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Comparative Genetic Activity of Samples Collected from Two Different Urban Waste Incinerators

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Incineration of industrial and urban waste materials is an important problem for the environmental contamination and therefore for human health.

Environmental contaminants spread by urban incinerators can contain a complex mixture of toxic compounds such as dioxin, benzofurans, alogenate acids (Olie et al., 1977).

These compounds may interact in a synergistic or antagonistic way with regard to their biological effects.

For this reason it is important to evaluate the genetic damage induced by complex mixtures widespread in the environment. Short-term tests provide important information on the carcinogenic potential of such substances.

In the present work, the genotoxic activity of samples obtained from the urban incinerator of Florence was analyzed. The results were compared with those obtained with samples drawn from the urban Snamprogetti incinerator of Schio (Vicenza), where halogenated acids contained in the smoke are neutralized with lime wash in a salification column.

Samples were tested using prokaryotic (Salmonella typhimurium TA98, TA100 and TA102 strains) and eukaryotic(Saccharomyces cerevisiae, D7 strain) microorganisms. These systems permit one to obtain rapid, reproducible and reliable results in order to evaluate the genotoxic activity of substances present in the environment (McCann et al., 1975).

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MATERIALS AND METHODS

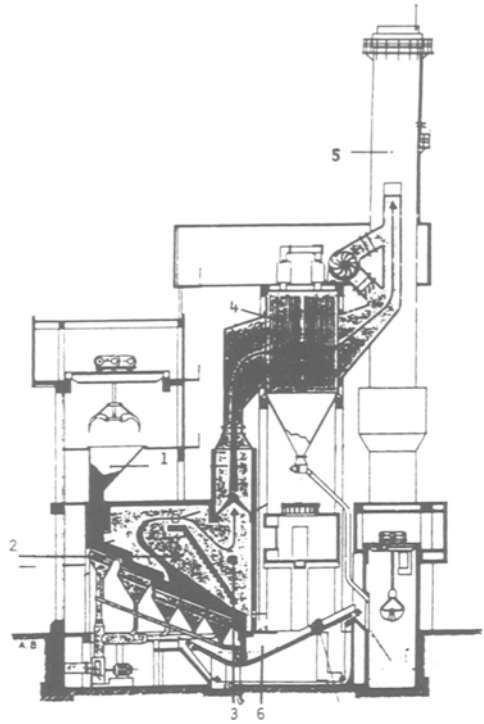
Samples drawn from two urban waste incinerator are: cinders, fly ash, dusts from an electrostatic separator and condensate smokes. Condensate smokes from the Schio incinerator are mixtures of 16 samples drawn upstream and downstream of the salification column (Fig.1 and Fig.2). Solid samples were extracted in Soxhlet apparatus for 24 hours in 100 ml of Toluene. The extract was then evaporated with a rotary evaporator and the residue taken up in 10 ml of dimethylsulfoxide (DMSO) (Commoner et al., 1978). Each extract was tested for genotoxic activity.

Mutagenesis testing was performed using the Salmonella typhimurium TA98, TA100 and TA102 strains, obtained from Dr. B.N.Ames. The samples were tested according to the standard methods described by Ames (Ames et al., 1975). The diploid D7 strain of Saccharomyces cerevisiae obtained from F.K. Zimmermann was used to determine the frequency of mitotic gene conversion at the trp5 locus and point reverse mutation at ilv1 locus (Zimmermann et al., 1975). Since the samples were dissolved in DMSO, all the negative controls were performed in the presence of an equal volume of DMSO.

S9 hepatic fraction for metabolic activation was prepared following the standard procedure as previously described (Bronzetti et al., 1983). Protein concentration of the S9 fraction (32 mg/ml) was determined according to Lowry, as reported by Bailey (1967). S9 mix concentration used in the Salmonella plate test corresponded to 1,6 mg total protein/plate. S9 concentration in yeast test was 8 mg/ml total protein of incubation mixture.
Data obtained were submitted to the Student's't' test with computer assistance.

RESULTS AND DISCUSSION

Table 1 and 2 show the number of S.typhimurium revertants/plate following treatment with condensate smokes, fly ash and dust of electrostatic separator. A significant increase of TA98 revertant number following treatment with fly ash and dust of electrostatic separator extracts in the presence of S9 mix was observed only for the samples collected from the



Fiure 1. Plant of the Urban Waste Incinerator of Florence

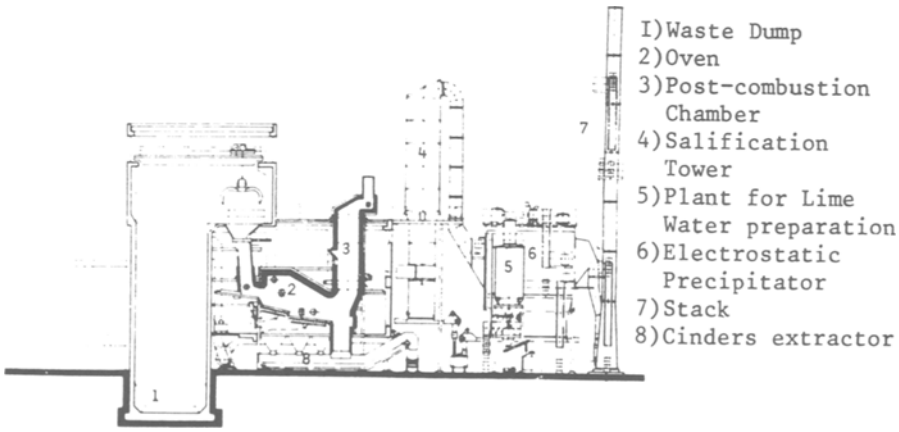


Figure 2. Plant of the Urban Waste Incinerator or Schio (VI

Table 1. Induction of His^+ revertans in TA98, TA100 and TA102 Salmonella typhimurium strains by samples collected from the Florence incinerator. Incubation were performed with and without S9 mix. Values are the mean of three independent experiments ± S.D.

SAMPLES ml/plate	TA98		TA100		TA102	
	-S9	+S9	-S9	+S9	-S9	+S9
Condensate[a] of smokes						
0.05	67.5±4.8	47.2±2.6	127.3±3.2	119.0±6.4	197.4±9.2	170.1±6.7
0.10	32.3±1.7	29.1±6.3	78.5±7.5	143.1±3.1	116.8±7.4	142.3±5.1
0.20	31.0±5.4	37.4±2.8	98.2±5.7	146.7±2.3	204.1±8.1	224.3±8.3
Fly ash[b]						
0.005	TOX	92.5±8.4	165.2±3.4	133.0±14.3	170.4±11.3	146.0±7.4
0.010	TOX	229.9±13.4***	71.5±6.8	115.5±5.7	168.3±9.3	128.9±15.7
0.020	TOX	TOX	TOX	TOX	TOX	TOX
Dust of electrostatic separator[c]						
0.025	41.1±2.7	43.9±3.4	130.1±11.4	133.1±7.7	189.7±7.5	150.0±7.7
0.035	37.0±5.1	140.3±8.7***	147.7±8.7	166.8±10.8	174.3±6.2	122.1±6.9
0.050	TOX	TOX	139.2±6.1	153.4±4.2	193.3±8.4	147.6±7.3
Controls	34.0±6.3	30.4±7.3	121.7±12.3	131.4±6.5	187.9±21.2	212.3±17.8
Positive controls						
BP 1mg/p		197.0±3.4		1022.1±31.7		569.0±19.7
NQO 0.5µg/p	292.5±9.4		3906.0±20.4		609.7±7.1	

a) 1 ml of sample corrisponds to $8.57 \cdot 10^{-3}$ Nm^3 of smokes give out from stack.
b) 1 ml of sample contains 0.1gr of fly ash extract.
c) 1 ml of sample contains 0.3gr of dust extract.
Benzo(a)pyrene (BP); 4-Nitroquinoline 1-Oxide (NQO);
***$p<0.001$ with respect to control.

Table 2. Induction of His^+ revertans in TA98, TA100 and TA102 Salmonella typhimurium strains by samples collected from the Schio incinerator. Incubation were performed with and without S9mix. Values are the mean of three independent experiments ± S.D.

SAMPLES ml/plate	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	TA102 -S9	TA102 +S9
Condensate of smokes [a]						
0.05	29.0±2.3	44.9±4.8	162.7±7.1	144.1±12.3	224.1±12.3	292.1±12.1
0.10	31.5±4.5	47.7±6.3	163.8±10.3	159.7±19.4	260.7±19.1	351.3±28.2
0.20	19.7±3.4	29.9±4.1	102.5±9.2	184.8±17.3	191.4±15.3	324.1±19.4
Fly ash [b]						
0.05	35.7±6.1	56.4±4.5	163.1±15.1	168.2±13.1	283.3±10.4	332.5±19.4
0.10	20.5±3.7	34.5±3.9	185.5±16.0	195.4±14.2	276.7±17.1	350.1±17.7
Dust of electrostatic separator [c]						
0.05	50.7±8.5	46.3±5.2	173.0±13.2	164.5±9.1	261.5±21.3	247.4±7.4
0.10	39.9±6.3	54.1±6.3	147.5±17.8	188.3±15.2	288.5±15.7	253.7±21.4
Controls	34.0±6.3	30.4±7.3	121.7±12.3	131.4±6.5	107.9±21.2	212.3±17.8
Positive controls						
BP 1mg/p		197.0±3.4		1022.1±31.7		569.0±19.7
NQO 0.5μg/p	292.5±9.4		3906.0±20.4		609.7±7.1	

a) 1 ml of sample corrisponds to $8.24 \cdot 10^{-3} Nm^3$
b) 1 ml of sample contains 0.4gr of fly ash extract
c) 1 ml of sample contains 0.3gr of dust extract
Benzo(a)pyrene (BP); 4-Nitroquinoline 1-Oxide (NQO).

incinerator of Florence (Tab.1). None of the samples collected from Schio incinerator induced a significant revertant increase both in the presence and in the absence of S9 mix (Tab.2).

Table 3 shows data obtained in cells of S.cerevisiae, D7 strain, treated with samples collected from Florence incinerator. All the samples induced a weak but significant increase of both gene conversion and point mutation in the presence of S9. Moreover, a significant induction of gene conversion, in the absence of S9, was observed in cells treated with fly ash and dust of electrostatic separator (Tab.3).

Table 3. Induction of mitotic gene conversion and point reverse mutation in D7 strain of Saccharomyces cerevisiae by samples drawn from the incinerator of Florence. Incubation were performed with and without S9 fraction. Concentrations are expressed as ml of sample in 4ml of incubation mixture. Value are the mean of five independent experiments ± S.D.

SAMPLES	Locus $trp5_5$ convertants/10^5surv.		Locus $ilv1_6$ revertants/10^6surv.	
	-S9	+S9	-S9	+S9
Control	0.65±0.21	0.73±0.18	0.20±0.06	0.23±0.01
Condensate of smokes[a]				
1.5	0.73±0.31	1.13±0.44	0.29±0.11	0.45±0.18*
2.0	1.36±0.41	1.03±0.26	0.44±0.09***	0.38±0.14
2.5	1.83±1.24	2.19±0.85**	0.33±0.30	0.87±0.07***
Cinders[b]				
0.1	1.03±0.44	1.57±0.24***	0.29±0.17	0.32±0.01
0.2	1.09±0.76	1.57±0.53***	0.57±0.36	0.87±0.59***
Dust of electrostatic separator[c]				
0.1	1.37±0.53	1.51±0.70	0.35±0.25	0.36±0.08*
0.2	1.46±0.49***	1.72±0.20***	0.62±0.23	0.55±0.28
Fly ash[d]				
0.1	1.25±0.19***	1.60±0.27***	0.25±0.11	0.37±0.09*
0.2	1.92±0.90*	1.52±0.23***	0.41±0.32	0.45±0.06***
Positive controls				
CP 30mM		6.21±0.75		3.22±0.75
MMS 2mM	21.7±5.2		4.27±0.43	

a) 1 ml of sample corrisponds to $8.57 \cdot 10^{-3}$ Nm^3 of smokes give out from stack.
b) 1 ml of sample contains 0.05gr of cinders extract
c) 1 ml of sample contains 0.3gr of dust extract
d) 1 ml of sample contains 0.1gr of fly ash extract.
*p<0.05, **p<0.01, ***p<0.001 with respect to control
Cyclophosphamide (CP); Methyl methanesulfonate (MMS).

Table 4 shows the results obtained using the D7 strain treated with the corrispondent samples drawn from Schio incinerator; no samples induced either gene conversion or point mutation with or without metabolic activation.

The present work demonstrates the presence of genotoxic agents formed during solid urban waste incineration (Fanelli et al., 1986, Farneti et al., 1987). Our results point out a clear difference in the genotoxic activity of the samples drawn from the two urban waste incinerators examined. This can be due either to the different cycles of incineration or to the different composition of the urban wastes to be incinerated. However, the former hypothesis, i.e. the presence of a more complete purification cycle in the Schio incinerator, seems to be the most likely.

Table 4. Induction of mitotic gene conversion and point reverse mutation in D7 strain of Saccharomyces cerevisiae by samples drawn from the Schio Incinerator. Incubation were performed with and without S9 fraction. Concentrations are expressed as ml of sample in 4ml of incubation mixture. Value are the mean of five independent experiments ± S.D.

SAMPLES	Locus trp5 convertants/10^5surv.		Locus ilv1 revertants/10^6surv.	
	-S9	+S9	-S9	+S9
Control	0.88±0.10	0.79±0.12	0.59±0.09	0.45±0.08
Condensate of smokes [a]				
2.0	0.62±0.13	0.70±0.15	0.25±0.06	0.38±0.10
Cinders [b]				
0.2	1.20±0.15	1.05±0.18	0.75±0.12	0.88±0.21
Dust of electrostatic separator [c]				
0.2	1.38±0.41	0.98±0.17	0.86±0.09	0.87±0.11
Fly ash [d]				
0.2	0.86±0.37	1.18±0.25	0.99±0.20	0.76±0.19
Positive controls				
CP 30mM	-	4.45±0.82	-	2.78±0.31
MMS 2mM	23.86±4.9		5.29±0.64	

a) 1ml of sample corrisponds to 8.24×10^{-3} Nm^3
b) 1ml of sample contains 0.04gr of cinders extract
c) 1ml of sample contains 0.3gr of dust extract
d) 1ml of sample contains 0.4gr of fly ash extract.
Cyclophosphamide (CP); Methyl methanesulfonate (MMS).

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