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Mutagenic Effect of Extracts from Particulate Matter Collected with Sediment Traps in the Archipelago of Stockholm and the Open Northern Baltic

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In recent reviews it has been pointed out that the load of various hydrophobic organic compounds (HOCs) on the Baltic Sea aquatic environment is considerable (Jansson et al. 1990; Olsson et al. 1990; Svanberg et al. 1990). The sampling area of this investigation, namely the water area around Stockholm, is of special concern since it is one of the most densely populated urban areas in the Baltic region, inhabited by more than one and a half million people. Further, Stockholm houses several power plants, municipal waste incinerators, waste water treatment plants, ports and oil terminals. The runoff from a large lake also passes through the estuarine-like archipelago of Stockholm on its way towards the open sea.

Due to the high particulate-water partition coefficients (K_p) of most ecotoxicologically relevant HOCs, particulate matter (PM) in water becomes very important for their occurrence and distribution in the aquatic environment. Further, this PM acts as the basic food source for many important organisms in the benthic, pelagic and littoral parts of the aquatic ecosystem.

The load of various HOCs such as petrogenic hydrocarbons (PHCs) (Broman et al. 1987), various polynuclear aromatic compounds (PACs), (Broman et al. 1987; 1988; Näf et al. 1992) and chlorinated hydrocarbons such as polychlorinated biphenyls (PCBs) (Brunström et al. 1992) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) (Broman et al. 1989; Näf et al. 1992) in association with PM in the aquatic environment of the Stockholm area is relatively well documented. Generally, the concentration of various HOCs associated with PM decrease with increasing distance from the city of Stockholm. However, the ecotoxicological relevance of organic extracts of PM, including the above mentioned identified compounds and various unidentified HOCs, is far from fully evaluated.

In an attempt to evaluate the genotoxic potential of extracts of PM, collected with sediment traps in the Stockholm water area and in the open northern Baltic, we used the Ames test on *Salmonella typhimurium* strain TA100, with and without a

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metabolizing system. After extraction and before the mutagenicity tests all PM samples were fractionated on an HPLC-system into three fractions containing aliphatic/monoaromatic-, diaromatic, (containing, e.g., PCDD/Fs and PCBs) and polyaromatic compounds (containing various PACs). The relative mutagenic potential of these fractions at the different sediment trap sampling stations are discussed and evaluated.

MATERIALS AND METHODS

Samples of particulate matter (PM) from four sediment trap stations have been used in the study, namely upstream from the city of Stockholm in Lake Mälaren, downstream from the city of Stockholm in the inner and middle parts of the estuarine-like archipelago, and the open northern Baltic. A detailed description of the investigation area is given elsewhere (Broman et al. 1988; 1989). In summary, the relatively shallow Lake Mälaren is one of Sweden's largest fresh water lakes with a total surface area of 1140 km^2. The archipelago of Stockholm is a shallow and wide, estuarine-like non-tidal area comprised by several thousand islands, sounds and bays.

The sediment trap station that is located in the inner part of the Stockholm archipelago is subjected to a high degree of runoff from hard surfaces of the city in addition to the direct deposition of airborne HOCs (Broman et al. 1988). At the other stations in the archipelago the importance of the direct deposition of airborne HOCs increases in importance with increasing distance from the city (Broman et al. 1991). The station in the open northern Baltic is primarily subjected to direct deposition of airborne HOCs but likely also to some HOCs transported with the south-going surface stream that passes the relatively heavy industrialized Swedish coast of the Bothnian Sea, on its way to the Baltic proper. The sediment trap station in Lake Mälaren upstream from the city of Stockholm is also primarily influenced by the deposition of airborne HOCs but also to some extent by HOCs in runoff from a few small cities but, probably more important, the agricultural district that dominates its drainage area.

The sediment trap technique that has been used in the study has been described previously by Broman et al. (1988). Briefly, the sediment traps contain three gimbal mounted glass collection cylinders with an aspect ratio of five and a surface area for collection of PM of 78.5 cm^2. All sediment traps were placed at a depth of about 15 m from the surface and 15 m above the bottom. The PM was collected from mid September 1987 to the end of May 1988 except for the sediment trap in the open northern Baltic in which the material was collected between May and July 1988. One sediment trap was deployed at each of the four sampling stations, and the PM collected in one of the three cylinders of each trap was used in this study. Chloroform was used as a preservative in all collection cylinders during the collection periods.

All PM samples were Soxhlet extracted for 24 hr with toluene, cleaned-up on an open silica column, and fractionated on an HPLC-system with an amino column that separates according to the number of aromatic rings into three fractions, aliphatics/monoaromatics, diaromatics and polyaromatics, according to a method described by Zebühr et al. (1989).

The three HPLC-fractions from the four sampling sites were carefully evaporated to nearly dryness under nitrogen and then dissolved in dimethyl sulfoxide (DMSO) and tested on *Salmonella typhimurium* strain TA100 according to Maron and Ames (1983). Each fraction was tested in three different concentrations corresponding to 50, 100, and 200 mg dry weight, respectively, using three plates per concentration. The only modification done was that biotin and histidine were added to the minimal medium instead of to the soft agar. The results are given as the number of revertants per gram dry PM initially extracted, taken from the linear regression analysis of the dose response curve. The statistical significance of the slope was examined by Student's *t*-test with the following significance levels: * $0.01<P<0.05$; ** $0.001<P<0.01$; *** $P<0.001$.

RESULTS AND DISCUSSION

The aliphatic/monoaromatic, diaromatic and polyaromatic fractions of the extracts from the PM collected with sediment traps upstream from the city of Stockholm in the Lake Mälaren, downstream from the city of Stockholm in the inner and middle parts of the archipelago, and in the open northern Baltic were tested for mutagenicity in *Salmonella typhimurium* both in the presence (+S9) and in the absence (-S9) of a metabolizing system.

The results (Table 1) showed that the polyaromatic fraction (containing PAHs among various other PACs) displayed the highest mutagenicity for all stations. For all samples, except the open Baltic sample, this high effect was seen in the presence of a metabolizing system. The sediment sample from Lake Mälaren gave 344 revertants per gram extracted material while the samples from the inner and middle parts of the archipelago induced 4 and 5 times higher mutagenicity, resulting in 1410 and 1720 rev/g, respectively. However, this may not be correlated with parent PAHs and it is likely that other polyaromatic compounds, e.g., alkylated, halogenated and other differently substituted PAHs, also contribute to the effects. This is further supported by the fact that the effect of the polyaromatic fraction of the sample from the open Baltic showed the highest mutagenic effect, in the absence of the metabolizing system. This station also showed the highest effect for the aliphatic/monoaromatic fraction in the absence of S9. Moreover, the results from this station revealed the highest effect for the diaromatic fraction both with and without the metabolising system. The mutagenicity profile of the sample from this station is thus clearly different from the other sampling sites. A possible explanation is that the sources of HOCs

Table 1. The mutagenicity in *Salmonella typhimurium* TA100 ± S9 of the aliphatic/monoaromatic (I), diaromatic (II) and polyaromatic (III) fractions of extracts from particulate matter collected with sediment traps at different locations in the Stockholm archipelago and in the Baltic Sea, expressed as number of revertants/g dry weight PM±S.E.

Sampling Station	Fraction Type	TA 100-S9	TA 100+S9
Lake Mälaren	I	92.2 ± 32.2*	31.9 ± 23.7
	II	42.7 ± 40.5	68.8 ± 25.3*
	III	53.5 ± 31.0	343.9 ± 51.5***
Inner Archipelago	I	228.9 ± 126.1	283.0 ± 66.9**
	II	213.3 ± 63.1*	262.1 ± 63.5**
	III	-36.6 ± 34.3	1412 ± 315.3**
Mid Archipelago	I	92.2 ± 48.9	82.4 ± 29.8
	II	249.0 ± 64.3**	164.7 ± 60.0*
	III	193.1 ± 81.5*	1720 ± 50.5***
Baltic Sea	I	580.0 ± 212.3*	160.0 ± 221.4
	II	393.3 ± 280.0	380.0 ± 96.6**
	III	880.0 ± 154.8***	46.7 ± 285.8

influencing the open Baltic might be different from those influencing the other stations located much closer to Stockholm. The reason for this can be the differences in distances to Stockholm but also that the station in the open Baltic might be influenced by the surface current from the north, as mentioned earlier (in Materials and Methods), which can contain contaminants from other sources along the Swedish coast of the Bothnian Sea.

In a recent study by Brunström et al. (1992), PM was collected during one year at some of the same stations (inner-, middle- and outer archipelago) as described in this study and the HPLC fractionated extracts were tested for embryolethality, immunotoxicity and EROD-inducing potency. Generally the toxic potency of the tested extracts decreased with increasing distance from the city of Stockholm, i.e., highest potency was found for the inner archipelago extract and the lowest for the outer archipelago extract. Further, the polyaromatic fraction from the inner archipelago extract was the most potent of all fractions tested. In this study the results are not as clearly geographically related as in the study by Brunström et al. (op cit.) which to some extent probably can be explained by the differences in the tests used in the two studies. In both studies, however, it was a polyaromatic fraction that showed the highest potency. In the study by Brunström et al. (op cit.) the contents of some selected HOCs in the PM samples were determined in order to evaluate the contribution of these substances to the

measured potencies of the same samples. The result showed that the contribution of, for example, the seventeen 2,3,7,8-substituted PCDD/Fs and the three non-ortho substituted PCBs (most potent congeners among each group) could only explain a few percent of the measured potency of the diaromatic fraction. Likewise could the 15 polycyclic aromatic hydrocarbons (PAHs) analyzed only explain a very small portion of the potency of the polyaromatic fractions that were tested. Apart from the PM samples analyzed in the study by Brunström et al. (op cit.), which to some extent can be compared with the samples in the present study, it can be mentioned that the concentrations of PCDD/Fs have been determined in the same samples as the present study (parallel collection cylinders of the same sediment traps) (Broman et al. 1989). Further, the content of some chloro-substituted PAHs has recently been determined on sub samples of the PM sample from the inner station of the present study (Ishaq et al. 1993). Finally, the concentrations of about fifteen PAHs in PM collected during different time periods along the transect from the inner archipelago of Stockholm out towards the open sea, have been determined earlier (Broman et al. 1988; Näf et al. 1992) as well as the concentrations of more than 150 PAHs and about 20 sulphur heterocyclic aromatic compounds (S-PACs) in a PM sample from the same inner station as in the present study (Broman et al. 1987).

To fully evaluate the results of the present study in terms of what substances give rise to the genotoxic effects far more compounds or classes of potent compounds than those HOCs mentioned above have to be qualitatively identified and quantitatively determined. Still, having done this time-consuming and complex analytical task the question of how much the identified compounds can explain of the measured genotoxic effects compared with the effects of the unidentified compounds still remains to be solved.

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