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# Biological transformation and detoxification of 7,12-dimethylbenz(a)anthracene in soil systems

Kap S. Park, Ronald C. Sims, William J. Doucette, John E. Matthews

**ABSTRACT:** Biological transformation and detoxification of 7,12-dimethylbenz(a)anathracene (DMBA) were studied in a nonacclimated sandy loam soil. Parent $^{14}C$ DMBA biodegraded extensively (62% to 20%), accompanying an increase of metabolite $^{14}C$ fraction (4% to 53%). Incorporation of DMBA into non-extractable soil residue $^{14}C$ increased from 12 to 17%, but the increase was not statistically significant. DMBA was transformed into several metabolic products in the soil system, including 4-hydroxy-, 5-hydroxy-, and 10-hydroxy-DMBA and 7,12-dihydro-12-methyl-7-methylene-benz(a)anthracene-12-ol. High polarity transformation products of DMBA demonstrated a negative mutagenic response with the Ames mutagenicity assay, strain TA-100, for both low and neutral pH soils. Moderate and low polar metabolites, however, induced mutagenicity for both soil samples. The mutagenicity of these metabolites decreased with incubation time in the soil, suggesting detoxification and assimilation of this polyaromatic hydrocarbon in soil systems. Mutagenic responses for the metabolites formed from low and neutral pH soil were similar. *J. Water Pollut. Control. Fed.* **60**, 1822 (1988).

**KEYWORDS:** (biodegradability), (detoxification), soil, hazardous waste, (polyaromatic hydrocarbon), (7,12-dimethylbenz(a)anthracene).

Many polyaromatic hydrocarbon (PAH) constituents in hazardous waste/soil systems have mutagenic and carcinogenic characteristics.[1] Biological transformation is believed to be the principal process responsible for the detoxification of the hazardous PAH constituents.[1] The transformation and detoxification potential of PAH compounds, therefore, is critical for the design and management of hazardous waste/soil systems to treat and dispose of wastes without causing environmental degradation and public health hazards.

The control of soil pH may provide an engineering management technique for the detoxification and assimilation of PAH constituents in hazardous waste/soil systems without causing mutagen formation. Fungi that predominate in low pH soil habitats produce epoxides, which initiate cancerous tumors, as initial metabolic intermediates of PAH biodegradation in soil systems.[2] However, in neutral pH soil, bacteria dominate, and they do not form these intermediates.[3]

One important PAH compound with high carcinogenic potency is 7,12-dimethylbenz(a)anthracene (DMBA).[4,5] of this compound by mammalian preparations[6] and microorganism cultures.[7] Transformation and detoxification characteristics of DMBA in soil systems have not been previously published.

This study investigated the biological transformation of DMBA in a sandy loam soil. Transformation was evaluated directly by determining the metabolic intermediates in soil extracts and indirectly by measuring major loss mechanisms of organic compounds in soil systems, including volatilization and abiotic losses. Detoxification characteristics of the transformation metabolites were also evaluated at low and neutral pH soil conditions.

## Materials and Methods

**Soil incubation of DMBA.** Transformation studies were performed with McLaurin sandy loam soil at low pH and with the same soil adjusted to a neutral pH soil condition. The McLaurin soil was selected on the basis of its low pH (pH = 4.8). For the neutral pH incubation, pH was adjusted to 7.5 by adding 70 mg of $CaCO_3$ to the soil. DMBA was chosen based on reported genetic toxicity[4,5] and the rate and extent of degradation measured in laboratory studies.[8]

One hundred grams (dry weight) of soil at the incubation water potential of −33 J/kg were placed in a 500-mL glass beaker. Following 14 days of incubation, 100 mg of DMBA were added to the soil (1000 $\mu$g/g) in a methylene chloride solution. After the methylene solution evaporated from the soil (approximately 24 hours), water was added to adjust the soil moisture content to −33 J/kg soil-water potential. To control soil water content, the soil beakers were covered with polyethylene film, which is permeable to oxygen and is effective for reducing the loss of soil water while maintaining aerobic conditions.[9] Evaporative water losses during incubation were compensated by water addition about every 2 weeks to the beakers to maintain the soil-water potential in the range of −100 to −33 J/kg. The soil beakers were incubated at 20°C in the dark to prevent photodegradation of the PAH compound. Control experiments were performed under identical incubation conditions, while soil blanks were incubated without DMBA. Poisoned controls (2% $HgCl_2$) with and without DMBA

were also prepared to monitor and account for possible abiotic transformations of soil humus and DMBA, respectively, in soil systems.

**DMBA analysis.** Soil beakers were withdrawn from the incubation units 0, 14, and 28 days after DMBA addition. The schedule of sampling times ensured that a sample would be taken beyond the DMBA half-life in soil. Each beaker was extracted with 200 mL of methylene chloride using a homogenization technique, and the extracts were then dried over anhydrous sodium sulfate and evaporated to 1 mL under an aerated hood.

An aliquot of the extract was injected into a high performance liquid chromatography system fitted with a 4.6 mm I.D. × 250 mm 15-$\mu$ octadecylsilane column and eluted with a water/acetonitrile gradient (from 35 to 100% of acetonitrile) at a flow rate of 0.9 mL/min. The eluate was monitored using a 254-nm UV detector. Seven reference standards of hydroxylated derivatives of 7,12-dimethylbenz(a)anthracene were used, including 1-hydroxy-, 2-hydroxy-, 3-hydroxy-, 4-hydroxy-, 5-hydroxy-, 8-hydroxy-, and 10-hydroxy-7,12-dimethylbenz(a)anthracene. These compounds were analyzed by HPLC using the same conditions used for soil extracts.

**Experiments with radiolabeled DMBA.** Experiments with radiolabeled DMBA were conducted as previously described except 2 $\mu$Ci (12-$^{14}$C) DMBA was added to 10 g (dry weight) of the McLaurin sandy loam soil (0.2 $\mu$Ci/g). The (12-$^{14}$C) DMBA had a specific radioactivity of 8.3 mCi/mmol, and radiochemical purity of 97%.

The distribution of $^{14}CO_2$ between evolved $CO_2$, soil extracts, and soil residue components was measured to construct a DMBA mass balance. Soil samples were extracted 0, 14, and 28 days after application of DMBA, air dried, and stored in a refrigerator before analysis for soil residue $^{14}$C. The extract was dried over anhydrous sodium sulfate and concentrated to 1 mL under an aerated hood.

The sample extracts (100 $\mu$L) were chromatographed on an HPLC system using the same conditions and methods as described in the analysis of DMBA. Fractions of the HPLC eluate (0.25 mL) were collected at 1 minute intervals and added to tubes containing 3.5 mL of scintillation liquid. The radioactivity present in each fraction was determined in a liquid scintillation counter. Corrections were made for machine efficiency and quenching.

An extracted soil sample (0.1 g) was combusted at 700°C in an $O_2$ stream in a biological material oxidizer. After catalytic oxidation of combustion gases, the $CO_2$ produced was introduced into a trapping solution. The trapping solution was analyzed for the $^{14}$C fraction.

Soil mineralization of $^{14}$C DMBA was determined using a flask with a well for $CO_2$ trapping liquid. 0.1 N KOH was used for trapping the evolved $CO_2$. The trapped $^{14}CO_2$ was quantified by liquid scintillation counting. The KOH solutions in the $CO_2$ traps were changed once a week during the 28-day incubation period.

**Mutagenicity evaluation.** Soil samples were incubated and extracted as described in the DMBA soil incubation except 1000 g (dry weight) of DMBA treated soils (1000 $\mu$g/g) were placed in a 3-L glass beaker. To obtain sufficient amounts of DMBA metabolites for the Ames mutagenicity assay, 1000 g/soil was used. Soil extracts were separated into metabolite fractions based on HPLC retention time (polarity); 0- to 15-minute, 15- to 33-minute, and 35- to 45-minute retention times demarcated the three fractions which were isolated by preparative scale HPLC on a 21.2 mm I.D. × 250 mm 15-$\mu$ octadecysilane column using a water/acetonitrile gradient (35 to 100%) at a flow rate of 8 mL/min. The HPLC fractions were evaporated to dryness under an aerated hood and reconstituted with dimethylsulfoxide.

Mutagenicity activities of DMBA and metabolite fractions were measured with the Ames assay[10] using the *Salmonella tryphimurium* TA-100, which detects mutagens causing base-pair substitutions. Samples were tested on triplicate plates in the standard plate incorporation assay at four dose levels with enzyme activation. 2-Aminofluorene (10 $\mu$g/plate) was used as a positive control. Mutagenic potential of test samples was expressed as the mutagenic ratio, which is the ratio of the number of colonies in the presence of a test sample to the number of colonies on a control growth plate in the absence of the test sample.

## Results and Discussion

The distribution of $^{14}$C from the incubation of (12-$^{14}$C) DMBA with McLaurin sandy loam soil is summarized in Table 1. Total $^{14}$C recoveries varied from 78 to 90% over the 28-day incubation period for both nonpoisoned and poisoned (2% $HgCl_2$) samples. Recovery efficiencies were typical for a $^{14}$C tracer study.[11]

With a half-life of 17 days, parent $^{14}$C DMBA was extensively biodegraded. Microbial transformation half-life was determined from the decrease of the DMBA $^{14}$C fraction over time, which was corrected for abiotic loss and

**Table 1—Transformations of ($^{14}$C) DMBA by McLaurin sandy loam soil.[a]**

| | $^{14}$C appearing in each fraction, percent | | | | |
|---|---|---|---|---|---|
| | Soil extract | | | | |
| Time, days | DMBA, parent compound | Metabolites | Soil residue | $CO_2$ | Total |
| 0 | 62 (69) | 4 (6) | 12 (13) | 0 (0) | 78 (88) |
| 14 | 26 | 43 | 16 | 0 | 85 |
| 28 | 20 (60) | 53 (11) | 17 (16) | 0 (0) | 90 (87) |

[a] Poisoned (control) data in parentheses.

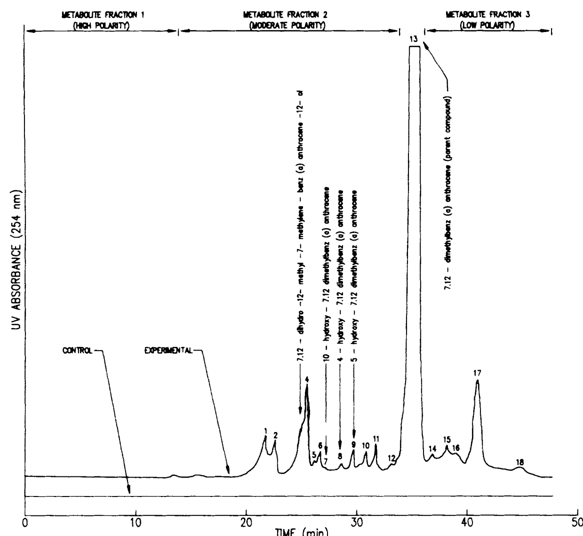


**Figure 1—HPLC chromatogram of soil extract formed from DMBA by McLaurin sandy loam soil (low pH).**

volatilization. These results are consistent with the results obtained for the non-radiolabeled DMBA degradation study, which showed a biodegradation half-life of 20 to 28 days.[8] Abiotic loss of $^{14}C$ DMBA from soil samples poisoned by 2% $HgCl_2$ was statistically not significant ($p < 0.05$), while $^{14}C$ DMBA volatilization was not detected during the 28-day soil incubation period.

Table 1 also shows that the decrease in parent $^{14}C$ is accompanied by an increase in the metabolite $^{14}C$ fraction. Incorporation of $^{14}C$ DMBA into a non-extractable soil residue $^{14}C$ increased from 12 to 17%; the increase, however, was not statistically significant ($p < 0.05$). Evolution of $^{14}CO_2$ was not detected during the 28 days of incubation, but this does not demonstrate that none of the parent compound was metabolized to $CO_2$ because $^{14}C$ DMBA used was radiolabeled only at the 12-position carbon. If evolution of $^{14}CO_2$ did occur, the benzene ring that contained the carbon-12 had to be mineralized to $CO_2$.

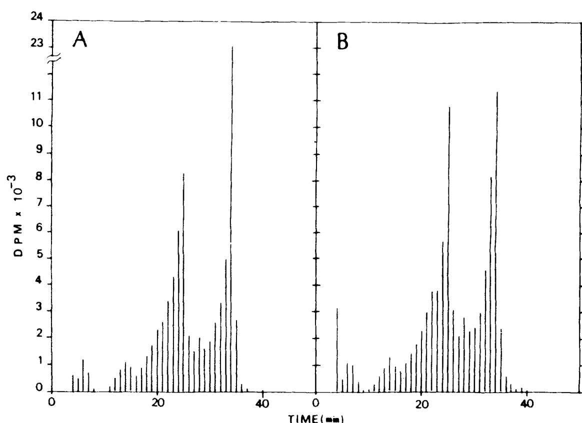


**Figure 2—Elution profile of metabolites formed from ($^{14}C$) DMBA in McLaurin sandy loam soil; A = 14-day incubation time, B = 28-day incubation time.**

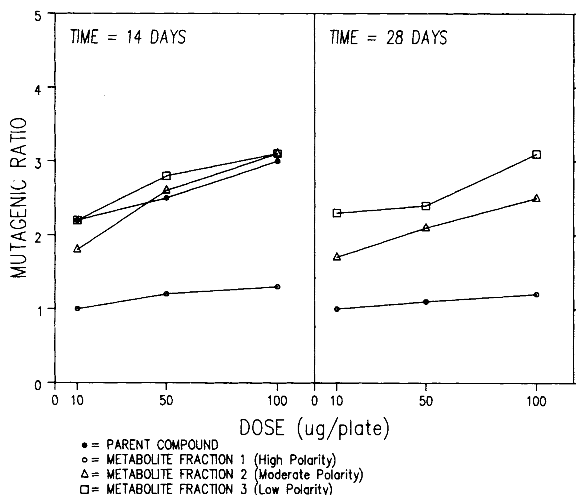


**Figure 3—Mutagenicity of DMBA metabolites from McLaurin sandy loam soil (low pH).**

The HPLC chromatogram of DMBA metabolites revealed several metabolic products (Figure 1). Peaks 7, 8, and 9 were tentatively identified as 10-hydroxy-, 4-hydroxy-, and 5-hydroxy-DMBA, respectively. HPLC retention times of these metabolites were identical with those given by reference standards. The mass spectrum of Peak 3 displayed ions at m/e values of 272 (molecular ion), 257 (loss of $CH_3$), and 226 (loss of $CH_3$, OH, and $CH_2$) and indicated that the metabolite is 7,12-dihydro-12-methyl-7-methylene-benz(a)anthracene-12-ol.

The HPLC elution profile (Figure 2) from the incubation of $^{14}C$ DMBA revealed a complex mixture of metabolic products that was similar to the HPLC chromatogram (Figure 1) for non-radiolabeled PAH metabolites. The elution profile also shows the formation of highly polar metabolic products that eluted before the HPLC retention time of 15 minutes.

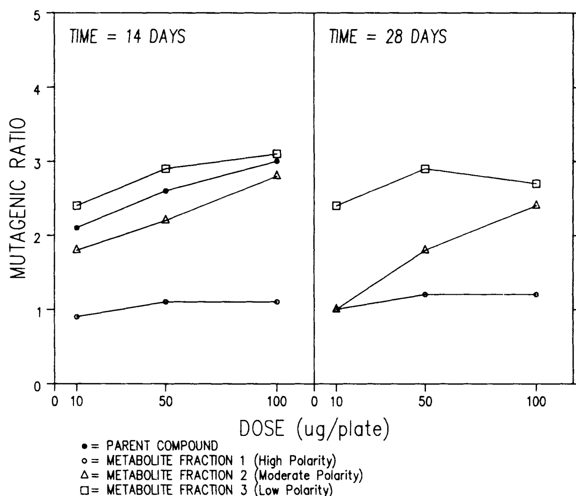


**Figure 4—Mutagenicity of DMBA metabolites from McLaurin sandy loam soil adjusted to neutral pH soil condition.**

Results for Ames assay testing with strain TA-100 for DMBA metabolites in low and neutral pH soil are presented in Figures 3 and 4, respectively. The highly polar metabolic fraction (HPLC retention time of 0 to 15 minutes) was mutagenically inactive towards TA-100 for both soil pH conditions, suggesting that these metabolites may be the detoxified conjugation products of soil microbial enzymes. Others[2] who studied the oxidation of $^{14}C$ benzo(a)pyrene by *Cunninghamella elegans* demonstrated that the metabolites eluting in the very polar HPLC region were mostly sulfate conjugates of dihydroxy benzo(a)pyrene metabolites and benzo(a)pyrene phenols.

Moderate and nonpolar (HPLC retention times of 15 to 33 minutes and 35 to 45 minutes, respectively) metabolite fractions induced a positive response[12] (mutagenic ratio greater than 2) for both soils. The mutagenic potential of these metabolite fractions, however, decreased during the 28-day soil incubation time. This potential for detoxification of DMBA may be important for engineering management and control of hazardous wastes containing this PAH compound because toxicity reduction as a function of incubation time in the soil can be used to assess treatment success. Detoxification was reported by another investigator,[13] who found that the mutagenicity of polar transformation products of benzo(a)pyrene increased, and then decreased, with incubation time (or treatment time) in the soil.

Mutagenic responses for the metabolites formed from low and neutral pH soil were not different (Figures 3 and 4). Soil pH adjustment changed the microbial populations by an order of magnitude, but significant and equivalent amounts of bacteria and fungi still existed in both soils (Table 2). Thus, differences in the metabolic products between low and neutral pH soil are not likely, and similar mutagenic responses between the two soil treatments may have resulted.

## Conclusions

Parent $^{14}C$ DMBA biodegraded extensively with a half-life of 17 days. The decrease in the parent $^{14}C$ DMBA (62 to 20%) was accompanied by an increase in metabolite $^{14}C$ fraction (4 to 53%). Soil residue $^{14}C$, however, did not increase significantly ($p < 0.05$) during the 28 days of soil incubation. DMBA was transformed into several metabolic products in the McLaurin soil. Compounds that were tentatively identified included 4-hydroxy-, 5-hydroxy-, and 10-hydroxy-DMBA and 7,12-dihydro-12-methyl-7-methylene-benz(a)anthracene-12-ol. High polarity transformation products of DMBA were not mutagenic for both low and neutral pH soil treatments. However, moderate and low polarity metabolites induced a positive response for both soil treatments, but the mutagenic potential of these fractions decreased with increasing incubation time.

**Table 2—Effect of soil pH on microbial populations in McLaurin sandy loam soil.**

| Soil pH | Bacteria, cfu/g soil | Fungi, cfu/g soil |
|---|---|---|
| 4.8 | $1.1 \times 10^5$ | $5.3 \times 10^4$ |
| 7.5 | $2.5 \times 10^6$ | $4.0 \times 10^3$ |

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