene and pyrene, indicating that nitrated PAHs are easily metabolized. However, the decomposition rate of 1-aminopyrene, which is a intermediate of 1-nitropyrene, was


i) phenobarbital sodium
100
50
10
5
3
remaining %
1) 1-nitropyrene
2) 2-nitrofluorene
3) 9-nitroanthracene
4) 2-nitronaphthalene
5) 1-aminopyrene
6) benzo(a)pyrene
7) pyrene
ii) 3-methylcholanthrene
0
20
40
60
80
100
120
Incubation time (min.)

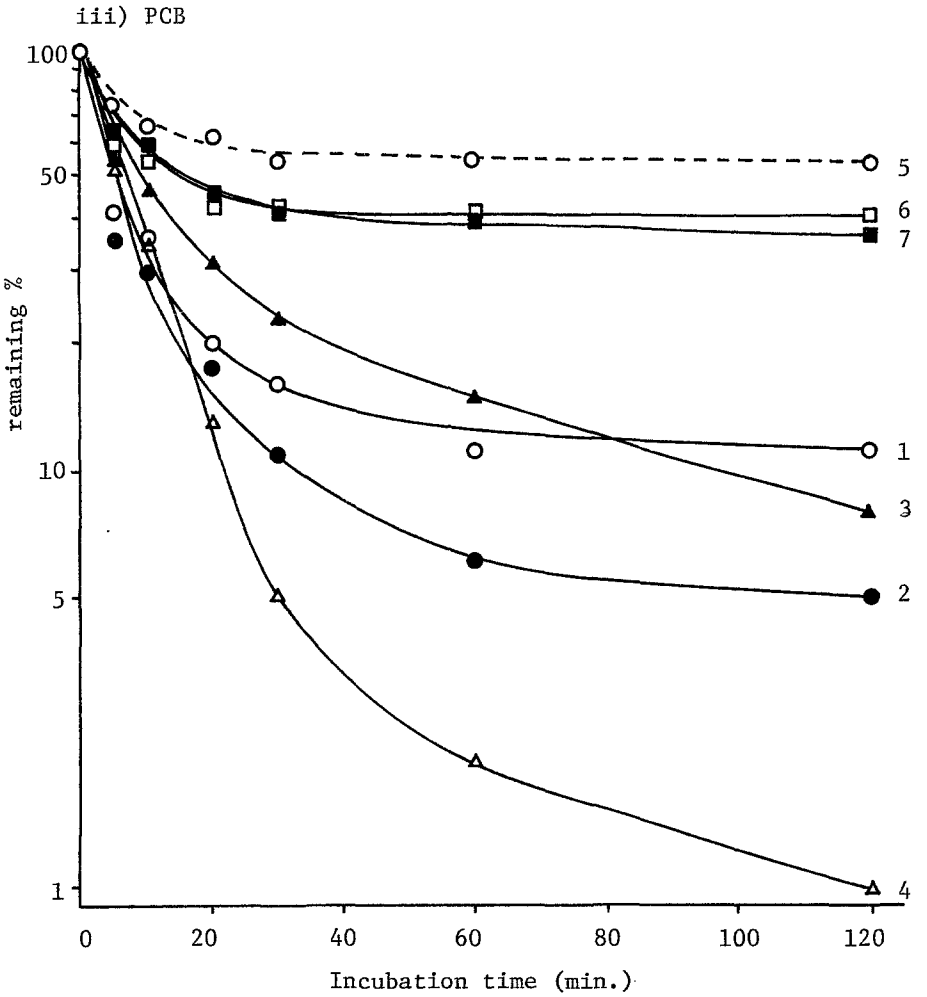


Figure 2. Decomposition rate of nitrated PAHs by rat liver homogenate treated with 3 different inducers.

considerably slow compared with nitrated PAHs.

The result on the micronucleus test in mice is summarized in Table 1. Over the entire dose range tested, 1-nitropyrene, 2-nitrofluorene and 1-aminopyrene did not induced a statistically significant increase in the number of micronucleated erythrocytes in male mice bone-marrow nor had any effect on the frequencies of reticulocytes. 1-nitropyrene and 2-nitrofluorene are highly mutagenic to *Salmonella typhimurium* TA 98 without S9 mix, that is, reduction of the nitro function to corresponding arylhydroxylamine is required of mutagenic potential (Rosenkranz and Mermelstein 1983) and 1-aminopyrene is slightly mutagenic to *Salmonella typhimurium* TA 98 with S9 mix (Tokiwa et al. 1981). In addition, some nitrated PAHs have been detected in diesel-engine exhausts and airborne particulate matters, and the carcinogenic potential

Table 1. Micronucleus test on mouse bone marrow

chemical	individual doses (mg/kg)	no. injection	mortality (dead/tested)	mean of reticulocytes (min./max.) %	erythrocytes with micronuclei	
					no.	%
1-nitropyrene	50	5	3/5	39 (37/41)	5	0.13
	25	5	0/5	38 (23/46)	11	0.11
	?.5	5	0/5	50 (46/53)	5	0.05
		1	0/5	55 (42/60)	12	0.12
	1ɔ	1	0/5	48 (42/52)	6	0.06
	50	1	0/5	54 (40/66)	17	0.17
2-nitrofluorene	50		2/5	40 (39/40)	1	0.02
	25		1/5	36 (33/40)	11	0.14
	12.5	-	0/5	39 (25/49)	4	0.04
	200	1	0/5	40 (35/44)	14	0.14
	100	1	0/5	40 (30/52)	12	0.12
	50	1	0/5	47 (39/53)	10	0.10
1-aminopyrene	50	5	2/5	33 (13/60)	6	0.10
	25	5	0/5	41 (30/45)	13	0.13
	12.5	5	0/5	54 (48/60)	10	0.10
	200	1	3/5	36 (30/41)	7	0.18
	100	1	1/5	24 (12/30)	6	0.08
	50	1	0/5	33 (26/48)	5	0.05
mitomycin C	3	1	0/6	46 (34/52)	326	2.72
DMSO	2.5 ml/kg	5	0/3	50 (49/53)	6	0.10
	2.5 ml/kg	1	0/5	47 (45/49)	20	0.20

of some nitrated PAHs have been demonstrated in animal experiments (El-Bayoumy et al. 1982; Ohgaki et al. 1982; Takemura et al. 1974). Therefore, it is important to evaluate the role of these chemicals as carcinogenic hazards to human population, although they were negative in micronucleus test in mice.

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Received May 21, 1984; accepted July 30, 1984