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A comparative analysis of mutagenic activities of air samples collected from Riyadh before, during and after the Gulf War

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Samples of air particulates were collected from the city of Riyadh in 1988 before the Gulf War, a few months after the torching of oil wells in Kuwait during 1991 and several months (in 1992) after the burning oil wells were fully capped. The air samples were collected on fibre glass filters (GF-A, pore size 1.6 μm) by using a RADECO air sampler. Filters were shredded, soaked and sonicated in acetone to dissolve the organic contents. The solid residues obtained after evaporation of acetone were redissolved in 5 ml volumes of fresh acetone and tested for mutagenic activity in the Ames Salmonella assay using the tester strain TA98 for histidine reversion (Ames 1979, Maron and Ames 1983). The mutagenicity assay was performed with and without metabolic activation provided by the S-9 fraction of an Aroclor-induced rat liver homogenate and co-factors. The results of this study showed that the pre-war samples of air particulates from Riyadh induced 11 histidine revertants/10 m^3 of air analyzed, while the 1991 samples (during war) induced 40–50 revertants/10 m^3 of air (4–5 times higher). However, the number of histidine revertants induced by the post-war (1992) samples came down to 10–11/10 m^3 of air. These data, thus, strongly suggested that there was an increase in air particulate mutagenicity due to the atmospheric pollution of Gulf areas caused by the burning oil wells in Kuwait. This notion is supported by the fact that the mutagenic activity of air samples collected months after the burning oil wells were capped assumed the pre-war level. In view of the strong correlation between mutagenicity and carcinogenicity (McCann and Ames 1976) it would be expected that air pollution with mutagenic agents enhanced by the Gulf War would increase the carcinogenic risk for upper aerodigestive tracts in the exposed population in Kuwait and the surrounding areas. Such adverse health effects of mutagenic air pollutants generated by the burning oil wells in Kuwait should be assessed by proper epidemiologic studies.

Keywords: Mutagenicity; air pollution; Gulf War; Salmonella assay; Kuwait; carcinogenicity; oil burning; Saudi air.

Introduction

Air pollution has unquestionable adverse effects on human health. Air-borne toxic agents are produced by industrial and automobile emissions as well as by burning of various organic substances (Suess *et al*, 1985). As the potential carcinogenic effects of air pollutants are of major concern, attempts have been made to determine and monitor genotoxic properties of air particulates in various cities of the industrialized countries (Sutou *et al*., 1980, Athanasiou *et al*,

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1986, 1987, Crebelli *et al*, 1988, Hoyer *et al*, 1992, Nardini and Clonfero 1992, Nardini *et al*, 1994). These studies have demonstrated the usefulness of Ames' histidine reversion assay in suitable tester strains of *Salmonella typhimurium* for screening organic extracts of air samples showing mutagenic activity. There is a paucity of data on air particulate mutagenicity in Gulf countries like Saudi Arabia where a rapid industrialization, increasing use of automobiles and changing lifestyle may significantly alter the environmental quality. A sudden deterioration of environmental quality in this region was believed to have resulted from the recent Gulf War during which a large number of oil wells in Kuwait were set on fire. Heavy smokes containing various unknown materials travelled over the sky of Saudi Arabia including the city of Riyadh for several months until the burning oil wells were fully capped. Although a number of studies addressed the problems related to environmental impact of Kuwaiti oil well fires in a qualitative sense, few studies were carried out to obtain quantitative data on the nature of pollutants and their probable toxicological effects (El-Desouki and Abdulraheem 1991, Harrison 1991, Al-Saleh and Hannan 1993, Kelsey *et al*., 1994) but there was none to compare the level of war-time pollution with that of the pre- and post-war periods so as to assess accurately the environmental impact of the oil fires particularly for long-term health effects. As we had initiated a study on the mutagenicity of air particulates in different cities of Saudi Arabia before the Gulf War, we collected and analyzed air samples from the city of Riyadh when the oil wells in Kuwait were in flame and several months after the wells were capped, and compared the war-time air mutagenicity data with those of the pre- and post-war periods. The results presented here showed a 4–5-fold increase in mutagenic activity of air particulates collected from Riyadh in 1991, which could be attributed to the pollution generated by the burning oil wells in Kuwait.

Material and methods

Sample collection

Air samples were collected on glass fiber filters type A (Whatman, GF-A) with a pore size of 1.6 µm. The sampling was carried out for 24 h continuously with a flow rate of 2 ft^3 of air per min. Four filters, each representing a 24-h collection, were shredded, soaked and sonicated in acetone. The mixture was filtered using 0.2 µm organic millipore filters. The acetone of the filtrate was evaporated at 45°C under a stream of nitrogen gas. The solid residue thus obtained was redissolved in 5 ml of fresh acetone (test material) and tested for mutagenicity in the Ames' Salmonella assay.

Ames assay

The mutagenicity assay of the 'test material' was performed using the Ames *Salmonella* tester strain TA98 with and without metabolic activation, following the plate incorporation method described by Maron and Ames (1983). Briefly, 0.1 ml of an overnight bacterial culture was added to 2 ml of soft agar containing 10% of 0.5 mM histidine/biotin, in each of 12 × 75 mm sterile capped culture tubes held at 45°C in a heating block. Different concentrations of the 'test material' were added to these tubes. For metabolic activation, an S-9 mix consisting of Aroclor-induced rat liver homogenate (Litton Bionetics, USA) combined with nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate and inorganic salts in 0.2 M sodium phosphate buffer (pH 7.4) was added at a concentration of 0.5 ml/tube. Bacterial cells and the 'test material' added to soft agar (2 ml aliquotes) with and without S-9 mix were vortexed and quickly poured onto minimal glucose agar plates. The assay was carried out in triplicate. The plates were incubated at 37°C for 72 h and the number of histidine revertants per plate were then

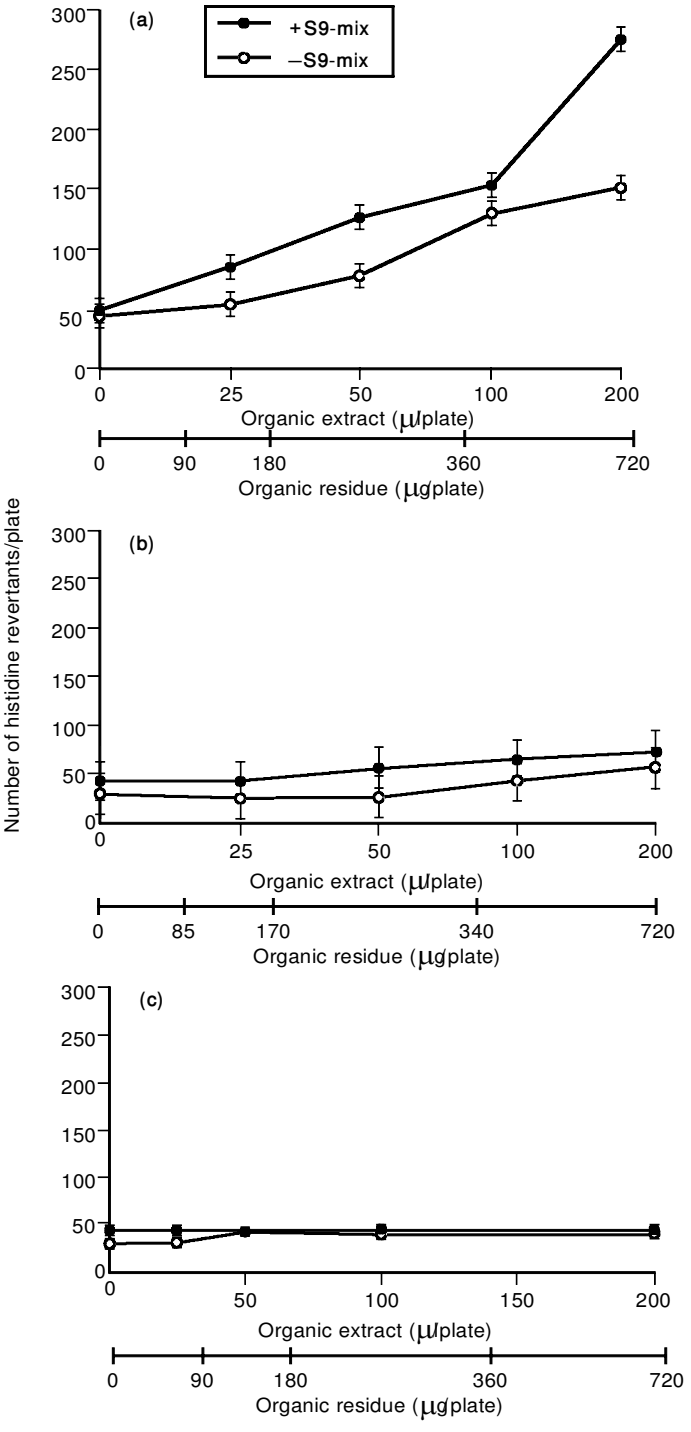

Fig. 1. Dose-response curves for histidine revertants/plate resulting from different concentrations of an organic extract of air sample obtained from: (a) Ras Tanurah; (b) Al-Qaseem; (c) Riyadh.

counted. Dose-response curves were drawn based on the number of histidine revertants/plate observed at different concentrations of the 'test material' used in μg/plate. Using back calculations, the amounts of air (in m^3) representing different concentrations of the 'test material' were estimated and the number of revertants/m^3 of air determined. Appropriate concentrations of acetone (solvent) alone were mixed with bacterial cells in soft agar with and without S-9 mix to serve as controls.

Results

Organic residues of air samples collected from several cities of Saudi Arabia before the Gulf War were tested for mutagenicity using the tester strain TA98. Both direct and indirect mutagens (detected respectively without and with metabolic activation) were found in some cities included in these studies. Mutagenicity of air samples varied depending on the environmental conditions of the sites investigated. There was a general indication that air samples from areas near heavy industry, oil refineries, automobile traffic and construction sites were the most mutagenic. The dose-response curves obtained for histidine revertants/plate with different concentrations of organic extracts of air samples collected, before the Gulf War, from Ras Tanurah, Riyadh and Al-Qassem are shown in Fig. 1 (a–c). It can be seen that the number of histidine revertants/plate

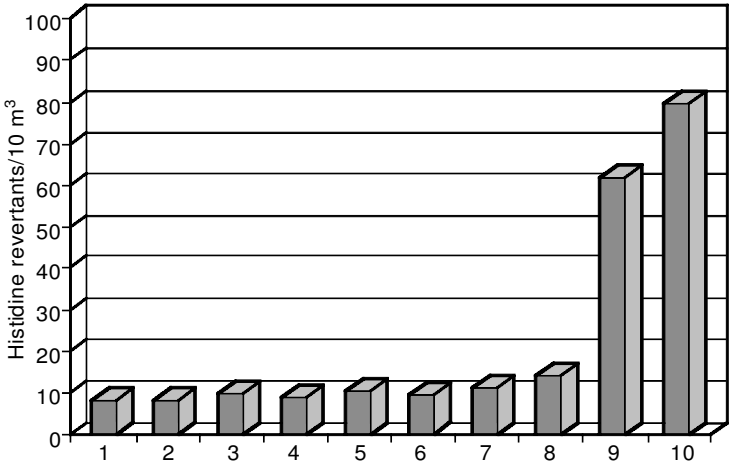

Fig. 2. Rank ordering of the investigated areas according to the mutagenicity of their air samples (revertants/m^3 of air) without S9-mix (nos 1–21 represent the areas as follows):

1. Dharan (Dharan/Dammam Highway).
2. Dharan (Aaramco Compound).
3. Al-Salm Ávenue (Riyadh).
4. KFSH&RC roof (Riyadh).
5. Al-Kharj City.
6. Al-Mulaida farmland (Qassem).
7. Batha Street (Riyadh).
8. KAC roof (Riyadh).
9. Ras Tanurah (residential area).
10. Ras Tanurah (oil refinery).

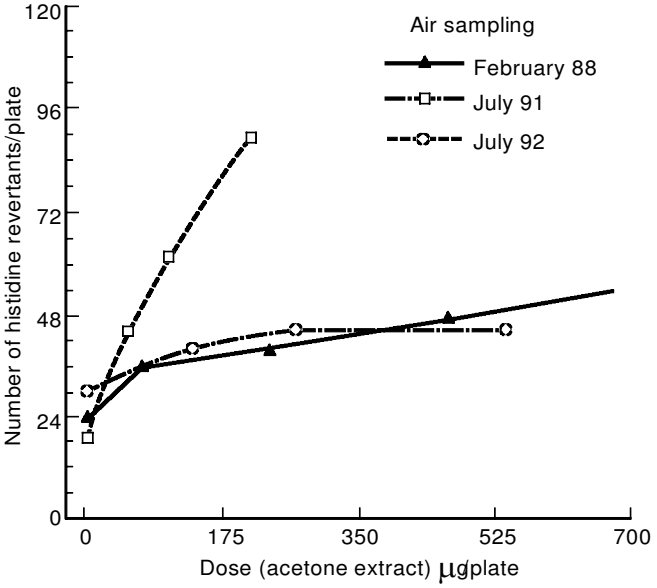

Fig. 3. Dose-response curves for histidine revertants/plate induced by organic extracts of air samples collected from Riyadh before (1988) during (1991) and after (1992) the Gulf War (without S9-mix).

induced by different concentrations of the air samples is low in Riyadh and Al-Qassem which have few industrial sites; whereas Ras Tanurah – a site of oil refineries – has a high number of histidine revertants/plate. In fact, a comparison of air mutagenicity data based on the number of histidine revertants induced per m^3 of air showed that air samples from Ras Tanurah produced the highest mutagenic effects, observed in this study, representing a 7–8-fold increase in air particulate mutagenicity relative to those obtained with air samples from Dhahran, Al Salam Avenue in Riyadh, Al-Qassem, Al Mulaida farms, and Al Kharj areas (Fig. 2). The areas such as Al-Kharj showing the lowest air particulate mutagenicity represent the sites of the least industrial development, little construction activities and low automobile traffic on the roads. Air samples collected from the city of Riyadh before the Gulf War showed an average of only 11 histidine revertants/$10m^3$ of air analyzed. A comparison of the dose-response curves for histidine revertants/plate obtained with different concentrations (in µg) of organic residues of air samples collected before, during and after the Gulf War is illustrated in Fig. 3, clearly showing an increased mutagenic activity of the war-time samples. This is further indicated by the fact that the samples collected from the city of Riyadh during oil well burning in Kuwait at the time of Gulf War produced 40–50 revertants/$10m^3$ of air representing more than a 4-fold increase in mutagenic activity compared to the pre-war time. The increased mutagenic activity of war-time air samples reflected a sudden and marked deterioration of the environmental quality. However, a study conducted 2 months after the oil wells were capped showed a decrease in air mutagenicity in Riyadh indicating that the mutagenic activity of post-war samples assumed the pre-war level. The increase in air particulate mutagenicity in Riyadh during the Gulf War could, almost certainly, be attributed to the airborne particles and smoke arising from the torching of oil wells in Kuwait.

Discussion

These studies on air particulate mutagenicity not only enabled the determination of the levels of ambient air pollution by potentially carcinogenic agents in different cities of Saudi Arabia, but also helped in comparing the data obtained from air samples collected in the city of Riyadh before, during and after the Gulf War in order to assist in calculating the environmental impact of oil well fires in Kuwait. The results of mutagenicity testing of air samples collected before the war indicated that, in general, the level of air pollution by potentially carcinogenic agents was relatively low in Saudi Arabian cities compared to the data obtained with urban air of major industrial countries (Tokiwa *et al.* 1977, Athanasiou *et al.* 1987). However, there was a clear indication that the histidine revertants/m^3 of air varied considerably with air samples from different areas of Saudi Arabia depending on the presence or absence of industrial sites, oil refineries, automobile traffic and construction activities. The air samples from areas with relatively less industrial development and automobile movements produced the least increase in histidine revertants/m^3 of air while those from Ras Tanurah, a site of old petrochemical refineries, showed the highest number of histidine revertants/m^3 of air. These data are generally in agreement with the notion that industrial and automobile emissions are the major contributing factors for urban air pollution (Crebelli *et al.* 1991, Scarpato *et al.* 1993, Nardini *et al.* 1994).

The city of Riyadh, having no major industrial activities, showed a low air particulate mutagenicity compared to Ras Tanurah. However, during the Gulf War, the city was affected by the black soot and dark cloud travelling from Kuwait while the oil wells were on fire. This atmospheric smoke disappeared after the oil wells were fully capped. The dose-response curves for histidine revertants/plate as well as the number of histidine revertants/m^3 of air samples from Riyadh analyzed during the time of Kuwait oil well fires showed a notable increase in air particulate mutagenicity compared to the data obtained with pre-war and post-war air samples. These data are clearly indicative of the deterioration of air quality resulting from pollution, particularly by potentially carcinogenic agents released by the burning oil wells in Kuwait.

Theoretical predictions made about possible climatological disasters (Hoffman 1991, Horgan 1991, Pain 1991) instigated the commencement of several studies to determine the overall impact of environmental pollution caused by the Kuwaiti oil well fires (Ayres 1991, El-Desouky and and Abdulraheem 1991, Harrison 1991, Al-Saleh and Hannan 1993, Kelsey *et al.* 1994). By comparison, studies addressing the toxicological effects, particularly the genotoxic effects of the war time air samples that would be relevant to the assessment of long-term health effects, were very few. Kelsey *et al.* (1994) reported that the genotoxic effects (induction of sister chromatic exchange in human blood lymphocytes and mutation at the hprt locus in a human lymphoblast cell line AHH-1) of soot from the 1991 Kuwaiti oil fires were similar in magnitude to those found by testing a standard air particulate sample from the Washington DC area. Such studies, unfortunately, do not fully reflect the environmental impact of the oil burning accurately as there is no comparison of the genotoxicity of air samples collected from the same area before the war or after the burning oil wells were capped. We believe that our data will be more useful in ascertaining long-term health effects of 1991 air pollution caused by the Gulf War as they clearly demonstrate that increased exposure to genotoxic air particulates did occur during the time of oil well fires. However, the air quality assumed a pre-war level after the oil wells were capped, indicating that the human inhalation of increased air particulate-borne genotoxic agents occurred only

during the oil fires, although exposure to the pollutants that settled on the ground or entered the food chain and water cycle may continue to occur over a longer period. In view of the correlation between genotoxicity and carcinogenicity (McCann and Ames 1976) it is likely that an increased exposure to genotoxic agents would increase the risk of cancer and perhaps other health problems in the exposed population. Only appropriate epidemiological studies will prove or disprove this hypothesis in the future.

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