Biochemical Pharmacology, Vol. 34, No. 4, pp. 545–551, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00
Pergamon Press Ltd.

EFFECT OF ARYL HYDROCARBON HYDROXYLASE INDUCTION ON THE *IN VIVO* COVALENT BINDING OF 1-NITROPYRENE, BENZO[*a*]PYRENE, 2-AMINOANTHRACENE, AND PHENANTHRIDONE TO MOUSE LUNG DEOXYRIBONUCLEIC ACID

CHARLES E. MITCHELL

Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM 87185, U.S.A.

(*Received* 28 *February* 1984; *accepted* 21 *June* 1984)

Abstract—The effect of aryl hydrocarbon hydroxylase induction on the covalent binding of 1-nitropyrene (1-NP), benzo[*a*]pyrene (BaP), 2-aminoanthracene (2-AA), and phenanthridone (PNDO) to mouse lung DNA was investigated. Cytochrome P-450-dependent monooxygenases were induced in mouse lung by intratracheal instillation of BaP, Aroclor-1254, or coal gas condensate (CGC) 24 hr before instillation of [^{3}H]BaP, [^{3}H]-2-AA, [^{14}C]-1-NP, or [^{14}C]PNDO. All inducing agents increased the amount of radioactivity of [^{3}H]BaP, [^{3}H]-2-AA, and [^{14}C]-1-NP or metabolites bound to DNA. However, pretreatment with BaP resulted in the highest amounts of radiolabels covalently bound to DNA. At 4 hr after instillation of radiolabels in BaP-induced mice, the amounts of [^{3}H]BaP, [^{3}H]-2-AA, and [^{14}C]-1-NP bound to DNA were increased 5.4-, 5.2-, and 160-fold above that of control levels; the amount of 1-NP bound to DNA was fifty times higher than the amount bound by BaP. Labeled compounds were still bound to DNA 1 week after administration. [^{14}C]PNDO was not bound to DNA in uninduced or induced mice. Based on the amount of labeled compounds bound to DNA, pretreatment of mice with BaP and CGC induced enzymes with similar specificities; however, enzymes induced by Aroclor were less effective in the metabolism of labeled compounds to DNA-bound products. These data show that specific cytochrome P-450-dependent monooxygenases are inducible in mouse lung and suggest that pre-exposure to inducing agents may be important in the potential toxicity to proximal tissues in direct contact with inhaled xenobiotics.

Man is exposed to airborne environmental chemicals that are emitted into the environment at an increasing rate. The sources of many pollutants are chemical manufacturing plants, refineries, energy production facilities, and automotive vehicle exhaust [1]. There is special concern about the potential health effects of emissions, products, and by-products that are released into the environment after combustion or conversion of fossil fuel [2]. In addition, the relationship between cancer and aromatic compounds and fossil fuel energy sources is of concern. This is documented in epidemiological studies of coke oven workers, gas works operators, and workers in experimental coal liquefaction plants, where high incidences of lung tumors were found [1].

Although the exact molecular mechanisms of toxicity, mutagenicity, or carcinogenicity are not well understood, it is known that many environmental chemical carcinogens are metabolically activated to electrophilic reactants that subsequently bind to cellular macromolecules before exerting their effects [3]. The enzyme system responsible for the metabolism of many xenobiotic agents is the microsomal cytochrome P-450-dependent monooxygenase system. Microsomal enzymes are induced in man and animals after exposure to pure or complex mixtures of chemicals such as cigarette smoke, diesel exhaust, coal gas condensate (CGC), polychlorinated biphenyls, and petroleum pollutants [4–10]. It has also been reported that extracts of particulate urban air samples were effective in inducing lung microsomal monooxygenases [11]. The induced enzymes can both activate and detoxify xenobiotic materials [12]. The spectrum of chemicals metabolized by the microsomal enzymes indicates their broad specificity for xenobiotics [13]. Additional studies have shown that the levels of specific enzymes are increased by different inducing agents [14]. These enzymes may have an increased capacity to metabolize the inducing agent to metabolites that bind to DNA [8]. Polycyclic aromatic hydrocarbons, nitroaromatics, heterocyclic aromatics, and aromatic amines are among the most toxic, mutagenic, and carcinogenic materials produced by combustion and conversion of fossil fuels and are likely to interact with cellular DNA [15–18].

Although the exact molecular mechanism of chemical carcinogenesis are not fully understood, interaction of chemicals with cellular macromolecules is probably related to the event (or events) leading to neoplastic transformations. A positive correlation has been found between cancer and chemicals covalently bound to DNA [19]. The purpose of the present studies was to determine how induction of microsomal enzymes by benzo[*a*]pyrene (BaP), Aroclor-1254, and CGC affects the binding of aromatic hydrocarbons of specific chemical classes to mouse lung DNA.

MATERIALS AND METHODS

Male laboratory-reared CD-1 mice, specific pathogen-free, 8- to 10-weeks-old, were used. They were fed Wayne Lab Blox (Allied Mills, Chicago, IL) and provided water *ad lib.* BaP (98% pure) and 2-aminoanthracene (98% pure) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Aroclor-1254 was obtained from Analabs, Inc. (North Haven, CT). CGC was obtained from an experimental low Btu coal gasifier at Morgantown, WV. CGC contains primarily aliphatic hydrocarbons and polycyclic aromatic hydrocarbons with their alkylated derivatives, nitrogen heterocycles and polycyclic aromatic hydrocarbons with polar functional groups [20]. [1,3,6-^{3}H]BaP (98% radiochemical purity; 50 Ci/mmole) was obtained from the New England Nuclear Corp. (Boston, MA). 2-Amino-[G-^{3}H]anthracene (98% radiochemical purity; 3.2 Ci/mmole) and 1-nitro[4,5,9,10-^{14}C]-pyrene (98% radiochemical purity, 43 mCi/mmole) were obtained from the Amersham Corp. (Arlington Heights, IL). Radioactive dinitropyrene was undetectable in this preparation by radioanalysis of appropriate high pressure liquid chromatographic fractions or mass spectrometry. [9-^{14}C]Phenanthridone (96% radiochemical purity, 1.1 mCi/mmole) was synthesized and purified as described by Dutcher and Mitchell [21]. All other chemicals and organic solvents were analytical reagent grade and were used without further purification.

A microcrystalline suspension of BaP or an emulsion of Aroclor-1254 or CGC was prepared in 0.2% gelatin in saline by brief exposure to an ultrasonic probe (Branson model S125, Danbury, CT). BaP (10 mg/kg body wt), Aroclor-1254 (10 mg/kg body wt), or CGC (10 mg/kg body wt) was intratracheally instilled into mice to induce microsomal enzymes 24 hr before administration of labeled compounds. Labeled compounds, with specific activities as described above, were prepared as microcrystalline suspensions and intratracheally instilled into mice at a dose of 0.5 mg/kg body wt. The intratracheal instillations method was essentially that described by Brain *et al.* [22] with several minor modifications. Briefly, the particle suspension or emulsion was delivered to the lungs through the trachea of an anesthetized mouse with a no. 22-guage needle, 40 mm long, inserted between the vocal folds. All animals received 0.100 ml suspension or emulsion per 100 g of body weight and were kept upright on the slanted board for 1 min to make sure no material was regurgitated.

Microsomal aryl hydrocarbon hydroxylase (AHH) was determined according to the method of Nebert and Gelboin [23]. Enzyme activity was determined in duplicate under conditions of linearity, both in respect to time and protein concentration. The enzyme activities are expressed as picomoles of 3-OH-BaP formed per milligram of protein per minute. Protein concentration was determined using a biuret method [24]. Bovine serum albumin was used as a standard.

Three mice from each treatment group were killed at 4 hr, 1 day, and 7 days after administration of labeled compound. At the time of sacrifice, the lungs were removed and homogenized in 10 vol. of 1% sodium tri-isopropylnaphthalene sulfonate/6% sodium 4-aminosalicylate/2% sodium chloride/6% 2-butanol [25] using a tissumizer (Tekmar Co., Cincinnati, OH). The homogenates were shaken twice with aqueous-saturated phenol, and the residual phenol and unbound radioactivity were removed from the aqueous solution by extensive diethyl ether extractions, which continued until radioactivity in the ether reached background levels. The DNA was isolated by precipitation in ethanol. After washing in ethanol, the DNA was purified by dissolving in 30 mM sodium chloride/3 mM sodium citrate and sequentially digested for 1 hr with 50 μg/ml ribonuclease (preheated at 80° for 10 min) and 1 hr with 100 μg/ml proteinase K. The DNA was further shaken twice with chloroform–isoamyl alcohol (5 : 1, v : v), precipitated in ethanol, washed repeatedly with ethanol, dried, and resuspended in 10 mM Tris/10 mM $MgCl_2$ (pH 7.0). The DNA was quantitated by the absorbancy at 260 nm (A_{260}), and covalently bound radioactivity was determined by scintillation counting (Packard Tri-Carb 2660 Packard Instruments, Downers Grove, IL). The quantity of labeled compound bound to DNA was calculated from the specific activity of the labeled compound and expressed as femtomoles of covalently-bound chemical per microgram of DNA.

To determine the amount of labeled compound that was bound non-covalently to DNA after isolation and purification, a procedure similar to that described by Lutz [26] was done. Lungs from control animals were homogenized in buffer containing a detergent as described earlier; 0.0175 mg of labeled compounds was added to the homogenates. It has been shown that the use of a detergent contained in the homogenizing buffer was effective in inhibiting enzymatic activity [25]. It was assumed that under these conditions metabolic activation of test compounds to reactive derivatives did not occur. The incubation was carried out in the dark to exclude photo-oxidative reactions, which might also lead to covalently-bound chemical. After 0.5 hr shaking at 37°, the DNA was isolated and purified, and the specific activity was determined as described previously. These experiments indicated that little or no radioactivity was bound non-covalently to DNA after isolation and purification. The radioactivity associated with DNA after incubation of lung homogenates with labeled chemical in the enzyme denatured system was always less than 0.5% of the amount in the metabolically active system.

RESULTS

The dose–response of chemicals on the induction of microsomal hydroxylase activity (production of 3-OH-BaP from BaP) in mouse lung is shown in Fig. 1. BaP, Aroclor-1254, and coal tar induced AHH activity to maximal levels at doses of approximately 8 mg/kg. AHH activity induced by BaP remained at about the same level with doses up to 30 mg/kg. The level of AHH activity induced by Aroclor-1254 was lower with higher doses of Aroclor. At higher doses of CGC, enzyme activity was markedly lower than in controls at doses of 50 mg/kg. The decreased

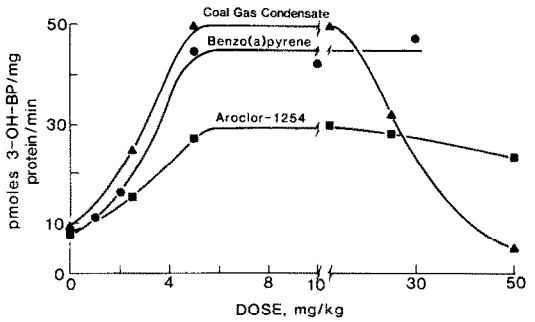

Fig. 1. Lung microsomal AHH activity in CD-1 mice at 24 hr after instillation of different quantities of BaP, Aroclor-1254, or CGC. Mice were killed at 24 hr after instillations, and AHH activity was measured. Each point represents the average hydroxylase activity of four animals.

AHH activity with dose may be due to enzyme inhibition or cellular toxicity.

After instillation of [^{3}H]BaP, [^{3}H]-2-AA, [^{14}C]-1-NP and [^{14}C]PNDO in control animals, covalently-bound radioactivity was associated with lung DNA at 4 hr, 1 day, and 7 days after administration of labeled compound (Fig. 2). The highest molar equivalent amount of label bound to DNA was found with [^{14}C]-1-NP, where it exceeded the amount of [^{3}H]-BaP bound by 1.7-fold. After induction of microsomal enzymes by BaP, the amounts of [^{3}H]BaP, [^{3}H]-2-AA, and [^{14}C]-1-NP were all increased over the amounts bound in uninduced control animals (Fig. 3). The amounts bound in induced over control animals were 5.4-, 5.2- and 160-fold for [^{3}H]BaP, [^{3}H]-2-AA, and [^{14}C]-1-NP respectively. Increased amounts of labeled compound were still present 7 days after administration. At that time, the amount of [^{14}C]-1-NP bound to DNA was ~73 times that of

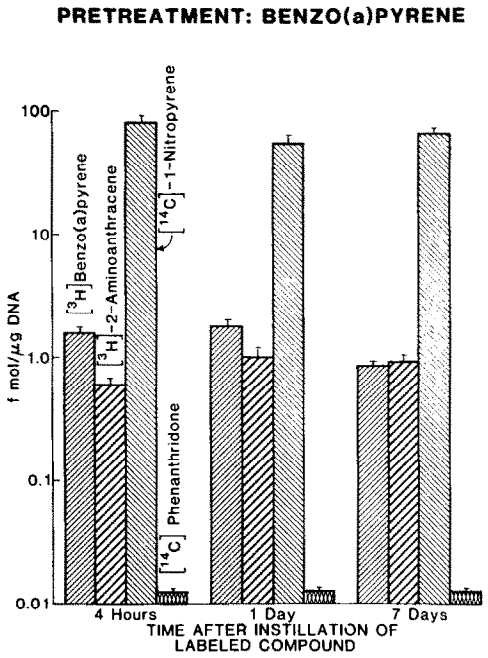

Fig. 3. Effects of BaP (10 mg/kg) pretreatment on the binding of radiolabeled BaP, 2-AA, 1-NP and PNDO to mouse lung DNA. Additional details are described in the legend of Fig. 2.

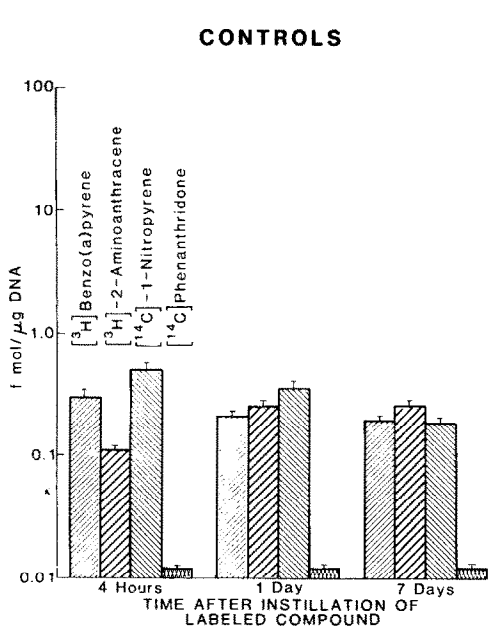

Fig. 2. Covalent binding of [^{3}H]BaP, [^{3}H]-2-AA, [^{14}C]-1-NP and [^{14}C]PNDO to mouse lung DNA. Mice were treated with vehicle (gelatin-saline) 24 hr before administration of labeled compounds (0.5 mg/kg). Bars represent the mean ± S.E. of three animals.

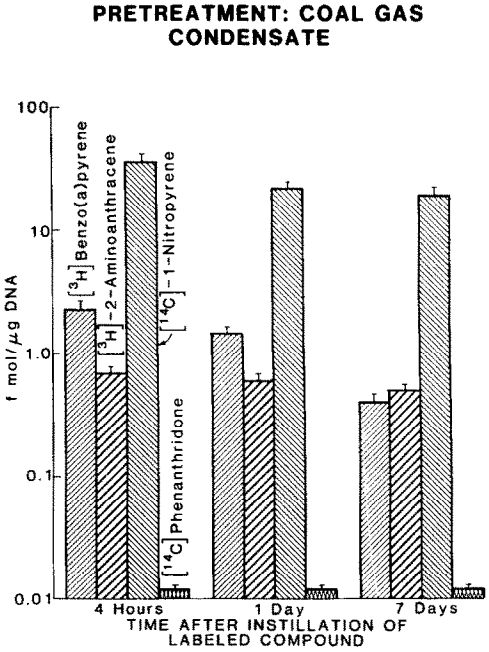

Fig. 4. Effect of coal gas condensate (10 mg/kg) pretreatment on the binding of radiolabeled BaP, 2-AA, 1-NP, and PNDO to mouse lung DNA. Additional details are described in the legend of Fig. 2.

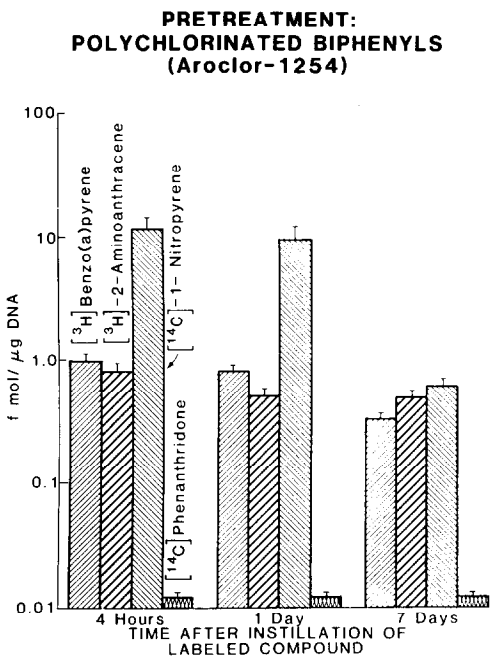

Fig. 5. Effect of pretreatment by polychlorinated biphenyls (Aroclor-1254; 25 mg/kg) on the binding of radiolabeled compounds to mouse lung DNA. Additional details are described in the legend of Fig. 2.

[^{3}H]BaP. Little [^{14}C]PNDO radioactivity was bound to DNA in uninduced or induced animals.

Pretreatment of animals with CGC also increased the amount of [^{3}H]BaP, [^{3}H]-2-AA, and [^{14}C]-1-NP bound to mouse lung DNA, but not as much as that bound after BaP induction, except for [^{3}H]BaP (Fig. 4). Lower amounts of [^{3}H]-2-AA and [^{14}C]-1-NP were found in CGC-pretreated mice than in BaP-pretreated mice although the amount of [^{14}C]-1-NP bound to DNA was still higher than for [^{3}H]-BaP and [^{3}H]-2-AA (~16 fold higher than for ^{3}H-BaP). Labeled compounds initially bound to DNA were still above control levels 1 week after administration.

Pretreatment of mice with Aroclor-1254 increased the amounts of [^{3}H]BaP, [^{3}H]-2-AA, and [^{14}C]-1-NP bound to lung DNA (Fig. 5). However, Aroclor-1254 induced the smallest increase in the amount of [^{14}C]-1-NP bound to DNA in induced mice. The amount of [^{14}C]-1-NP bound was still ~10-fold more than that of [^{3}H]BaP. In addition, the binding of [^{14}C]-1-NP after this induction method was not as persistent as for the other methods. Only 5% of the radioactivity bound at 4 hr after administration was present 1 week later, whereas in BaP-pretreated animals 80% of the bound activity present 4 hr after administration was still present 1 week later.

Because of the extremely high amounts of [^{14}C]-1-NP bound to lung DNA in induced animals, a range of 1-NP doses was instilled in mice induced with BaP to determine the binding over a wide range. A linear relationship was found for bound [^{14}C]-1-NP over a range from 0.25 to 25 mg/kg (Fig. 6). The lowest dose administered was 0.03 mg/kg, in which the amount of bound [^{14}C]-1-NP detected in mouse lung DNA was ~5 times background radioactivity levels.

DISCUSSION

The presence of airborne chemicals with the ability to induce microsomal enzymes suggests that increased microsomal cytochrome-dependent P-450 monooxidase activity could exist in populations with high environmental exposures to airborne chemicals

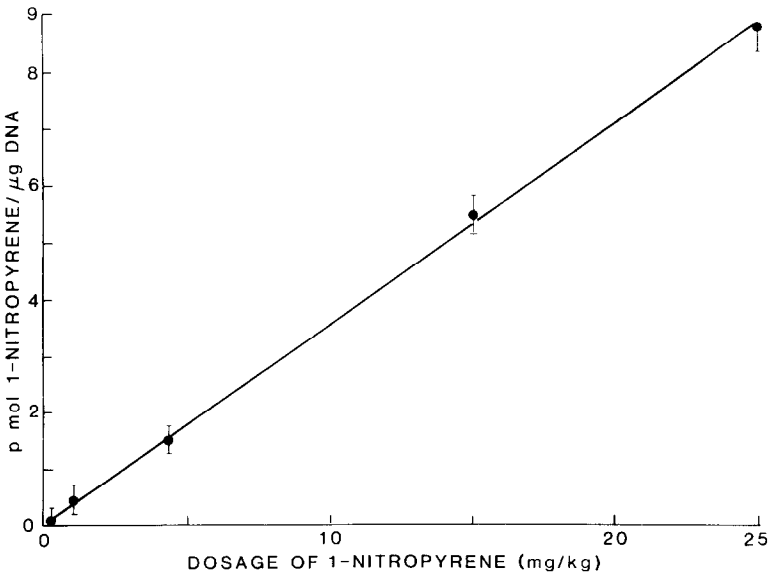

Fig. 6. Effect of dosage of 1-nitropyrene on the covalent binding of 1-nitropyrene metabolites to mouse lung DNA. Mice were treated with BaP 24 hr before administration of [^{14}C]-1-NP; covalent bound material was determined 4 hr later. Each point represents the mean ± S.E. of four mice.

and may contribute to an increase in carcinogenesis from environmental chemicals. A positive correlation was found between cancer and chemicals covalently bound to DNA [19]. It was reported that carcinogenicity is ~3 times better correlated with DNA covalent binding *in vivo* than to mutagenicity in the Ames test [27]. It had been shown previously that cytochrome P-450-dependent monooxidase is induced to maximal levels in mouse lung at 1 day after a single administration of BaP [28], CGC, and Aroclor-1254 [8] and that a positive correlation was found between induction of enzymes and binding of BaP to mouse lung DNA [8]. An excellent correlation has been found between the quantitative binding of chemicals to DNA and carcinogenicity [26]. Where sufficient data are available, the most striking correlation is seen between DNA binding and hepatocarcinogenesis with many different chemical classes [26]. Fewer studies in other target tissues do not show as good a correlation between DNA binding and carcinogenicity. Cohen *et al.* [29] have shown that pretreatment of mice with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits the carcinogenicity of both BaP and dimethylbenzanthracene (DMBA) when they are applied to mouse skin as tumor initiators. Pretreatment with TCDD also causes a marked decrease in the binding of [^{3}H]-DMBA to DNA, whereas the binding of [^{3}H]BaP to DNA is increased markedly, which suggests significant differences in metabolic activation and detoxification of BaP and DMBA. Additional studies showed that butylated hydroxyanisole, α-angelica lactone, and β-naphthoflavone (βNF), in general, inhibit DNA adduct formation in mouse liver and forestomach; however, adducts in the lung remain at the same level or increase slightly [30, 31]. Pretreatment with βNF and aroclor also results in a decrease and increase in total binding, respectively, but the binding does not correlate with specific adduct levels. In these studies the levels of pulmonary enzymes induced by pretreatment were not reported, although it was mentioned in one study that the level of induction was only slight [31]. Thus, it is difficult in these studies to assess the role of induced enzymes in the metabolic activation of xenobiotics in lung tissue. The present study is consistent with those of Eastman *et al.* [32, 33], which showed the appearance of a BaP diol-epoxide-DNA adduct and the persistence of 3-methylcholanthrene (3-MC) bound to DNA after pretreatment with aroclor as an enzyme inducer. Kouri *et al.* [34] reported that an increased formation of 3-MC-induced sarcomas was observed after enzyme induction by TCDD pretreatment. The present study showed that BaP and CGC pretreatments were also effective in increasing the binding of [^{3}H]BaP, [^{3}H]-2-AA and [^{14}C]-1-NP to mouse lung DNA. These data are consistent with Nebert's hypothesis [35], which suggests that tumors and toxicity after administration of carcinogenic polycyclic aromatic hydrocarbons can be expected to occur in proximal tissues of responsive strains, i.e. at sites that have come into direct contact with xenobiotics. Thus, when assessing the effect of enzyme induction on the activation of carcinogens, not only the dose and type of carcinogen but also the route of administration, the time, and the target organs of tumors or toxicity relative to the site of administration are all very important. A difference in enzyme specificity for metabolism and covalent binding of BaP metabolites to DNA after induction by different inducing agents was not apparent in the present study. This suggests that the enzymes induced were similar in specificity for the metabolism of BaP.

2-Aminoanthracene is a potent carcinogen [36]. Its most unusual characteristics are its specificity for epithelial tissues of different animal species and ability to produce malignant tumors of a variety of histological types in rodents [37]. 2-Aminoanthracene is also a potent bacterial mutagen after metabolic activation of microsomal preparations from mammalian organisms [38]. Although the carcinogenic and mutagenic properties of 2-AA are known, little information is available on the *in vivo* metabolic activation of 2-AA and its interaction with mammalian DNA [17]. The present study showed that pretreatment of mice with BaP, Aroclor-1254, or CGC induced microsomal enzymes that were effective in the metabolism of 2-AA to reactive intermediates that bound covalently to lung DNA. The amount of 2-AA or metabolites bound to DNA was similar to the amounts bound by BaP metabolites, but less than the amount bound by 1-NP metabolites. The binding was also persistent at 1 week after administration. Specific inducible enzymes for the metabolism of 2-AA to electrophilic agents were not apparent after induction with BaP, Aroclor-1254, or CGC. These data indicate that specific enzymes involved in the metabolism of primary amines are inducible to approximately the same degree by all three agents used in this study.

1-Nitropyrene is found in airborne particulate matter and in diesel exhaust emissions. Nitropyrenes are among the most mutagenic chemicals in bacteria [18]. 1-Nitropyrene is an animal carcinogen [39] and a direct acting mutagen in the Salmonella assay [18]. It is thought to be metabolically activated by nitroreductases present in bacteria [18]. It has been suggested that the mutagenicity is dependent upon the formation of adducts between DNA and nitropyrene metabolites [40]. The increased amounts of 1-NP covalently bound to lung DNA after pretreatment of mice with BaP, Aroclor-1254, or CGC and the persistence of the apparent 1-NP-DNA adducts indicate that cytochrome P-450 type enzymes are important in the metabolism of nitropyrene in mouse lungs to metabolites that bind to DNA. The positive correlation between the amounts of administered 1-NP and the binding of 1-NP metabolites to lung DNA also suggests that not only are 1-NP metabolites bound tenaciously to mouse lung DNA but that the enzyme system responsible for their metabolism is effective in metabolizing this compound over a wide dose range without any apparent evidence of binding site saturation. This is similar to compounds of many different chemical classes that have been reported to have a linear dose-binding relationship [26].

The increasing amounts of radioactivity bound to rat lung DNA after metabolism of [^{14}C]-1-NP are similar to the increase in radioactivity bound to DNA in bacterial cells after metabolism of 1-NP [40]. How-

ever, it is not known with certainty that nitroreductases are the primary enzymes involved in the metabolism of 1-NP to metabolites that bind DNA [40]. It was reported that xanthine oxidase, a mammalian nitroreductase, catalyzes the binding of [^{3}H]-1-NP to DNA [41]. The present study indicates that CGC contains primarily cytochrome P-448-inducing chemicals because similar levels of binding of 1-NP were found after BaP and CGC pretreatment. In contrast, the amounts of 1-NP bound at different times in Aroclor-1254-induced mice indicate that specific enzymes responsible for the metabolic activation of 1-NP were not induced by polychlorinated biphenyls to the extent induced by CGC or BaP. Aroclor-1254 is known to induce both cytochrome P-450 and P-448 enzyme systems [42]. It is not known which metabolites are formed by induced enzymes and covalently-bound to lung DNA. However, it is known that 3-MC-induced cytochrome P-448 and phenobarbital (and possible Aroclor-1254)-induced P-450 enzymes have different substrate specificities. Induced cytochrome P-448 enzymes have higher *O*-deethylase and monooxygenase activities, preferential metabolism of BaP to 7,8-diols, 9,10-diols and 9-hydroxy-BaP, and 2-acetylaminofluorene to 5-hydroxy, 7-hydroxy, and *N*-hydroxy metabolites [43]. In contrast, induced cytochrome P-450 enzymes have higher *O*- and *N*-demethylase activities and preferentially metabolize BaP to 4,5-diol and 1,6- and 3,6-quinones [42, 43]. Thus, the induction of cytochrome P-448 and P-450 enzymes by xenobiotics may affect the form and persistence of metabolites bound to DNA. Whether the particular enzyme induced is similar to one that has been reported for the metabolism of dinitropyrene in cultured mouse lymphoma cells will require further investigation [44].

The lack of any appreciable binding of phenanthridone (PNDO) indicates that microsomal enzymes important in the metabolism of nitrogen heterocyclics such as PNDO are not present in mouse lung, are present in very low concentration, or are not inducible by agents used in this study. The lack of PNDO binding also indicates that detoxification pathways may be more prevalent for PNDO or that the reactive species may bind to other macromolecules. It has been reported that PNDO has some direct and indirect mutagenic activity in the Ames Salmonella test [45].

Some progress has been made in the characterization of P-450 isoenzymes in rabbit lung [46] and nasal tissues of other species [47]. It was reported also that monooxygenases are induced in rabbit lung [46], in Clara cells, and in Type II cells after β-naphthoflavone treatment [48]. The cytochrome P-450 enzymes in the nose, trachea, and lung may play a major role in the metabolism, disposition, and fate of inhaled xenobiotic material. It has been reported that BaP, 2-AA, 1-NP, and PNDO are all rapidly cleared from the respiratory tract of rats after inhalation [21, 49, 50]. However, the amount retained by tissues may be covalently bound to macromolecules, including DNA [50]. It was found that the amount of parent compound (1-NP) remaining in lung tissue 1 week after instillation was very small; the amount of DNA covalently bound products that would be formed from this parent material would be only a fraction of the bound products actually observed 1 week after instillation of 1-NP.* Thus, it appears that the persistent binding 1 week after instillation is not due to the slow clearance of 1-NP from the lung but, more likely, due to the inefficient removal of 1-NP adducts, which may be affected largely by normal DNA turnover in target cells in the lung [51].

In summary, the present study showed that pretreatment of mice with BaP, Aroclor-1254, and CGC induced cytochrome P-450-dependent monooxygenases that increased binding of several chemicals to lung DNA. This information is consistent with the hypothesis that organs at risk to toxicity from xenobiotics in responsive strains are those that come into direct contact with the xenobiotics. Additional studies are needed to determine the role of organ-specific induced enzymes, their chemical specificity toward different chemicals, the specific DNA adduct or adducts formed, their repair, and their relationships to toxicity and binding of chemicals to DNA.

Acknowledgements—The author acknowledges the excellent technical assistance of Miss Frances Straus and Mr. Mark Binette. The author also thanks Drs. J. A. Bond, A. L. Brooks, A. R. Dahl, J. S. Dutcher, A. F. Eidson, R. F. Henderson, A. J. Howard, R. O. McClellan, S. G. Shami, and J. D. Sun for careful review of this manuscript. Research was performed under U.S. Department of Energy Contract DE-AC04-76EV01013. Research was conducted in facilities fully accredited by the American Association for the Accreditation of Laboratory Care.

* C. E. Mitchell, unpublished results.

REFERENCES

1. Committee on Biological Effects of Atmospheric Pollutants, National Research Council, *Particulate Polycyclic Organic Matter*. National Academy of Sciences, Washington, DC (1972).
2. Environmental Protection Agency, *Scientific and Technical Assessment Report on Particulate Polycyclic Organic Matter*, EPA Publication EPA-600/6-75-001. Environmental Protection Agency, Washington, DC (1975).
3. J. A. Miller and E. C. Miller, in *Biology of Radiation Carcinogenesis* (Eds. J. M. Yuhas, R. W. Tennant and J. E. Regan), p. 147. Raven Press, New York (1976).
4. E. T. Cantrell, G. A. Warr, D. L. Busbee and R. R. Martin, *J. clin. Invest.* **52**, 1881 (1973).
5. F. J. Akin and J. F. Benner, *Toxic. appl. Pharmac.* **36**, 331 (1976).
6. I. P. Lee, K. Suzuki, S. D. Lee and R. L. Dixon, *Toxic. appl. Pharmac.* **52**, 181 (1980).
7. J. F. Payne, *Science* **191**, 945 (1976).
8. C. E. Mitchell, *Fedn Proc.* **41**, 1563 (1982).
9. H. Mukhtar and D. R. Bickers, *Toxic. Lett.* **11**, 221 (1982).
10. G. M. Cohen, P. Votila, J. Hartiala, E-M. Suolinna, N. Simberry and O. Pelkonen, *Cancer Res.* **37**, 2147 (1977).
11. R. A. Mostardi, D. L. Ely, A. Liebelt, S. Grossmann and M. M. Fu, *J. Toxic. environ. Hlth* **7**, 809 (1981).
12. H. V. Gelboin, *Adv. Cancer Res.* **10**, 1 (1967).
13. A. H. Conney and J. J. Burns, *Science* **178**, 576 (1972).
14. R. E. Rasmussen and I. Y. Wang, *Cancer Res.* **34**, 2290 (1974).

15. R. L. Hanson, R. E. Royer, R. L. Carpenter and G. J. Newton, in *Third International Symposium on Chemistry and Biology, Carcinogenesis and Mutagenesis* (Eds. P. W. Jones and P. Leber), p. 3. Ann Arbor Science Publishers, Ann Arbor, MI (1979).
16. F. C. Combes, *Coal Tar and Cutaneous Carcinogenesis in Industry*, p. 15. Charles C. Thomas, Springfield, IL (1954).
17. R. Harpaz and C. E. Mitchell, *Chem. Biol. Interact.* **36**, 129 (1981).
18. R. Mermelstein, D. K. Kirizaides, M. Butler, E. C. McCoy and H. S. Rosenkranz, *Mutation Res.* **89**, 187 (1981).
19. P. Brooks and P. D. Lawley, *Nature, Lond.* **202**, 781 (1964).
20. J. M. Benson, J. O. Hill, C. E. Mitchell, G. J. Newton and R. L. Carpenter, *Archs environ. Contam. Toxic.* **11**, 363 (1982).
21. J. S. Dutcher and C. E. Mitchell, *J. Toxic. environ. Hlth* **12**, 709 (1983).
22. J. D. Brain, D. E. Knudson, S. P. Sorokin and M. A. Davis, *Environ. Res.* **11**, 13 (1976).
23. D. W. Nebert and H. V. Gelboin, *J. biol. Chem.* **243**, 6242 (1968).
24. R. F. Itzhaki and D. M. Gill, *Analyt. Biochem.* **9**, 410 (1964).
25. K. S. Kirby and E. A. Cook, *Biochem. J.* **104**, 254 (1967).
26. W. K. Lutz, *Mutation Res.* **65**, 289 (1979).
27. S. Parodi, M. Taningher, P. Boero and L. Santi, *Mutation Res.* **93**, 1 (1982).
28. C. E. Mitchell, *Res. Commun. Chem. Path. Pharmac.* **28**, 65 (1980).
29. G. M. Cohen, W. M. Bracken, R. P. Lyer, D. L. Berry, J. K. Selkirk and T. J. Slaga, *Cancer Res.* **39**, 4027 (1979).
30. Y. M. Ioannov, A. G. E. Wilson and M. W. Anderson, *Cancer Res.* **42**, 1199 (1982).
31. A. G. E. Wilson, H-C. Kung, M. Boroujerdi and M. W. Anderson, *Cancer Res.* **41**, 3453 (1981).
32. A. Eastman, J. Sweetenham and E. Bresnick, *Chem. Biol. Interact.* **23**, 345 (1978).
33. A. Eastman and E. Bresnick, *Cancer Res.* **39**, 2400 (1979).
34. R. E. Kouri, T. H. Rude, R. Jogiekar, P. M. Dansette, D. M. Jerina, S. A. Atlas, I. S. Owens and D. W. Nebert, *Cancer Res.* **38**, 2777 (1978).
35. D. W. Nebert, in *Host Factors in Human Carcinogenesis* (IARC Scientific Publications No. 39), p. 365. International Agency for Research on Cancer, Lyon, France (1982).
36. M. J. Shear, *J. biol. Chem.* **123**, Cvii (1938).
37. F. Bielschowsky, *Br. J. Path.* **27**, 54 (1946).
38. J. McCann, E. Choi, E. Yamaski and B. N. Ames, *Proc. natn Acad. Sci. U.S.A.* **72**, 5135 (1975).
39. H. Ohgaki, N. Matsukura, K. Morino, T. Kanachi, T. Sugimura, K. Morita, H. Tokiwa and T. Hirota, *Cancer Lett.* **15**, 1 (1982).
40. F. Messier, C. Lu, P. Andrews, B. E. McCarry, M. A. Quilliam and D. R. McCalla, *Carcinogenesis* **2**, 107 (1981).
41. P. C. Howard and F. Beland, *Biochem. biophys. Res. Commun.* **29**, 727 (1982).
42. B. V. Madhukar and F. Matsumura, *Toxic. appl. Pharmac.* **61**, 109 (1981).
43. A. Astrom, J. Meijer and J. W. DePierre, *Cancer Res.* **43**, 342 (1983).
44. J. Cole, C. F. Arlett, J. Lowe and B. A. Bridges, *Mutation Res.* **93**, 213 (1982).
45. J. M. Benson, R. E. Royer, J. B. Galvin and R. N. Shimizu, *Toxic. appl. Pharmac.* **68**, 36 (1983).
46. H. Vainio, in *Concepts in Drug Metabolism* (Eds. P. Jenner and B. Testa), Vol. 10, p. 251. Marcel Dekker, New York (1980).
47. W. M. Hadley, A. R. Dahl, J. M. Benson, F. F. Hahn and R. O. McClellan, *Proc. west. Pharmac. Soc.* **25**, 197 (1982).
48. K. G. Jones, J. F. Holland and J. R. Fouts, *Cancer Res.* **42**, 4658 (1982).
49. J. D. Sun, R. K. Wolff, H. M. Aberman and R. O. McClellan, *Toxic. appl. Pharmac.* **69**, 185 (1983).
50. C. E. Mitchell, *Toxicology* **28**, 65 (1983).
51. P. J. Abbott and F. Crew, *Cancer Res.* **41**, 4115 (1981).