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DNA Damage Caused By Pesticide-contaminated Soil

K. KRISHNAMURTHI[1], S. SARAVANA DEVI, AND T. CHAKRABARTI

Environmental Biotechnology Division, National Environmental Engineering Research Institute (NEERI), Nehru Marg, Nagpur-440020, India

Objective To determine the DNA damaging potential and the genotoxicity of individual compounds in pesticide contaminated soil. **Methods** In the present study, DNA damaging potential of pesticide-contaminated soil and the genotoxicity of individual compounds present in the soil were assessed using fluorimetric analysis of DNA unwinding assay. **Results** The contaminated soil sample showed 79% (P<0.001) of DNA strand break, whereas technical grade of major carbaryl and α-naphthol constituents of the contaminated soil showed 64% (P<0.01) and 60% (P<0.02) damage respectively. **Conclusion** Our results indicate that the toxicity caused by contaminated soil is mainly due to carbaryl and α -napthol, which are the major constituents of the soil sample analyzed by GC-MS.

Key words: Carbaryl; Contaminated soil; DNA damage; FADU- Fluorimetric analysis of DNA unwinding assay; Genotoxicity; α-Napthol and pesticide

INTRODUCTION

Synthetic organic pesticides have been widely used for more than four decades and during this period, their use has contributed greatly to increased worldwide food production. However, pesticides are also known to cause considerable negative effects on non-target organisms[1]. Some pesticides are found to be highly persistent in nature, thereby causing contamination of soil, ground and surface water[2-3]. Such contamination with low level of pesticides has resulted in serious environmental concern and some of the pesticides, though not showing an immediate effect *in vivo*, may pose long term health hazard to human beings. Their toxic effects are manifested in different ways such as bioaccumulation, bio-magnification, chronic toxicity, acute immune response, allergic reaction, and mutagenic, teratogenic and carcinogenic effects.

DNA is one of the most critical cellular targets for hazardous chemicals and wastes which may get damaged by alteration of bases or disruption of the sugar phosphate backbone[4-5]. Though low levels of base damages are difficult to be measured by physical or chemical means, DNA strand breaks can be detected with a great sensitivity by methods utilizing the observation that the rate of unwinding of the DNA strands in alkali is related to the covalent length of the strands[6-7] and as little as one break per chromosome can give a detectable increase in the rate of unwinding. Morgan and Pulleyblank[8] have reported that the fluorescent dye, ethidium bromide, could bind selectively to double stranded DNA in the presence of single stranded DNA when short duplex regions in single stranded DNA are destabilized by alkali.

Fluorimetric analysis of DNA unwinding (FADU) is one of the sensitive techniques for measuring DNA damage induced by chemicals *in vivo* as well as *in vitro*[9-10]. In the present investigation, this technique was used to assess DNA damaging potential of contaminated soil from a dumpsite near a carbamate pesticide manufacturing industry located in Central India. This procedure is very rapid and sensitive cells can be analyzed directly without culturing or radiolabeling. The above technique was used in the present study to evaluate the genotoxic potential of the pesticide-contaminated soil collected from a dumpsite near a carbamate pesticide manufacturing industry.

[1]Correspondence should be addressed to Dr. K. KRISHNAMURTHI, Tel: 91-712-2249757. Fax: 91-712-2249961. E-mail: twmneeri@ nagpur. dot.net.in; krishnamurthi_k@rediffmail.com; kmurthi_saravana@hotmail.com

Biographical note of the first author: Dr. K. Krishnamurthi is a scientist, majoring in sequestration of carbon from atmosphere using biological systems under cleaner development mechanism (CDM) and toxicogenomics genetic, polymorphism in Indian population exposed to industrial/environmental chemicals.

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MATERIALS AND METHODS

Materials

Sodium sulphate, ethidium bromide and D-glucose from Hi-Media Laboratories, India. Tris-Cl, dimethyl sulphoxide (DMSO), sodium hydroxide were from Sisco Research Laboratories (SRL), India. Methylene chloride and benzene were purchased from E-Merck India. All other chemicals and solvents were of the highest analytical grade available. Soil sample was collected from a pesticide manufacturing industry's dump site located at Central India.

Soil Extraction

Contaminated soil (2.5 g) was extracted with dichloromethane as solvent using soxhlet extraction (SW 846 method 3540)[11]. The extract was concentrated in a rotary vacuum evaporator and florisil cleanup was carried out. One portion of the cleanup fraction was subjected to GCMS analysis. The other portion of the extract was dissolved in DMSO at a final concentration of 0.1% DMSO for DNA strand break study.

Chemical Analysis

Chemical analysis was carried out by gas chromatography-mass spectrometer (GC-MS, Saturn Model Varian Associates, Walnut Creek, CA) with DB-5, ms column (M/s. J & W Scientific, USA 25 m × 0.25 mm id × 0.25 μ) and helium gas as carrier. The injector and transfer line temperature was 250℃ respectively. Ion trap temperature was 210℃ under EPA conditions for acquisition of MS data. The oven program was 50℃ for 1 min, raised to 150℃ at 25℃/min for 2 min, then to 320℃ at 5℃/min. The pesticides were identified by computer search of the National Institute of Science & Technology (NIST-1998) Library of Mass Spectra on the basis of mass fragmentation pattern.

Preparation of Peripheral Leukocytes

Human venous blood was collected from healthy donors using a plastic syringe containing sufficient heparin to give a final concentration of 60 IU/mL. Leukocytes were isolated as follows. Three mL of blood was mixed with 9 mL of solution A and kept at 0℃ for 30 min (or) until lysis of RBC was completed. The suspension was centrifuged (0℃, 20 min, 400×g) and suspended in 3 mL of solution A, and again centrifuged for 10 min. The pellet was suspended in 3 mL of balanced salt solution (BSS) to give total leukocyte concentration of 5×10^6/mL[4]. The viability of leukocyte was checked as 98% determined by trypan blue exclusion assay[12].

Cytotoxicity Test

Cytotoxicity of the soil extract was carried out using trypan blue exclusion assay[12]. Approximately 1×10^6 human polymorphonuclear leukocytes were incubated with different concentrations of the soil extract (10 μL, 25 μL, 50 μL, 100 μL, and 200 μL) for different time interval for up to six hours.

Fluorimetric Analysis of DNA Unwinding

Heparinized human venous blood was mixed with solution A (0.87% ammonium chloride, 10 mmol/L Tris pH 7.2) at the ratio of 1:3 and incubated at 0℃ for 20 minutes till RBC lysi. The mixture was centrifuged at 400×g for 20 minutes at 4℃. The supernatant was removed and the pellet containing peripheral blood mononuclear cells was washed and suspended in DMEM with the cell density of 5-6×10^6/mL. About 1 mL of cells (5×10^6) was treated with soil extract (50, 100, and 200 μL) while negative control (0.1% DMSO and distilled water) was treated for one h at 37℃. The treatment was terminated by the addition of ice-cold saline (0.9 % NaCl). The treated and control cells were centrifuged at 400×g for 10 minutes at 4℃ and resuspended in solution B (0.54 mmol/L EDTA in PBS) and the volume was made up to 2.0 mL. The suspended cells were processed for fluorimetric analysis of DNA unwinding as described by Brinbiom and Jevcak[4] with slight modifications in ethidium bromide dye concentration (0.006 mg/100 mL)[13].

Briefly about 1 million cells (0.2 mL) were distributed into three tubes (T, P, B) in duplicate according to the treatment group. To each group 0.2 mL of solution C (9 mol/L urea, 10 mmol/L NaOH, 2.5 mmol/L EDTA and 0.1% SDS) was added and incubated at 0℃ for 10 minutes to render lysis and chromatin disruption. After the lysis 400 μL of solution E (1 mol/L glucose, 14 mmol/L mercaptoethanol) was added to T tube only with mixing. To all the tubes 0.2 mL of solution D (0.2 nmol/L NaOH) was added without disturbing the tubes and incubated at 0℃ for 30 minutes during this time the alkali diffused into the cells to give the pH 12.8. After the incubation B tube of the treatment and control groups was sonicated for 1 minute in ice to ensure rapid denaturation of double stranded DNA. Thereafter all the tubes (T, P, and B) were kept at 20℃ for 60 minutes for denaturation and unwinding of double stranded DNA. After incubation the tubes were chilled at 0℃ for 10 minutes and P and B tubes were added with 400 μL of solution E, lowering the

pH to 11.0 and stopping denaturation. All the tubes were sonicated for a short duration to render homogeneity and added with 1.5 mL of solution F (0.006 mg of Ethidium bromide in 10 mmol/L Tris pH 8.0) and incubated at dark for 10 minutes. The fluorescence of the tubes was measured with a Hitachi make F-5000 model fluorescence spectrophotometer at the excitation wavelength of 520 nm and emission wavelength of 590 nm. The results were statistically analyzed using ANOVA one-way test.

RESULTS

GCMS analysis (Fig. 1) of contaminated soil showed the presence of carbaryl and α-naphthol. The concentration of carbaryl, 18.5 mg/g and α-naphthol 46 mg/g of soil (dry basis), respectively.

Figure 2 shows cytotoxicity caused by the soil extracts. Significant cytotoxicity was not observed after one hour exposure to the doses. However, significant cell death was seen in 100 and 200 μL dose groups from the 4th hour onward and significant cytotoxicity was observed from the 5th hour onward after treated with 50 μL, whereas 10 and 25 μL treatment did not cause significant cytotoxicity at all the time periods in control.

Figure 3 shows the DNA damage in human leukocytes induced by extracts from contaminated soil (79% DNA strand break) and different concentrations of standard grade carbaryl (57.61%), and α-naphthol (49.04%). The reported values were expressed as the percentage of DNA damage after the cells were exposed to carbaryl and α-naphthol.

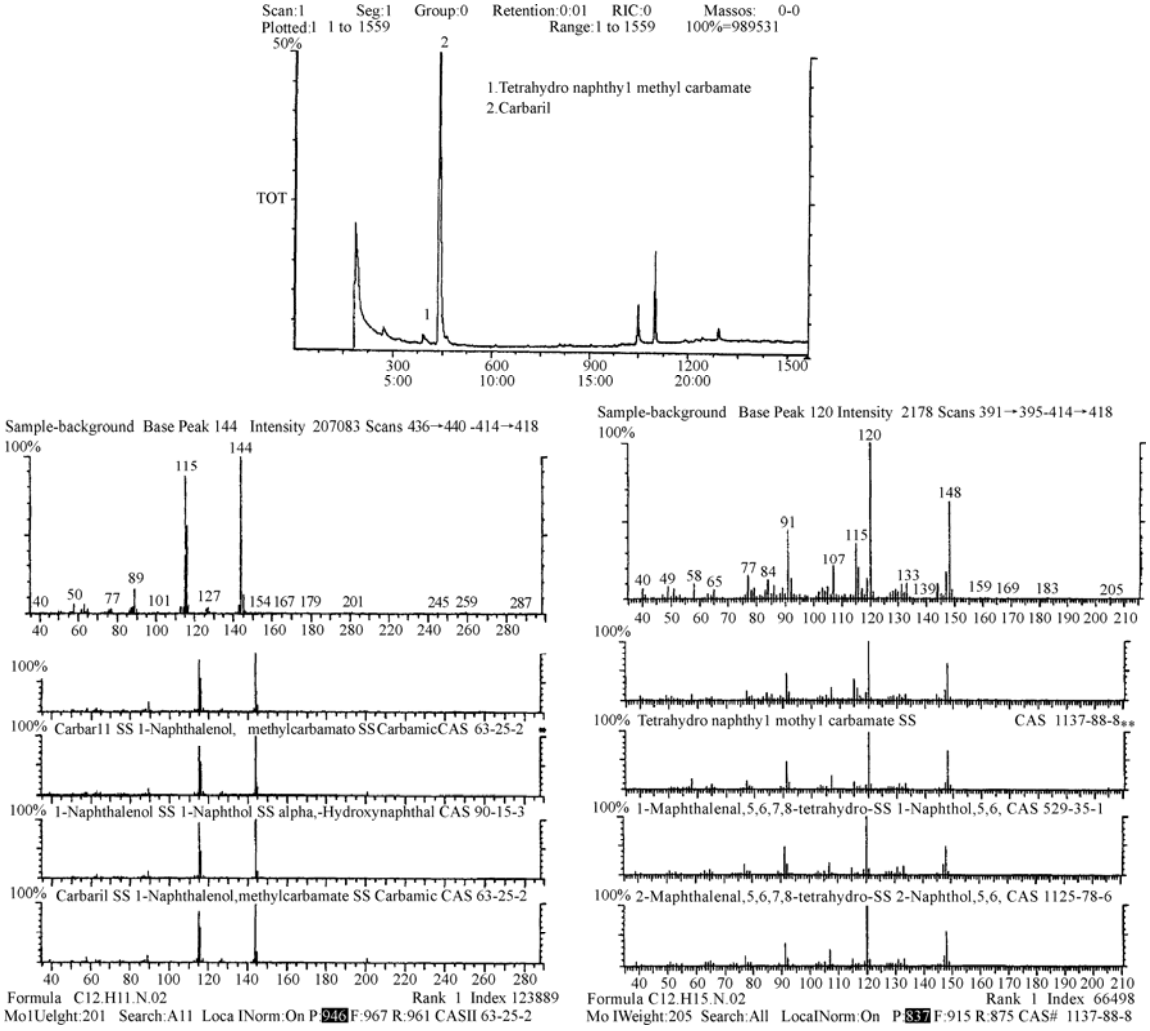

FIG. 1. Reconstituted total ion chromatograph and mass fragmentation pattern of soil extracts.

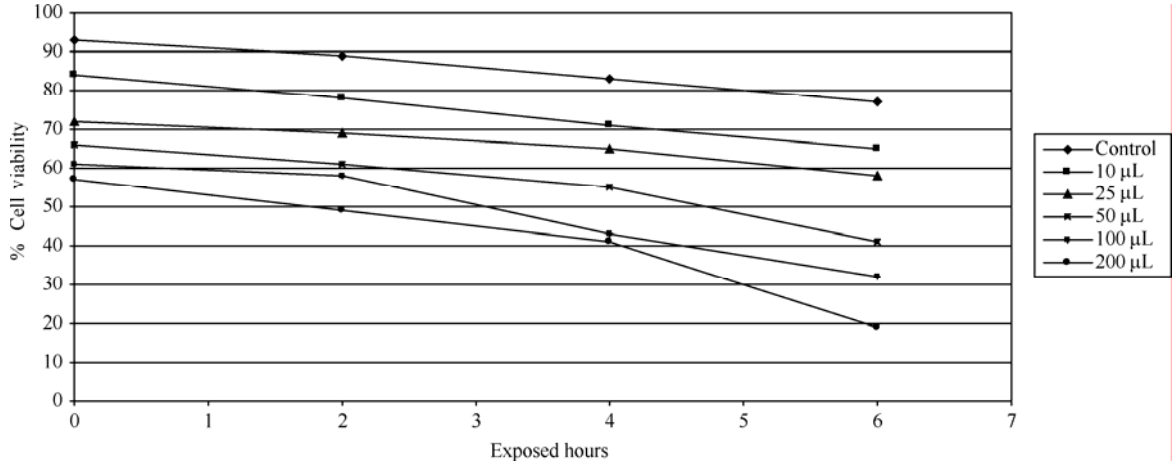

FIG. 2. Cytoxicity caused by soil extract in peripheral leukocytes.

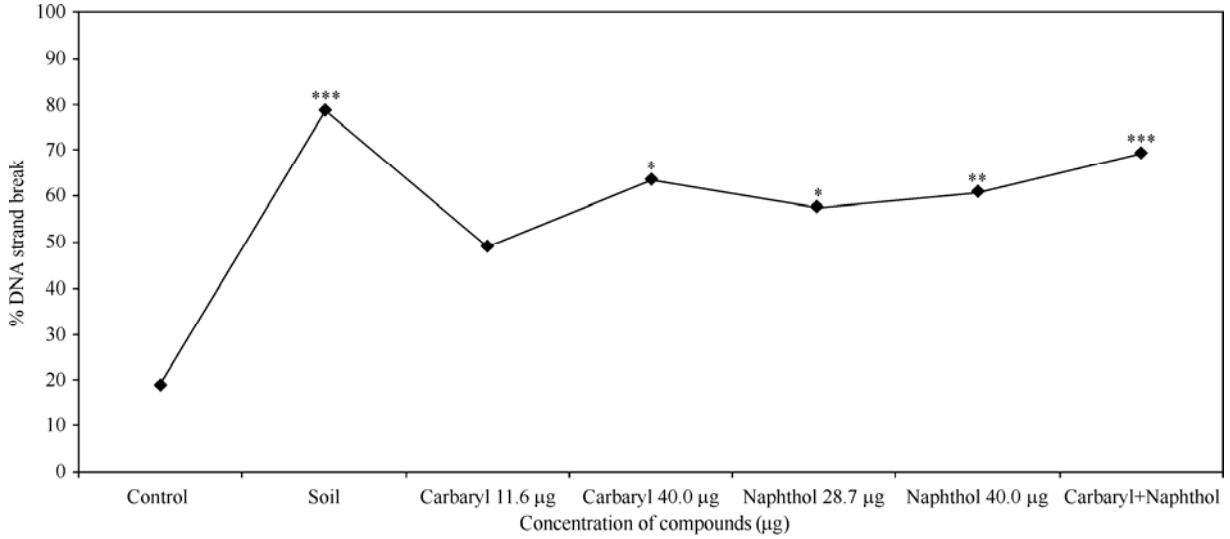

FIG. 3. DNA strand break induced by soil extract and pesticide standards. The results are average of the sets of experiments. **P*<0.02. ***P* <0.01. ****P* <0.001.

DISCUSSION

The DNA strand breaks induced by the extract (contaminated soil) were significantly higher than those of the individual components present in the soil (Fig. 2, *P*<0.001). Both technical grade of carbaryl and α-naphthol, singly or in combination, could induce *in vitro* DNA strand break in human leukocytes (carbaryl 57.61%, α-Naphthol 49.04% and 69.32% respectively). The extract from contaminated soil caused 79% DNA damage, whereas carbaryl and α-naphthol in combination, caused 69.32% DNA damage indicating that the major part of toxicity caused by the contaminated soil is due to carbaryl and α-naphthol. However, genotoxicity of other unknown contaminants in the soil extract could not be ruled out.

Our results are consistent with those obtained by Ahmed *et al.*[14], who reported that carbaryl, even at a concentration of 1 µmol/L, can induce DNA damage in human fibroblast cell line (VA-4). Lockand *et al.*[15] showed that carbaryl can rapidly inhibit DNA, RNA and protein synthesis in L-2 cells from rat lung. Experiments on Swiss Albino mice administered carbaryl intraperitoneally have shown clastogenic effect in a dose-dependent manner. Our studies on human lymphocytes also demonstrated that carbaryl induces chromosomal damage, leading to cell death[16].

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