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Mutagenicity of Organic Pollutants and Their Active Components in the Xi River Water at Shenyang

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Environmental pollution with organic compounds has increased at the city of Shenyang in the Northeast China due to the rapid development of petrochemical industry. Approximately several hundred thousand tons of wastewater were discharged into the Xi River per day. The water was seriously polluted, which affected adversely public health and industrial and aqricultural supplies.

Epidemic disease investigation and animal test data show that some organic compounds have mutagenic effects. This paper presented the research of mutagenicity of concentrated organic pollutants and their active components in the Xi River by the mouse bone marrow micronucleus test.

MATERIALS AND METHODS

Water samples were collected from March 7 to March 17, 1987 at two locations: Ganguan Bridge, a seriously polluted section (hereinafter referred to G), and the Quishou, a natural site away from the industrial area (hereinafter referred to D). Water samples were collected using a Van Dorn water sampler in glass bottles and transferred to the laboratory for analysis normally within 24 hr of collection. Samples were collected six times per day with an interval of 1 hr (see Figure 1) . Bulk samples were deposited and filtered immediately through 0.45 um filter membranes. The part of water samples (S) were then passed through three series of columns of GDX502 resins with the pH adjusted to neutral, acid, and basic conditions. The organic compounds retained by the resins were recovered with CH_2Cl_2 with the adjustment of pH value. The eluates were concentrated under reduced pressure and dried with nitrogen gas, then combined into neutral component (SN), basic component (SB) and acid component (SA). The water neutral component from Ganguan Bridge (GSN) was further passed through a silica gel column and eluted with CH_2Cl_2 (GXN1) and methanol (GSN2) respectively.

The particulate matter remaining on the filter membrane (X) was ground with anhydrous disodium sulfate and extracted with CH_2Cl_2 (Soxhlet extractor). The GX and DX were further eluted with following solvents: (I) n-hexane (the

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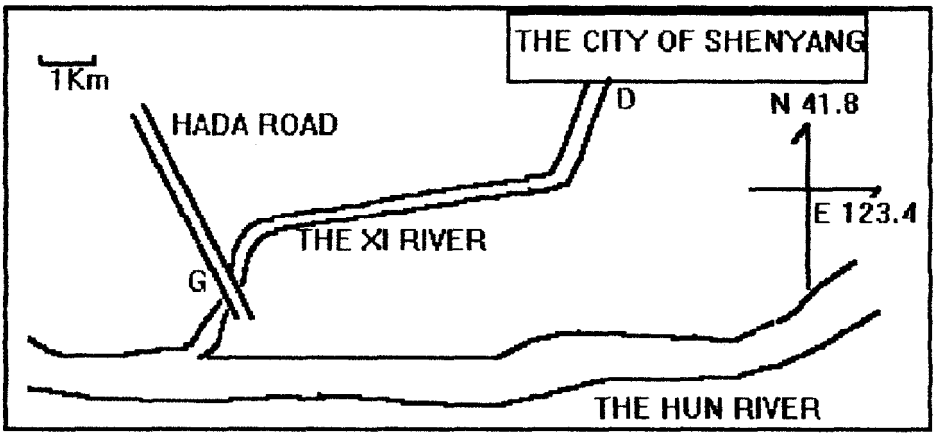

Figure 1. Sampling Sites at the Xi River

eluates were referred to as GX_1 and DX_1, respectively); (II) 10% dichloromethane in n-hexane (referred to as GX_{12} and DX_{12}); (III) dichloromethane (referred to as GX_2 and DX_2) ; and (IV) 5% methanol in dichloromethane (referred to as (GX_{23} and DX_{23}.

Subsamples and blank extracts were obtained for mutagenicity tests and chemical analysis with GC-MS. GC/MS analyses was performed on a Finnigan-MAT 4510 GC/MS equipped with an OV-101 capillary column: and a flame ionization detector. The temperature was kept at 80°C and raised to 280°C at a rate of 4°C/min; injector temperature was 280°C. EPA/NBS library was used to identify the organic pollutants presented in all extracts.

The micronuclei test was performed as proposed by Schmid(1977), and modified according to Kong(1987). China Kunming mice were obtained from the animal colour of the Department of Biology in Nanjing University. All adult mice were male and weighed between 18 and 22 g when tested, 5 animals per group. Tveen-80 was sclected for solvent control and MMC for positive control. The animals were given oral administration; and were killed 30 hr after treatment by cervical dislocation. Five bone marrow preparations per animal were made. Slides were stained with Giemsa and prepared for microscopic analysis. one thousand polychromatic erythrocytes (PCE) per slides were observed. The frequencies of micronucleus polychromatic erythrocytes per 1000 polychromatic erythrocytes (MNPCE‰) and the polychromatic erythrocytes per 100 erythrocytes (PCE%) were estimated. The percentage of nucleated cells is in bone marrow cells were also calculated. The results were statistically evaluated using Poison distribution. The significance was tested at $P<0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, the MNPCE‰, caused by GSN at the dose of 600 mg/kg body weight, is significantly different from the control ($p<0.05$), and the difference was highly significant at the dose of 1200 mg/kg($p<0.01$). The MNPCE‰ caused by SA at the dose of 1000 mg/kg is significantly different from the control ($p<0.05$). However, the MNPCE‰ caused by SB showed no

significant difference compared with the control ($p>0.05$).

Table 1. The effects of the fractionated water components on MNPCE‰ in mice bone marrow

samples	dose (mg/kg)	PCE(%)	nucleated cells(%)	frequencies of MNPCE(‰)
GSN	300	66.20±6.40	58.71±8.04	4.0±1.58*
	600	61.33±8.07	54.66±3.86	5.4±1.67**
	1200	52.26±4.90	54.19±5.33	10.0±4.06**
TWEEN80		59.44±5.40	58.44±6.91	2.6±1.34
GSA	500	55.46±7.39	57.73±7.31	3.6±1.14
	1000	51.46±4.99	57.13±7.73	4.4±1.14*
TWEEN80		59.80±8.43	59.90±9.02	1.8±1.43
GSB	300	59.86±8.15	62.78±4.19	1.67±0.58
	600	55.62±6.04	48.02±4.26*	2.0±1.0
TWEEN80		61.12±2.73	64.88±3.76	1.17±1.52
GSN1	500	59.28±8.31	61.10±11.35	2.8±1.30
	1000	53.80±2.78**	54.55±1.78*	5.2±2.17*
TWEEN80		65.96±3.30	60.34±4.11	2.4±1.34
GSN2	500	60.36±3.30*	56.45±8.78	2.2±1.30
	1000	51.35±3.36**	49.84±1.89**	2.0±1.30
TWEEN80		65.96±3.30	60.34±4.11	2.4±1.34
MMC	3	35.07±4.19**	20.09±5.43**	71.8±8.9**

* = significant difference ($p<0.05$)
** = very significant difference($p<0.01$)

Table 2 shows that the MNPCE‰, cause by suspended components GX_{12} at the dose of 1600 mg/kg body weight is signficantly different, compared with the control ($p<0.05$) . The other components did not increase the MNPCE‰, significantly ($p>0.05$).

Results from this study indicated that, the mutagenic agents mainly existed in the neutral component of water samples and secondarily in the acid component. The basic component caused almost no mutagenic effects, which corresponded to the report of GC-MS analysis (shown in Table 3): there were 116 organic compounds in GSN , 7 of those were the priority pollutants listed by U.S. EPA); there were 32 organic compounds in GSA, 1 of those was the priority pollutant; 17 organic compounds existed in GSB without the priority pollutants.

Table 2. The effects of the fractioned suspended components on MNPCE‰ in mice bone marrow

samples	dose (mg/kg)	PCE(%)	nucleated cells(%)	frequencies of MNPCE(%)
DX1	500	62.84±5.54	59.04±10.57	2.8±1.92
	1000	63.14±5.93	50.30±10.14	1.6±1.14
TWEEN80		62.72±8.12	67.11±6.84	2.6±0.89
GX1	900	57.37±8.58	59.41±7.05	2.0±2.35
	1800	55.36±4.68	48.74±6.71	3.4±1.67
TWEEN80		62.95±5.59	64.47±8.23	2.2±0.84
DX12	750	59.41±9.89	60.60±11.82	3.2±2.17
	1500	47.20±5.63*	47.82±7.28*	3.5±1.73
TWEEN80		63.87±9.61	63.77±2.87	1.8±1.10
GX12	800	51.70±3.36*	58.11±2.96*	2.2±1.30
	1600	50.34±2.49*	51.29±1.55*	4.8±2.95*
TWEEN80		65.17±8.84	68.98±7.41	1.6±1.14
DX2	800	58.72±6.97	63.90±8.32	1.4±1.34
	1600	48.31±3.74*	50.95±2.20*	2.0±0.71
TWEEN80		65.17±8.84	68.98±7.41	1.6±1.14
GX2	800	65.46±7.71	68.82±7.76	2.4±1.14
	1600	49.46±4.42*	52.28±2.94*	2.6±0.89
TWEEN80		65.17±8.84	68.98±7.41	1.6±1.14
DX23	1250	62.51±8.83	62.04±5.20	2.8±1.30
	2500	48.06±9.96*	52.05±2.25*	3.8±2.95
TWEEN80		63.17±3.01	63.96±9.84	4.2±1.64
GX23	1500	52.86±2.33	56.29±3.99*	1.8±0.84
	3000	49.41±6.69	48.89±5.46*	3.6±1.67
TWEEN80		59.17±9.74	68.29±5.74	2.4±1.34
MMC	3	29.64±5.83*	18.30±4.09*	61.0±11.8**

* = significant difference($p<0.05$)
**= very significant difference($p<001$)

Micronucleus test indicated that. the suspended components GX_{12} had mutagenic effects(see Table 4). The GC/MS analysis showed there were 67 organic compounds in GX_{12}, and 5 of those were priority pollutants; there were 52 organic compounds in DX_{12}, and 6 of those were priority pollutant.

Table 3. The priority organic pollutants listed by U.S.EPA in the different fractioned water components analyzed by means of GC-MS

Component	The priority organic pollutants	Result of MN test
GSN	Toluene, Acenaphthene, Chlorobenzene, BHC, Ethylbenzene, Naphthalene, Diethyl phthalate	++
GSA	Dichlorophenol,2,4-	+
GSB	*none*	-
GSN1	Toluene, Chlorobenzene, Ethylbenzene, Naphthalene, Acenaphthene	+
GSN2	BHC, Diethyl phthalate	-

Table 4. The priority organic pollutants listed by U. S .EPA in the different fractioned suspended components analyzed by means of GC-MS

Component	Number of organic compounds	The priority organic pollutants	Result of MN test
DX1	41	*none*	-
DX12	52	Hexachlorocyclohexane-alpha, -beta, -gamma, Pyrene, Anthracene	+
DX2	17	Dibutyl phthalate, Diethyl phthalate	-
DX23	8	*none*	-
GX1	89	Naphthalene, Acenaphthene,	-
GX12	67	Anthracene, BHC, Diethyl phthalate, Dibutyl phthalate, Pyrene	-
GX2	36	Dibutyl phthalate Diethyl phthalate	-
GX23	12	Dibutyl phthalate Diethyl phthalate	-

Micronucleus test, indicated that some fractionated components had not shown mutagenic effects, but. the PCE% and the frequencies of nucleated cells were significantly different from the control. It indicated some nongenotoxic agents could enter into mouse hone marrow and express the toxic effects on cell lethality.

Some authors (Hayashi 1984, Heddle 1977) thought that mutagenic agents could induce chromosome or chromatid break, and the formation of the MN arose from chromosomal fragment. Other authors (Heddle 1977, Hayashi 1984) thought spindle prisons such as colchicine could induce C-mitosis, and the MN arose from the lay chromosome or chromatid without centromere. This kind of MN usually had the larger dimension, so the clastogens and the spindle poisons could be judged by the comparison of the diameter of MN (d) and diameter of cell (D). If $d>1/4D$ then the effect of spindle poisons can be determined. Our study found that the most MN in the test had the small dimension, which indicated that the mutagenic agents in the Xi river had the clastogenic effects.

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