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Invited Review

Mutagenicity of Nitroaromatic Compounds

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Contents

1. Introduction

The pharmacological and toxicological properties of nitroarenes have been the subject of many studies for the past fifty years. As early as the 1950s, 4-nitroquinoline-1-oxide (4-NQO)[1] was reported to be a potent carcinogen (*1*). Studies of its binding to DNA and the mutagenic consequence of this interaction began in the 1960s (*2*−*4*). It is one of the first nitroaromatic compounds in which carcinogenic activity was thought to be initiated by nitroreduction (*5*). In the late 1970s, it became evident that nitroaromatic compounds are ubiquitous in nature. Pitts et al. (*6*) and Jager (*7*) were the first to show that polycyclic aromatic hydrocarbons react with oxides of nitrogen to form nitroaromatics under conditions that might be expected in polluted air and in combustion processes. As a result, nitroaromatic compounds are present in many mixtures such as cigarette smoke, coal fly ash, and diesel exhaust (*8*−*10*). They have

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[1]Abbreviations: 4-NQO, 4-nitroquinoline-1-oxide; 1-NP, 1-nitropyrene; 4-NP, 4-nitropyrene; 1,6-DNP, 1,6-dinitropyrene; 1,8-DNP, 1,8-dinitropyrene; 6-NC, 6-nitrochrysene; nitrofen, 2,4-dichlorophenyl-*p*-nitrophenyl ether; 2-NF, 2-nitrofluorene; DNP, dinitropyrene; 2,5-diNF, 2,5-dinitrofluorene; 2,7-diNF, 2,7-dinitrofluorene; 3-NBA, 3-nitrobenzanthrone; 4-NBP, 4-nitrobiphenyl; AA, aristolochic acid [i.e., 6-nitrophenanthro[3,4-*d*]-1,3-dioxolo-5-carboxylic acid (AAII) and its 8-methoxy dervivative (AAI)]; 8-OH-dG, 8-hydroxydeoxyguanosine; dG-C8-AP, *N*-(deoxyguanosin-8-yl)-1-aminopyrene; 2-NP, 2-nitropyrene; 6-AC-1,2-dihydrodiol, *trans*-1,2-dihydro-1,2-dihydroxy-6-aminochrysene; 2-AF, 2-aminofluorene; dG-C8-AF, *N*-(deoxyguanosin-8-yl)-2-aminofluorene; dG-C8-AAF, *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene; dG-*N*-Ac-ABA, *N*-acetyl-3-amino-2-(deoxyguanosin-8-yl)benzanthrone; dG-C8-4-ABP, *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl; AF2, furylfuramide; FANFT, formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide; ANFT, 2-amino-4-(5-nitro-2-furyl)thiazole; nitrofen, 2,4-dichlorophenyl-4-nitrophenyl ether; 1-NOP, 1-nitrosopyrene; CHO, Chinese hamster ovary; *hprt*, hypoxanthine guanine phosphoribosyl transferase; XP, xeroderma pigmentosum; HCMM, hereditary cutaneous malignant melanoma; 2-AAF, 2-acetylaminofluorene; DB[*a*,*h*]A, dibenz[*a*,*h*]anthracene; 7-NDB-[*a*,*h*]A, 7-nitrodibenz[*a*,*h*]anthracene; CASE, computer-assisted structure evaluation; QSAR, quantitative structure−activity relationship.

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also been detected in grilled foods (*11*, *12*). In addition, several nitroaromatic compounds found their use in the chemical industry, and a group of nitrofurans and nitroimidazoles has been marketed worldwide as drugs. Therefore, human exposure to one or more nitroaromatic compound could occur by a variety of routes.

The "Eighth Report on Carcinogens" published by the National Toxicology Program listed several nitroaromatic compounds as "reasonably anticipated to be a human carcinogen", which include *o*-nitroanisole, 1-nitropyrene (1-NP), 4-nitropyrene (4-NP), 1,6-dinitropyrene (1,6-DNP), 1,8-dinitropyrene (1,8-DNP), 6-nitrochrysene (6-NC), and nitrofen (2,4-dichlorophenyl-*p*-nitrophenyl ether) (*13*). In addition, the International Agency for Research on Cancer lists 2-nitrofluorene (2-NF) as possibly carcinogenic to humans (*10*). Although a convincing link based on epidemiological data is yet to be established, several members of the nitroaromatic compound family have been implicated in the etiology of breast cancer (*14*).

o-Nitroanisole is used mainly as a precursor to *o*-anisidine, a compound used in the synthesis of more than 100 different azo dyes. *o*-Nitroanisole is also used in the pharmaceutical industry as a synthetic intermediate.

The nitropyrenes are common environmental pollutants (*9*, *15*). These compounds have also been detected in food (*11*, *12*). 1-NP is one of the major mutagenic components of diesel particulates, whereas the dinitropyrenes (DNPs) (substituted at 1,3-, 1,6-, and 1,8-positions) are present in much lower concentrations (*9*, *15*). 1-NP accounts for approximately 25% of the mutagenicity of diesel emissions (*8*). On an equimolar dosage basis, however, the dinitropyrenes are much more mutagenic (*8*, *16*). All the mono- and dinitropyrenes are carcinogenic in experimental animals (*17*−*20*). 1,6-DNP is the most potent carcinogen within the group, whereas 1,8-DNP is the most mutagenic in several assays. The three DNPs have recently been detected in surface soil samples in Japan (*21*). Even though the unequivocal demonstration of the carcinogenicity of these compounds in humans is lacking, an association of 1-NP with human lung cancer has been shown (*22*, *23*). In experimental animals, 1-NP induces malignancy in various tissues, but an NIEHS study determined that it has a high likelihood of being carcinogenic to the respiratory tract (*23*). Lung specimens collected from lung cancer patients from China and Japan showed a significant concentration of benzo[*a*]pyrene and 1-NP (*22*).

6-NC, another component of combustion, has been detected in many mixtures, including coke oven emissions (*24*). It is weakly mutagenic in bacteria but a potent tumorigen in experimental animals (*25*, *26*). In newborn mice, it was the most active tumorigen among the nitroaromatics that were tested (*26*, *27*). 6-NC was shown to induce lung and liver tumors in mice and mammary and colon tumors in rats (*28*−*32*).

2-NF, like 1-NP, is one of the most abundant nitroaromatic compounds isolated from combustion processess. Other nitrofluorenes that have been identified in the environment include 2,5-dinitrofluorene (2,5-diNF), 2,7-dinitrofluorene (2,7-diNF), and 9-oxo derivatives of several nitrofluorenes. In addition, 9-oxo-2,4,7-trinitrofluorene is a fungicide and an industrial chemical. In Tokyo, the concentration of 2,7-diNF is 30-fold greater than 2-NF levels and 10-fold greater than 1-NP levels (*33*). 2-NF is mutagenic in bacterial and mammalian cells and carcinogenic in rodents (*34*−*36*). A review by Beije and Moller (*37*) provides the sources and biological effects of the nitrofluorenes.

3-Nitrobenzanthrone (3-NBA) has recently been identified as a member of a new class of mutagens isolated from organic extracts of diesel fuel and airborne particles in amounts similar to those of dinitropyrenes. 3-NBA ranks with 1,8-DNP among the strongest mutagens ever tested in the Ames assay.[2] Nitrobenzanthrones are most likely formed in combustion processes and from reaction of benzanthrone (whose presence in the environment is well documented) with nitrogen oxides. Dinitro derivatives of benzanthrone (such as 3,9- and 3,11-dinitrobenzanthrone) were found to exhibit lower mutagenicities. It is interesting that in the case of nitropyrenes and nitrofluorenes, the opposite trend has been observed (*38*).

Incomplete combustion also forms several nitrofluoranthenes. 2-Nitrofluoranthene and smaller amounts of 3- and 8-nitrofluoranthene have been identified in ambient air particulates (*39*). In addition, the 3,7- and 3,9-dinitrofluoranthene isomers have been detected in airborne particulates, although present in much smaller amounts (*40*). A review by Tokiwa et al. (*41*) describes in detail the mutagenicity and carcinogenicity data of these compounds.

4-Nitrobiphenyl (4-NBP), a chemical of industrial origin, is mutagenic in bacteria and exhibits carcinogenic activity in dog bladders (*42*, *43*). The reduced derivative of 4-NBP, 4-aminobiphenyl, is a bladder carcinogen in humans and dogs (*44*, *45*).

Nitrofen was used as a contact herbicide by growers of rice, broccoli, cauliflower, cabbage, brussels sprouts, onion, garlic, and celery. It was also used in nurseries. The estimated direct crop use of nitrofen was 882 000 lb in 1980. It was withdrawn from the market, however, after demonstration of its carcinogenicity in mammals (*46*, *47*).

Nitrofurans have been widely used in human and veterinary medicine, as antibacterials for topical and urinary tract infections, and as food preservatives. Many of these have been withdrawn from commercial use because of genotoxic and carcinogenic activities associated with this group of compounds (*48*).

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[2]The Ames test using reversion of histidine auxotrophs of *S. typhimurium* has been developed by Bruce Ames and co-workers (*207*, *208*). This is the most widely used short-term assay in screening for compounds for potential mutagenic activity. Reversion of TA1535 results from a base pair substitution in G46 of the *hisG* gene encoding phosphoribosyl ATP synthetase, which is required for histidine synthesis. A two-base deletion of CG from a CGCGCGCG sequence in the histidine dehydrogenase gene is responsible for the reversion of TA1538 (*207*). Reversion is detected by the appearance of colonies capable of growing in the absence of exogenous histidine. TA1535 and TA1538 also carry a *uvrB* deletion, which eliminates a DNA repair system that protects against damage from ultraviolet light. In addition, these strains carry a *rfa* mutation that changes the bacterial cell wall to make it more permeable to nonpolar compounds such as polycyclic aromatic hydrocarbons. These two mutations enhance the sensitivity of the assay by making the strains more susceptible to the mutagenic effects of activated chemicals. Strains TA100 and TA98 are derivatives of TA1535 and TA1538, respectively, which carry plasmid pKM101 (*208*). pKM101 carries the *mucAB* genes (*209*), which are active analogues of *E. coli umuDC* genes. It enhances the frequency of mutations by a number of DNA damages. Several *Salmonella* strains lacking one or more nitroreductase enzymes were developed to assess the relative contribution of nitroreduction toward the mutagenicity of nitroaromatic compounds (*210*−*213*). TA98NR and TA98/1,8-DNP_6 are derivatives of TA98 that lack the "classical" nitroreductase and *O*-acetyltransferase, respectively (*214*−*216*). Many assays incorporate a postmitochondrial supernatant fraction from rat liver, commonly termed "S9", as a preincubation or coincubation step. This homogenate contains a mixed-function oxygenation system that may influence the metabolism of the compounds tested in the Ames assay.

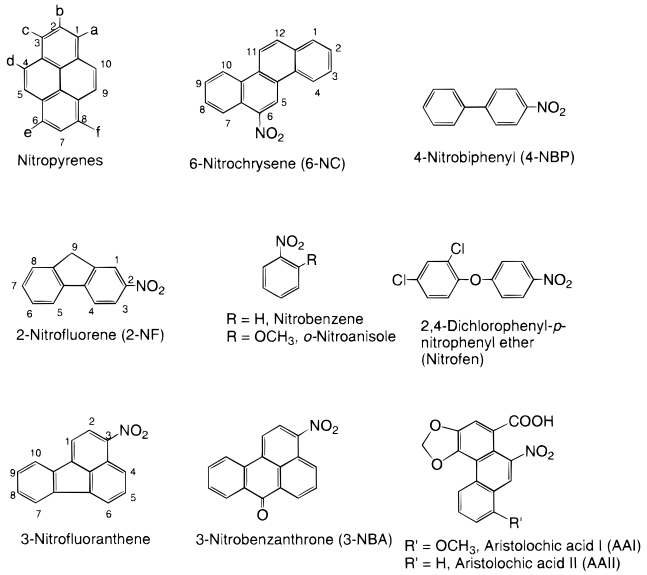


Figure 1. Chemical structures of the nitroaromatic compounds disccused in this article. For the nitropyrenes, a is NO_2 and b−f are H [1-nitropyrene (1-NP)], b is NO_2 and a and c−f are H [2-nitropyrene (2-NP)], d is NO_2 and a−c, e, and f are H [4-nitropyrene (4-NP)], a and c are NO_2 and b and d−f are H [1,3-dinitropyrene (1,3-DNP)], a and e are NO_2 and b−d and f are H [1,6-dinitropyrene (1,6-DNP)], and a and f are NO_2 and b−e are H [1,8-dinitropyrene (1,8-DNP)].

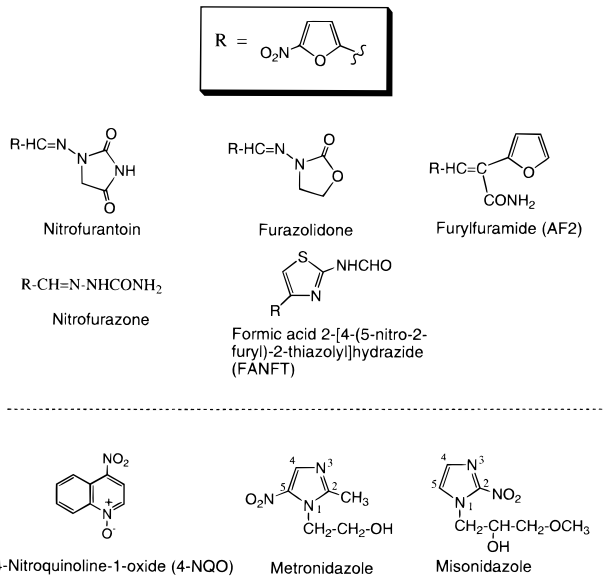


Figure 2. Chemical structures of representative nitrofurans and other heteroaromatic compounds discussed in this article.

Nitroimidazoles are a family of heterocyclic compounds that, like the nitrofurans, have been used as drugs in humans and animals. 5-Nitroimidazoles, such as metronidazole, have been widely prescribed to fight *Trichomonas vaginalis* and other infections in humans. Also, 2-nitroimidazoles, such as misonidazole, have been used as radiosensitizers to increase the effect of therapeutic radiation or as a diagnostic tool to assess the degree of hypoxia of a tissue (*49*). Many of these compounds are mutagenic in bacteria, but the mutagenic effect in mammalian systems appears to be greatly reduced (*50*).

Other nitroaromatic carcinogens include 1-nitronaphthalene, which is formed during gas-phase atmospheric reaction in the South Coast air basin of California (*51*), and aristolochic acid (AA), a mixture of naturally occurring carboxylic acids isolated from the leaves and roots of *Aristolochia* (a plant genus). A limited number of studies were performed with 1-nitronaphthalene. Even so, it was shown to be metabolized by P450 in both mouse and rat, and naphthylamine was one of the metabolites that was detected (*52*, *53*). Herbal therapies using extracts from the aristolochia plant have been used in Chinese herbal therapy for years. When these extracts were found to be both mutagenic and carcinogenic, licensing of drugs using these extracts was revoked in Germany in 1981. Antiinflammatory agents that used AA in their preparations were withdrawn from the market in 1982 (*54*−*57*).

As in the case of polycyclic aromatic hydrocarbons, the nitroaromatics exhibit widely different mutagenic and tumorigenic activities, which evidently stem from their differences in metabolism, DNA adduct formation, and repair. Even though many studies have been carried out in these areas, the data are extremely complex and dependent upon many factors. Recent progress in molecular biology and DNA technology is now beginning to influence the biomedical research of nitroarenes, and there is a need to examine the existing information base to identify future directions. We have therefore combined the data from many laboratories to provide an overview of the sequences that lead to mutagenesis by this group of chemicals. Structures of the compounds discussed in this article are shown in Figures 1 and 2. We wish to emphasize that, in addition to the ones already cited, there are several excellent reviews on the biological effects of nitroarenes, which include Sugimura and Takayama (*58*), Tokiwa et al. (*59*), Beland and Kadlubar (*60*), Fu (*61*), Beland and Marques (*62*), and Fu et al. (*63*). These articles should complement the current review.

2. Metabolism and DNA Binding

A common metabolic fate of most nitroaromatic compounds is nitroreduction [see earlier reviews by Rosenkranz and Howard (*64*), Beland and Kadlubar (*60*), Fu (*61*), and Beland and Marques (*62*)]. Oxidative pathways also contribute to the metabolism of many of these compounds. Considerable variation in the enzymes responsible for nitroreduction and oxidation has been observed in different organisms. In humans, xanthine oxidase and microsomal NADPH-cytochrome *c* have been identified as the enzymes involved in nitroreduction, whereas the cytochrome P450 family of enzymes is primarily responsible for the oxidative metabolism of these compounds.

2.1. 4-Nitroquinoline-1-oxide. 4-NQO is reductively activated to the proximate carcinogen 4-hydroxyaminoquinoline-1-oxide, which is acylated by a complex formed by the enzyme seryl-tRNA-synthetase in the presence of ATP, serine, and Mg^{2+} (*5*). This acetyl ester of 4-hydroxyaminoquinoline-1-oxide forms adducts at N^2 and C8 of guanine and N^6 of adenine (*65*, *66*). The N^2 guanine adduct is the major lesion formed both in vitro and in vivo (*67*). Intraperitoneal injection of 4-hydroxyaminoquinoline-1-oxide into Wistar rats led to the formation of 3-(deoxyguanosin-N^2-yl)-4-aminoquinoline-1-oxide (**A** in Figure 3, 50%), *N*-(deoxyguanosin-8-yl)-4-aminoquinoline-1-oxide (**B** in Figure 3, 30%), and 3-(deoxyadenosin-N^6-yl)-4-aminoquinolone-1-oxide (**C** in Figure 3, 10%) (*65*). The reaction of 4-acetoxyaminoquinoline-1-oxide

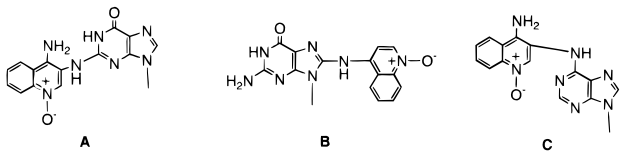
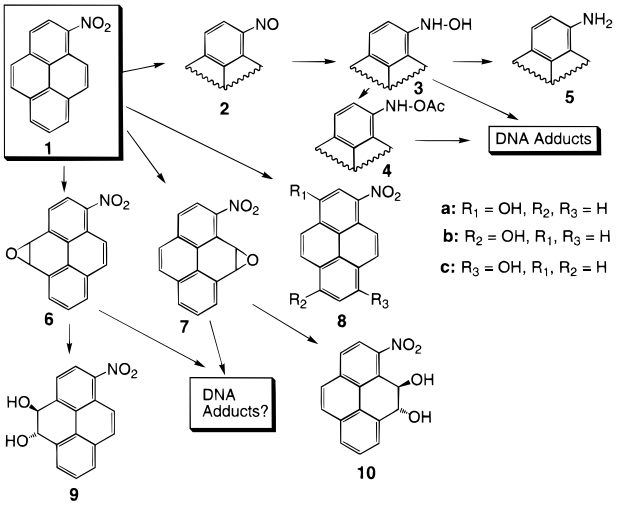


Figure 3. DNA adducts formed by 4-NQO.

with native DNA at 0 °C gives >75% of the N^2 guanine adduct, whereas the reaction with denatured DNA forms mainly the C8 guanine adduct (*68*). 4-Hydroxyaminoquinoline-1-oxide also generates reactive oxygen species, which in turn induce oxidative base damage such as 8-hydroxyguanine and induce strand breakage (*69*, *70*). 4-NQO is a strong inducer of the *soxRS* regulatory system of *Escherchia coli* (*71*). It has been suggested that the 4-NQO-induced oxidative stress might contribute to the tumor-promoting activity of 4-NQO. Part of the mutagenicity of 4-NQO may indeed arise from 8-hydroxydeoxyguanosine (8-OH-dG) formation as a result of 4-NQO metabolism. Increased levels of 8-OH-dG were found following treatment of calf thymus DNA with 4-hydroxyaminoquinoline-1-oxide in the presence of seryladenosine monophosphate (*69*). Furthermore, increased mutation rates were found in *Salmonella* strains deficient in 8-hydroxyguanine DNA glycosylase. Similar results were observed with 2-NF. It was suggested that in a uvr^+ background, 8-OH-dG contributes more to mutagenicity than the adducts derived from the activated forms of 4-NQO (*72*). For additional information about the metabolism and DNA adduct formation of 4-NQO, see Bailleul et al. (*73*).

2.2. Nitrated Pyrenes. 1-NP (**1**), the most abundant nitroaromatic compound in many environmental samples, undergoes both ring oxidation and nitroreduction (*6*, *9*, *74*). In bacteria, nitroreduction of 1-NP is a critical pathway that leads to mutagenesis. In *Salmonella typhimurium*, 1-NP is slowly converted to 1-aminopyrene (**5**) (*75*). A minor amount of *N*-acetyl-1-aminopyrene (**4**) was also detected. Mutation assays in nitroreductase-deficient strains of *S. typhimurium* resulted in reversion frequencies much lower than those from assays performed with nitroreductase-proficient strains (*8*) (vide infra). *N*-Hydroxy-1-aminopyrene (**3**) or its *O*-esterified derivative (e.g., **4**) formed by an enzymatic conjugation is believed to be the ultimate carcinogen in mammals (Scheme 1).

Scheme 1. Metabolic Pathways of 1-NP



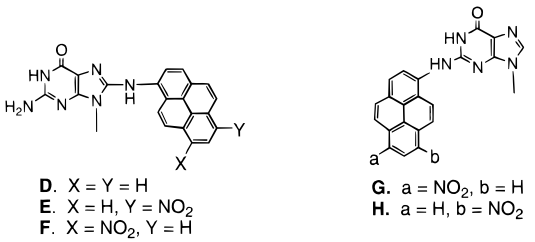


Figure 4. Structures of DNA adducts formed by 1-NP, 1,6-DNP, and 1,8-DNP: **D**, dG-C8-AP; **E**, dG-C8-amino-6-NP; **F**, dG-C8-amino-8-NP; **G**, 6-dG-N^2-AP; and **H**, 8-dG-N^2-AP.

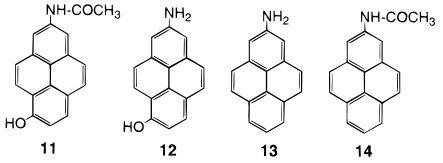


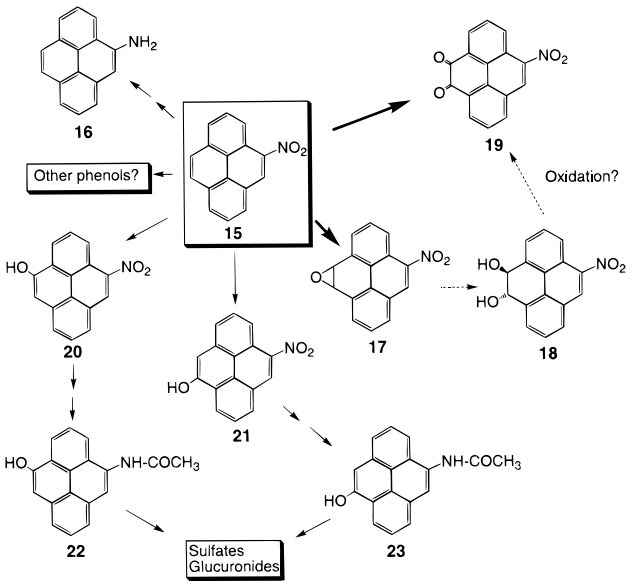
Figure 5. Fecal metabolites following 2-NP administration to female Sprague-Dawley rats.

The major DNA adduct formed by reductively activated 1-NP both in vitro and in vivo is *N*-(deoxyguanosin-8-yl)-1-aminopyrene (dG-C8-AP) (**D** in Figure 4) (*76*). Two minor adducts isolated in one in vitro study are 6- and 8-(deoxyguanosin-N^2-yl)-1-aminopyrene (6- and 8-dG-N^2-AP) (**G** and **H** in Figure 4) (*77*).

In mammals, 1-NP also undergoes oxidative metabolism through the cytochrome P450 family. Major metabolites isolated following 1-NP incubation with the rat liver S9 fraction include 1-nitropyren-3-ol (**8a**), 1-nitropyren-6-ol (**8b**), 1-nitropyren-8-ol (**8c**), and *trans*-4,5-dihydro-4,5-dihydroxy-1-nitropyrene (**9**) (Scheme 1) (*78*). Analysis of metabolites isolated from incubations of 1-NP with guinea pig liver microsomes revealed the presence of two additional metabolites, 1-nitropyrene-4,5-oxide (**6**) and 1-nitropyrene-9,10-oxide (**7**) (*79*). Although both oxides are strongly mutagenic in TA98, it remains to be established whether the oxidative pathway contributes to mutagenicity and tumorigenicity in mammals.

Two other mononitropyrenes, 2-NP and 4-NP, have been detected in urban air, albeit at a much lower level than 1-NP. Like 1-NP, the other two mononitropyrenes undergo both reduction and ring oxidation, and sulfate and glucuronide derivatives of some of the metabolites have been isolated (*80*−*82*). The mutagenic and tumorigenic potencies of the three mononitropyrenes are very different. In bacteria, 4-NP is significantly more mutagenic than 2-NP, which in turn is more mutagenic than 1-NP (*83*). 4-NP is the strongest tumorigen in the lung and liver of male newborn mice as well as in mammary pads of weanling female CD rats (*27*, *84*, *85*). 2-NP undergoes reductive metabolism like 1-NP. Four fecal metabolites were detected following administration of 2-NP in female Sprague-Dawley rats: 6-hydroxy-2-acetylaminopyrene (**11**, 19.5%), 6-hydroxy-2-aminopyrene (**12**, 10.4%), 2-aminopyrene (**13**, 10%), and 2-acetylaminopyrene (**14**, 0.8%) along with unmetabolized 2-nitropyrene (10%) (Figure 5) (*81*). Analysis of the DNA hydrolysate of rats treated with radiolabeled 2-NP showed that only a small proportion (∼2%) of the radiolabel is associated with isolated *N*-(deoxyguanosin-8-yl)-2-aminopyrene and *N*-(deoxyadenosin-8-yl)-2-aminopyrene. Unlike 1-NP and 2-NP, which form phenols and *trans*-dihydrodiols as major metabolites, 4-nitropyrene-9,10-dione (**19** in Scheme 2) is a major metabolite of 4-NP in rat liver microsomes

Scheme 2. Metabolic Pathways of 4-NP



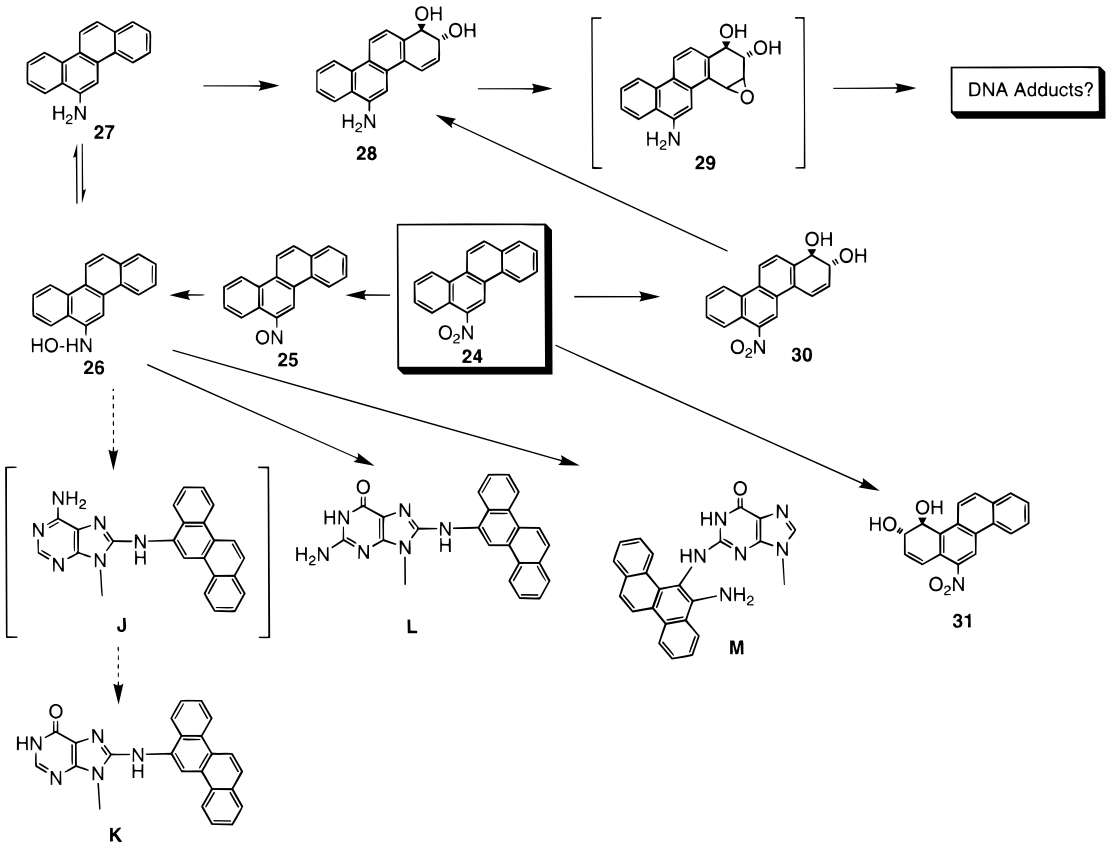
(*82*). Although it was not established how 4-NP-9,10-dione is formed, a likely route involving hydrolysis of 4-NP-9,10-oxide (**17**) to *trans*-9,10-dihydro-9,10-dihydroxy-4-nitropyrene (**18**) followed by oxidation of the latter has been suggested (Scheme 2).[3] In female Sprague-Dawley rats, 1- and 2-NP metabolites and unreacted parent compounds are excreted mainly via the intestinal tract, whereas with 4-NP, the level of urinary excretion is slightly higher than that of fecal excretion products (*19*, *81*, *82*). The excretion products of 4-NP included 9- and 10-hydroxy-4-nitropyrene[4] (**21** and **20**, respectively) and 4-aminopyrene (**16**). DNA adducts from the mammary gland of female CD rats following ip injection of 4-NP have been isolated (*86*). These adducts are chromatographically similar to those isolated from incubations of 4-NP with xanthine oxidase and calf thymus DNA, indicating that nitroreduction is the principal pathway responsible for 4-NP DNA adduction. However, the isolated adducts are not similar to the chemically synthesized C8 guanine adduct formed from the reaction of *N*-acetoxy-4-aminopyrene with dG (*87*). The isolated adducts also exhibit properties different from those of adducts derived from incubation of 4-NP-9,10-oxide (**17**) with calf thymus DNA. One of the adducts isolated from rats was proposed to be a deoxyinosine adduct. A rationale suggested for the latter was that nitroreduction of 4-NP is accompanied by deamination of adenine to inosine in a manner similar to that observed for 1-NP incubation with xanthine oxidase in which deamination of cytosine was observed (*88*). In a recent study, the metabolism of 1-, 2-, and 4-NP by human hepatic and pulmonary microsomes was examined (*89*). The metabolic profiles from both microsomal preparations were similar. However, the levels of metabolites were much lower for pulmonary microsomes. The major metabolites of 1-NP were *trans*-4,5-dihydro-4,5-dihydroxy-1-nitropyrene (**6** in Scheme 1) and 3-, 6-, and 8-hydroxy-1-nitropyrene (**8a**−**c** in Scheme 1).

The dinitropyrenes undergo a reductive activation pathway similar to that of 1-NP. Unlike 1-NP, however, it was determined that the role of the *O*-esterification enzyme is critical for the mutagenic activity of 1,6- and 1,8-DNP in *Salmonella* after experiments showed that both nitrosonitropyrenes exhibited a sharp reduction in the number of revertants in strains that lack the esterification enzymes relative to normal and nitroreductase-deficient strains (*90*). The major DNA adduct of reductively activated dinitropyrenes is 1-*N*-(deoxyguanosin-8-yl)amino-8(6)-nitropyrene (*91*−*93*) (**E** and **F** in Figure 4). The ability of human and rat liver microsomes to detoxify the three dinitropyrene isomers was shown by assessing *umu* gene expression (as measured by β-glactosidase activity) of incubations in *Salmonella* TA1535/pSK1002. The genotoxicities of all three DNPs were reduced to a similar extent following incubation with rat liver microsomes. In human microsomes, the ability to detoxify 1,3-dinitropyrene (1,3-DNP) was greatly enhanced compared to the 1,6 and 1,8 isomers. In rats, it appears that P450 IA1 and IA2 are responsible for detoxification of the three DNPs. However, in humans the picture is more complex in that different P450s appear to be responsible for the detoxification of the three DNPs (*94*). There has also been some work correlating nitroreduction of 1,6-DNP with the generation of reactive oxygen species (*95*−*97*). Although 1,6-DNP reduction does not appear to be oxygen sensitive, reduction of 1-nitro-6-nitrosopyrene results in the formation of metabolites which can react with oxygen (*96*). These findings correlate with a study in which increased levels of 5-(hydroxymethyl)deoxyuridine were observed in rats following a single injection of 1,6-DNP (*98*).

2.3. 6-Nitrochrysene. The metabolism of 6-NC is unique in comparison to most of the nitroaromatic compounds. The extent of the in vitro reaction of DNA with *N*-hydroxy-6-aminochrysene (**26** in Scheme 3) was 2−10 times greater than those of several other *N*-hydroxyarylamines, and this reaction resulted in the formation of *N*-(deoxyinosin-8-yl)-6-aminochrysene (**K**), *N*-(deoxyguanosin-8-yl)-6-aminochrysene (**L**), and 5-(deoxyguanosin-N^2-yl)-6-aminochrysene (**M**) (Scheme 3) (*99*). The deoxyinosine adduct was presumed to be derived from spontaneous oxidation of the corresponding deoxyadenosine adduct (**J**). However, the major DNA adduct isolated from preweanling mice did not cochromatograph with any of these three adducts (*100*). The major adduct detected in mice is chemically similar and chromatographically identical to that obtained from incubation of *trans*-1,2-dihydro-1,2-dihydroxy-6-aminochrysene (6-AC-1,2-dihydrodiol) (**28**), calf thymus DNA, and liver microsomes isolated from rats fed 3-methylcholanthrene to induce metabolic enzymes. On the basis of these observations, 6-AC-1,2-dihydrodiol appears to be the most critical metabolite of 6-NC that leads to DNA adduct formation in the preweanling mouse (Scheme 3). Metabolic activation of 6-NC in rats involves both nitroreduction and a combination of ring oxidation and nitroreduction (*101*). DNA adducts derived from both pathways were detected in the target organ, the colon, and in nontarget organs such as lung, liver, and mammary tissues, although the adducts derived from the combination pathways predominated. The major metabolites identified following incubation of 6-NC with human hepatic and pulmonary microsomes were *trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene (**30**), *trans*-9,10-dihydro-9,10-dihydroxy-6-nitrochrysene (**31**), 6-AC-1,2-dihydrodiol (**28**), 6-aminochrysene (**27**), and chrysene-5,6-quinone. P450s IA2

[3]Compound **18** was not isolated in this study.
[4]Elution times for compounds **20** and **21** were similar under the experimental conditions that were employed, and therefore, the relative ratio of each could not be determined.

Scheme 3. Metabolism of 6-NC



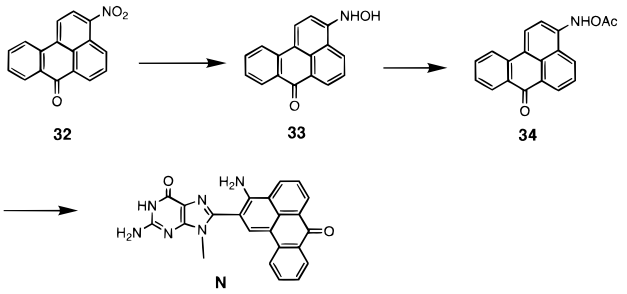
and IA1 are the major forms responsible for the metabolism of 6-NC to *trans*-1,2-dihydro-1,2-dihydroxy-6-nitrochrysene (**30**) in hepatic and pulmonary microsomes, respectively. The reductive metabolism of 6-NC to 6-aminochrysene (**27**) has been attributed to P450 IIIA4 and IIE1 (*102*).

2.4. 2-Nitrofluorene. The metabolic fate of 2-NF is complex. In *Salmonella*, it has been suggested that part of the mutagenicity of 2-NF is a result of metabolism that produces 8-OH-dG. *Salmonella* strains deficient in 8-hydroxyguanine DNA glycosylase were shown to exhibit enhanced mutagenicity when incubated with 2-NF. It may be the case that 2-NF undergoes redox cycling of the nitro group producing reactive oxygen species which form lesions such as 8-OH-dG (*72*). 2-Aminofluorene (2-AF), the fully reduced form of 2-NF, has been shown to share some common intermediate metabolites with 2-NF. The only adducts of 2-NF that have been fully characterized are identical to those obtained from 2-AF metabolism. The metabolism, DNA binding, and mutagenesis that result from 2-AF have been extensively studied [see Heflich and Neft (*103*) for a review]. In rats, intestinal microflora are believed to be responsible for much of the nitroreductive pathway. Administration of 2-NF to rats resulted in the formation of several adducts in rat liver. Even though the major adduct is yet to be characterized, one of the two minor adducts was identified as *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF). The identical major adduct was isolated after administration of 2-NF to bile duct-catheterized rats, indicating that intestinal microflora are probably not responsible for formation of this adduct. In vitro experiments, in which inhibitors of epoxide hydrolase were used, suggest that epoxidation may be involved in its metabolic activation (*104*). In a related study, Moller et al. (*105*) have observed a 6-fold increase in the mutagenicity of urine excreted by germ free rats compared to conventional rats when both were treated with 2-NF. This increase in mutagenicity is attributed to an increase in the level of hydroxylated 2-NFs present in the urine of germ free rats. These findings support the notion that the major mutagenic metabolites are not derived from microflora metabolism. In another study, following oral administration of 2-NF to rats, the major adduct isolated from rat liver was characterized as dG-C8-AF, while the minor adduct was *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-C8-AAF) (*106*). In a subsequent study, long-term administration of 2-NF to rats produced four adducts. Two of the adducts were identified as dG-C8-AF and the N^2 guanine adduct of acetylaminofluorene (*107*). While nitroreduction appears to be the primary metabolic pathway when 2-NF is orally administered to rats, the oxidative pathway is the major pathway when 2-NF is administered intratracheally or intravascularly (to mimic inhalation) (*108*).

2.5. 3-Nitrobenzanthrone. The direct-acting mutagenicity of 3-NBA in TA98 is comparable to that of 1,8-DNP, one of the strongest mutagens tested in this assay. Its mutagenicity in YG1024, which contains a plasmid expressing enhanced *O*-acetyltransferase activity, was shown to be 30 times greater, suggesting that acetylation may be an important step in activation (*38*). It is therefore likely that 3-NBA undergoes nitroreduction and acetylation, which either directly or after formation of a nitrenium ion reacts with dG and dA. Incubation of the acetoxyacetyl derivative of 3-NBA with calf thymus DNA

produced one major adduct that was identified as *N*-acetyl-3-amino-2-(deoxyguanosin-8-yl)benzanthrone (dG-*N*-Ac-ABA) (**N** in Scheme 4). This adduct with a covalent

Scheme 4. A Likely Route to dG Adduction by 3-NBA



linkage at the carbon next to the amino group probably results from stabilization of the nitrenium ion with a charge at the C-2 position of 3-NBA (Scheme 4) (*109*). Incubation of 3-NBA with rat liver S9 homogenate under aerobic conditions produced an adduct profile similar to that of incubation of 3-NBA with xanthine oxidase, a mammalian nitroreductase, and incubation with zinc, a reducing agent. The observation that incubation with the homogenate is similar to incubation with substances capable of nitroreduction suggests that nitroreduction is the major pathway for adduction (*110*).

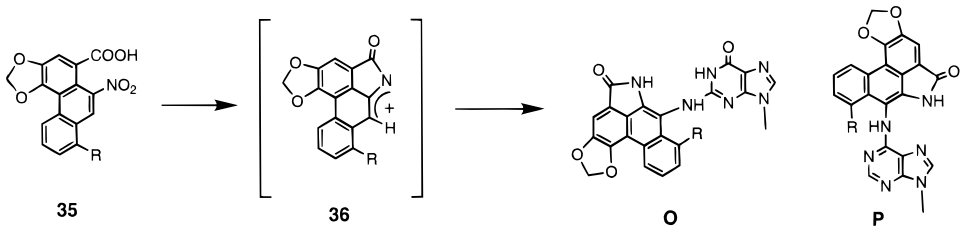
2.6. 4-Nitrobiphenyl. 4-NBP, like its reduced derivative 4-aminobiphenyl, is a bladder carcinogen. Its metabolic activation pathway has been postulated to be nitroreduction. The level of reversion induced by 4-NBP is much lower in TA98NR than in TA98. However, preincubation of 4-NBP with NADH and xanthine oxidase results in a dose-dependent increase in reversion frequency in TA98NR. Furthermore, the mutagenic potency of *N*-hydroxy-4-aminobiphenyl is equal in TA98 and TA98NR, suggesting that it may be the proximate carcinogen. Also, 4-NBP mutagenesis in TA98NR is activated by rat liver and dog bladder cytosol and inhibited by allopurinol, a xanthine oxidase inhibitor, suggesting a link between the chemical and its target organ (*111*). 4-NBP reductase activity was measured in both human lung microsomes and cytosol. The major metabolite that was detected was 4-aminobiphenyl, although a small amount of *N*-hydroxy-4-aminobiphenyl was also observed. The major adduct derived from *N*-hydroxy-4-aminobiphenyl is *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-4-ABP) (70%) with two other minor adducts detected, the N^2 adduct of guanine and the C8 adduct of adenine. Another possible pathway for metabolic activation involves reduction to 4-aminobiphenyl and subsequent peroxidation by myeloperoxidase. However, the dG-C8-4-ABP adduct accounts for only 10−20% of the 4-aminobiphenyl adducts derived from peroxidative activation; the remainder of the adducts have not yet been characterized (*112*).

2.7. 2,4-Dichlorophenyl-*p*-nitrophenyl Ether. Nitrofen falls into a general category of 4-nitrodiphenyl ethers that have been used as herbicides. Several of these 4-nitrodiphenyl ethers are mutagenic (*113*). Nitrofen is carcinogenic and teratogenic in rats (*46*, *47*). In rats, nitrofen is metabolized primarily through a nitroreduction pathway. Products isolated include amino and acetylamino derivatives (*46*). As with other nitroaromatic compounds, it is believed that these nitrodiphenyl ethers exhibit mutagenicity upon interaction of a nitroreduction intermediate with DNA. The observation that the mutagenicity of the *N*-hydroxy derivative is higher than that of the nitroso derivative, which in turn is higher than that of the parent nitro compound, is consistent with this hypothesis (*114*).

2.8. Nitrobenzene. Nitrobenzene is mutagenic in *Salmonella* only with preincubation with S9 homogenate and norharmon, a component of cigarette smoke (*115*). The mutagenic mechanism probably involves formation of a conjugate with norharmon followed by metabolic activation of the conjugated species with S9 as was demonstrated in the case of aniline (*116*). The observation that both nitrobenzene and aniline require norharmon implies that these compounds are not mutagenic, but are capable of forming conjugates with compounds present in the environment which, after metabolic activation, exhibit mutagenic activity. However, many derivatives of nitrobenzene (e.g., nitrotoluene, nitroanisole, nitrobenzaldehyde, etc.) are weakly mutagenic in the Ames test or by a *rec* assay (*117*). The precise route by which nitrobenzene exhibits its carcinogenic and toxic effect is still unclear. Recently, Ohkuma and Kawanishi (*118*) have shown that nitrosobenzene, a metabolite of nitrobenzene, causes oxidative DNA damage in the presence of NADH and Cu(II). The DNA damage included 8-OH-dG and DNA cleavage at C and T residues. ESR studies confirmed that nitrosobenzene is reduced by NADH (nonenzymatically) to the phenylhydroxide radical. However, no DNA damage is observed in the absence of Cu(II). A possible mechanism of DNA damage involving Cu(II) and hydrogen peroxide has been proposed.

2.9. Nitrofurans. Because of their antibacterial activity, nitrofurans have found widespread use as human and veterinary medicines, and as food preservatives. In many countries, nitrofurantoin continues to be used in humans for the treatment of urinary tract infections and nitrofurazone is used for topical infections. Although nitrofurantoin did not induce tumors in female rats, nitrofurazone is tumorigenic and both these nitrofurans are mutagenic (*48*). In 1964, nitrofurazone was shown to be mutagenic in *E. coli* (*119*), although later it was found to be nonmutagenic in mammalian cells (*120*). Furazolidone, another nitrofuran used for gastrointestinal infections in humans and animals, is mutagenic in bacteria and tumorigenic in experimental animals (*121*, and references therein), whereas furylfuramide (AF2), a food additive widely used in Japan, is mutagenic but nontumorigenic (*122*). The discovery that formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide (FANFT) is carcinogenic in rats (*123*) triggered testing of a large number of nitrofurans, and many of them have been found to be tumorigenic in experimental animals. Nitroreduction and esterification are suspected to be involved in the metabolic activation since *Salmonella* strains deficient in these activities exhibited reduced mutagenicity (*124*). As early as 1960, it was shown that nitrofurans are reduced by pyridine nucleotide-dependent enzymes (*125*). Two groups of nitroreductases have been detected in *E. coli*. Type I (molecular weight of ∼50000) is active in the presence of oxygen and uses both NADPH and NADH (*126*). Type II, on the other hand, has a higher molecular weight, uses only NADH, and works only under hypoxic conditions (*127*). In *E. coli* strains that contain type I reductase, nitrofuran treatment causes alkali-labile damage in DNA. The extent of alkali-labile damage roughly

Scheme 5. DNA Adduction by AA[a]



parallels the carcinogenic potency of these nitrofurans (*128*). Type II reductases in both bacteria and mammalian cells carry out a single electron transfer to generate a nitro radical anion that, under hypoxic conditions, disproportionates to form an equimolar mixture of nitrosofuran and nitrofuran. In the presence of oxygen, the nitro radical anion undergoes oxidation to regenerate the nitrofuran and to form superoxide ion. With labeled nitrofurans, covalent binding of these compounds to DNA in *E. coli* cells has been demonstrated. For example, in one study incubation of 10 μM 2-amino-4-(5-nitro-2-furyl)-thiazole (ANFT) resulted in its DNA binding at a level of 1 for every 6200 nucleotides (*129*). The lack of mutagenic activity of the furan analogues of two mutagenic nitrofurans clearly indicated the importance of the nitro substituent (*130*). For some time, intestinal microflora have been thought to be responsible for the nitroreduction of nitrofurans. However, similar metabolites have also been detected following administration of nitrofurazone to germ free rats, which suggests that mammalian enzymes can also reduce the nitro group. Incubation of AF2 is toxic to Chinese hamster V79 and human fibroblast cells. The toxicity increased significantly in a hypoxic environment, suggesting that nitroreduction is likely involved in activation. Only a few studies have attempted analysis of the different metabolites formed from the nitrofurans. In one study, the ring-opened derivative, 3-(4-cyano-2-oxobutylideneamino)-2-oxazolidinone, was identified as a major metabolite of furazolidone isolated from *E. coli* and rat liver homogenate (*131*). This metabolite was also isolated from rat liver microsomes (*121*). Similarly, nitroreduction of nitrofurazone by bacteria produces the open chain nitrile, 4-cyano-2-oxobutyraldehyde semicarbazone (*132*). There are additional reports showing that mammalian enzymes, including xanthine oxidase and NADPH-cytochrome *c*, activate these compounds by nitroreduction, which results in DNA strand breaks and cytotoxicity (*133*). For additional information about the metabolism and mutagenicity of nitrofurans in bacterial and mammalian cells, see McCalla et al. (*134*).

2.10. Nitroimidazoles. Nitroimidazoles such as metronidazole are another group of drugs that have been used in humans and animals as antibacterial agents. In addition, 2-nitroimidazoles, such as misonidazole, are used as radiosensitizers for therapeutic radiation [see Weissler (*49*)]. In bacteria, these compounds are reduced to hydroxylamines that react with the nucleophilic sites of cellular macromolecules. Reduction of the nitro group appears to be related to the antibacterial properties of these compounds, which may be a consequence of their ability to damage DNA. In bacteria, DNA damage results from both covalent adduct formation and single- and double-strand breaks (*135*). In humans, however, metronidazole is primarily metabolized by the oxidative P450 enzymes to generate hydroxy and acetic acid metabolites. P450 IA is believed to be primarily responsible, although the IIB, IIC, and IIIA subfamilies seem to contribute as well (*136*). It is noteworthy that 97% of all urinary metabolites of metronidazole contain an intact nitro group, which suggests that nitroreduction is not the primary metabolic pathway in humans (*137*). Reduction of metronidazole with liver microsomes results in the formation of a nitro anion radical that, under aerobic conditions, produces superoxide and regenerates metronidazole. Under anaerobic conditions, the generated radical undergoes further reduction to nitroso, *N*-hydroxy, and amino derivatives (*138*). Isolation and tentative identification of a N^2 guanine adduct with metronidazole has been reported using sodium dithionite as a reducing agent (*139*).

2.11. Aristolochic Acid. Aristolochic acid is a mixture of related nitrophenanthrene carboxylic acids that consists mainly of 6-nitrophenanthro[3,4-*d*]-1,3-dioxolo-5-carboxylic acid (AAII) and its 8-methoxy dervivative (AAI) (*57*). AAI and AAII have been shown to be mutagenic in bacterial and mammalian cell assays. The nitro group appears to be critical for the mutagenic activity of these compounds. Both AAI and AAII have reduced mutagenicity in nitroreductase-deficient strains of *S. typhimurium* (*55*). The major adducts derived from in vitro nitroreduction of these compounds exhibit binding with the exocyclic amino group of nucleotides (Scheme 5). The ultimate carcinogenic species may involve a unique cyclic *N*-acylnitrenium ion with a delocalized positive charge (**36**). Adenine and guanine N^2 adducts of AAI and AAII (**O** and **P** in Scheme 5) were isolated from rat organs after oral administration of AA (*49*, *140*).

3. Mechanism of Mutagenesis and Repair in Bacteria

3.1. *S. typhimurium*. Because of simplicity of the assay, the Ames *Salmonella* tester strains[2] have been used extensively to screen and to determine the types and frequencies of mutations induced by nitroaromatic compounds. These strains have been used to elucidate the effect of metabolizing enzymes such as nitroreductases and transesterificases, and whether mutagenesis is dependent on error-prone repair systems. Table 1 summarizes reversion frequencies of some of these compounds in several tester strains from experiments carried out in different laboratories.

The following section illustrates some of the important conclusions that can be made from these studies. [For additional information, please refer to an early review by Rosenkranz and Mermelstein (*9*).] While some of the bicyclic compounds revert base substitution strains more efficiently, all the nitroaromatics with three or more fused ring systems induce much more reversion in frameshift tester strains such as TA98 than in the base pair substitution strains such as TA100. Nitroreduction plays

Table 1. Mutagenicity of Nitroarenes in *S. typhimurium*[a]

	TA100	TA98	TA98 with S9	TA98NR	TA98NR with S9	TA98/1,8-DNP$_6$	TA98/1,8-DNP$_6$ with S9
4-NQO	1399[217]	126[217]		124[217]			
4-NOQO[b]	590[218]						
4-HAQO[c]	36[217]	8[217]		8[217]			
1-NP	148[219]	237[79]	1731[79]	11[79]	685[79]	285[79]	127[79]
1-NOP		27000[90]		26000[90]		26000[90]	
1-NP-4,5-ox[d]		295[79]	371[79]	19[79]	164[79]	259[79]	25[79]
1-NP-9,10-ox[e]		982[79]	79[79]	146[79]	152[79]	819[79]	18[79]
2-NP	741[83]	2223[83]					
4-NP	582[83]	2475[83]					
1,3-DNP	42658[219]	144760[9]		24750[9]		2750[9]	
1,6-DNP	12303[219]	255000[90]		209000[90]		32000[90]	
1,6-NONP[f]		34000[90]		33000[90]		9000[90]	
1,8-DNP	54954[219]	734000[90]		401000[90]		8000[90]	
1,8-NONP[g]		82000[90]		103000[90]		2000[90]	
6-NC	166[219]	269[203]					
2-NF	13[36]	18[36]	21[36]	0.8[220]			
5-OH-2-NF[h]		9[35]	2[35]				
7-OH-2-NF[i]		34[35]	15[35]				
9-OH-2-NF[j]		4[35]	7[35]				
2,7-diNF	620[36]	3990[36]	640[36]	34[220]			
2-nitrofluorenone	17[36]	34[36]	30[36]				
3-NBA	29700[38]	208000[38]					
3,9-diNBA[k]	4300[38]	46000[38]					
3,11-diNBA[l]	360[38]	3000[38]					
4-NBP		0.51[221]				0.43[221]	
nitrofen	negative[222]	negative[222]					
NO-nitrofen[m]	3.7[219]						
NHO-nitrofen[n]	2.7[219]						
NH_2-nitrofen[o]	neg.[219]	0.003[222]					
o-nitroanisole	0.029[117 p]	0.0029[117 p]					
nitrobenzene		0.023[115 q]				0.049[115 q]	
nitrofurazone		10.6[124]	11.4[124]	3.8[124]	5.0[124]	1.5[124]	1.3[124]
furazolidone		173.4[124]	122.3[124]	26.8[124]	25.9[124]	41.4[124]	64.1[124]
nitrofurantoin	200[219]	10.8[124]	10.1[124]	0.1[124]	0.2[124]	2.6[124]	10.0[124]
AF2	2010[208]	203[208]					
metronidazole	0.72[114]						
AAI	0.81[55 p]						
AAI (32%) and AAII (68%)	0.60[55 p,r]						

[a] The numbers of revertants (per nanomole) are calculated from the linear portion of the dose−response curve. Numbers as superscripts correspond to the references from which the data were obtained. [b] 4-NOQO, nitroso derivative of 4-NQO. [c] 4-HAQO, *N*-hydroxy derivative of 4-NQO. [d] 1-NP-4,5-ox, 1-nitropyrene-4,5-oxide. [e] 1-NP-9,10-ox, 1-nitropyrene-9,10-oxide. [f] 1,6-NONP, 1-nitroso-6-nitropyrene. [g] 1,8-NONP, 1-nitroso-8-nitropyrene. [h] 5-OH-2-NF, 5-hydroxy-2-nitrofluorene. [i] 7-OH-2-NF, 7-hydroxy-2-nitrofluorene. [j] 9-OH-2-NF, 9-hydroxy-2-nitrofluorene. [k] 3,9-diNBA, 3,9-dinitrobenzanthrone. [l] 3,11-diNBA, 3,11-dinitrobenzanthrone. [m] NO-nitrofen, nitroso derivative of nitrofen. [n] NHO-nitrofen, *N*-hydroxy derivative of nitrofen. [o] NH_2-nitrofen, amino derivative of nitrofen. [p] Values calculated from numerical data reported in the paper. [q] With preincubation with norharmon. [r] Nanomoles determined as the weighted average of nanograms of each component in the sample.

a major role in mutagenesis by most of these compounds. For example, all the mono- and dinitropyrenes are potent mutagens in TA98 in the absence of S9 homogenate, but the mutation frequency of each mononitropyrene drops sharply in TA98NR that lacks the "classical" bacterial nitroreductase. It is interesting, however, that the frequency of reversion induced by 1,6- and 1,8-DNP is only slightly lowered in TA98NR but is sharply reduced in TA98/1,8-DNP$_6$. Compared to the parent strain TA98, the number of revertants produced by exposure to 1,3-DNP is reduced 4−6-fold in TA98NR and more than 50-fold in TA98/1,8-DNP$_6$. The nitroreduction of 1-NP has been thought to proceed via a nitroso intermediate. 1-NP exhibits reduced (∼5% of that of TA98) mutagenicity in TA98NR, but it is fully effective in TA98/1,8-DNP$_6$. Therefore, different functions appear to be blocked in TA98NR and TA98/1,8-DNP$_6$. It was hypothesized that TA98/1,8-DNP$_6$ is deficient in a specific arylhydroxylamine esterification enzyme that is necessary for the expression of mutagenicity of the DNPs and 2-NF but not for that of 1-NP and 1-NOP. The mutagenicity of 1-NP, 2-NF, and 6-NC increases significantly in TA98NR upon addition of S9, suggesting the presence of nitroreductase(s) in S9. The role of *O*-acetyltransferase was explored by constructing derivatives of TA98 and TA100 that overproduce this enzyme (*141*). The reversion frequency induced by 2-NF, 1-NP, and 1,8-DNP was remarkably enhanced in this strain, although 4-NQO did not exhibit any increase in these strains. Indeed, it is important to point out that some of the nitroaromatic compounds (e.g., 4-NQO) are equally active in TA98, TA98NR, and TA98/1,8-DNP$_6$, which suggests that additional nitroreductases might be involved in their metabolism.

In *E. coli*, expression of the *umuDC* operon is required for mutagenesis by many lesions, including the bulky adducts formed by aromatic and nitroaromatic compounds. In *Salmonella* tester strains, the reversion frequency induced by most of the nitroarenes is greatly enhanced in the presence of plasmid pKM101, which is believed to encode a similar error-prone repair system. *Salmonella* also has two sets of *umuDC*-like operons,

umuDC$_{ST}$ on the chromosome and *samAB* on a 60 MDa cryptic plasmid. Introduction of a plasmid encoding *umuDC*$_{ST}$ increased the rate of reversion of *his*3052 by 1-NP and 1,8-DNP 10- and 5-fold, respectively (*142*). The *samAB* operon, on the other hand, has very little influence on the mutagenicity of these compounds. For 1-NP, the most frequent mutation among the revertants in TA98 is a −2 deletion of a GpC or CpG pair within a CGCGCGCG hotspot sequence upstream of the *hisD*3052 mutation (*143*). Sequence analysis confirmed that the presence of *umuDC*$_{ST}$ enhances the −2 deletion by 1-NP (*142*).

The positional effects of the mono- and dinitropyrenes are complicated, but appear to be related to their metabolic fate. This was discussed in detail by Rosenkranz and Mermelstein (*9*). The mutagenicity of most nitroaromatics is much higher in strains deficient in the *uvr* gene product(s), suggesting that the covalent adducts are subject to excision repair.

To further investigate the role of acetylation in mammalian metabolism, the cDNA sequences of human *N*-acetyltransferases NAT1 and NAT2 were introduced into NM6000, a *Salmonella* strain lacking *O*-acetyltransferase (TA1538/1,8-DNP$_6$/pSK1002). Using these new strains, NM6001 and NM6002, with human NAT1 and NAT2, respectively, the role that each NAT gene plays in the metabolic activation of certain nitroaromatics can be assessed. Recent studies indicate that NAT1 is involved in 2-NF activation and NAT2 is involved in 1,8-DNP and 6-aminochrysene activation (*144*).

It has been proposed that a contributing factor in the mutagenicity of nitroaromatics involves redox cycling that creates reactive oxygen species that form DNA lesions such as 8-OH-dG. To investigate the contribution of oxidative damage to observed nitroaromatic mutagenesis, several nitroaromatics have been tested in strains YG3001 and YG3002 in which the 8-hydroxyguanine DNA glycosolase gene was disrupted. A significant increase in the number of revertants in these strains compared to that in their parent strain indicated that part of the mutagenicity of 2-NF and 4-NQO may be attributable to 8-OH-dG lesions (*72*). These findings are supported by the work of Ritter and Malejka-Giganti (*145*) that showed that ascorbate-catalyzed one-electron reduction of 2-NF forms nitro anion radicals that react with molecular oxygen to produce superoxide.

3.2. *E. coli*. The *lacI* system in *E. coli* developed by Miller (*146*−*148*), which allowed detection of *amber* and *ochre* mutations in the *lacI* gene, was used to determine the sequence changes induced by 4-NQO. The major classes of mutations induced by 4-NQO are GC-to-AT transitions followed by much less frequent GC-to-TA transversions (*148*). It is unclear which adduct(s) might be responsible for these base substitutions.

1-NP is a potent frameshift mutagen in *E. coli*. In a forward mutation assay, DNA sequence analysis of 1-NOP-induced mutants in the lambda *cI* gene of *E. coli* *uvr*$^-$ lysogen showed that ∼70% of mutants are one-base deletions or additions (*149*). In a subsequent study in pBR322, −1 deletions and all the targeted base substitutions are observed, but +1 additions have not been detected (*150*). In another study, 1-NP mutagenesis in *E. coli* was studied in single-stranded DNA, which exhibited +1 and −1 frameshifts as the major types of mutations with SOS (*151*) [see Friedberg et al. (*152*) for a discussion of SOS]. However, deamination of cytosine occurs at a significant frequency in single-stranded DNA during nitroreduction of 1-NP, which results in C-to-T substitutions (*88*).

To demonstrate unequivocally if the 1-NP-induced frameshifts are due to dG-C8-AP, a single-stranded M13 genome was constructed in which the dG-C8-AP adduct was located at G$_2$ of an inserted CG$_1$CG$_2$CG$_3$ sequence (*153*). In *E. coli* strains with normal repair capability, the adduct induced approximately 2% CpG deletions, which is 20-fold of that of the control. With SOS, the frequency of frameshift mutations increased to 2.6%, even though the frequency of CpG deletions is reduced. The enhancement in mutagenesis is due to a +1 frameshift that occurs at a high frequency. In strains with a defect in methyl-directed mismatch repair, a 50−70% increase in mutation frequency is observed. When these strains are SOS-induced, the level of frameshift mutagenesis increased by approximately 100%. When transfections are carried out in *dnaQ* strains that are impaired in 3′-to-5′ exonuclease activity of DNA polymerase III, the level of frameshift mutagenesis increased 5−7-fold. The levels of dG-C8-AP-induced frameshifts in the (CG)$_3$ sequence, therefore, vary from 2 to 17% depending on the state of repair of the host cells. The conclusion, therefore, is that dG-C8-AP induces both −2 and +1 frameshifts in the CpG repetitive sequence and that these two mutagenic events likely result from competing pathways. The CpG deletion does not require SOS functions, whereas the +1 frameshift is SOS-dependent. Both types of frameshifts occur as a result of misalignment, and are corrected primarily by the proofreading exonuclease of the DNA polymerase. Misaligned structures that escape the exonuclease are repaired by methyl-directed mismatch repair, albeit with limited efficiency (*153*). In a subsequent study in *E. coli*, dG-C8-AP induced semitargeted one-base deletions and targeted G-to-T and G-to-C transversions in a nonrepetitive CGC sequence (*154*). This suggests that DNA sequence context plays an important role in dG-C8-AP adduct mutagenesis.

Mutagenesis of 1-nitroso-8-nitropyrene, the reductively activated form of 1,8-DNP, was examined in a nucleotide excision repair-deficient strain of *E. coli* (*155*). Approximately 95% of the DNA adduct formed in this study comigrates with the C8 guanine adduct, 1-*N*-(guanin-8-yl)amino-8-nitropyrene. Analysis of the *lacI* mutations reveals a mutational spectrum that consists of mainly −1 frameshifts in runs of G or C residues (110 of 159, 81%). More than 94% (104 of 110) of these events occurred in sequences containing at least two contiguous G or C residues. The numbers of −1 frameshift mutations per guanine in a run of given length are 0.019, 0.185, 0.869, and 3.50 for G, GG, GGG, and GGGG, respectively. This shows that the frequency of frameshift mutagenesis is highly dependent on the length of the reiterated sequence.

An extension of the above study attempted to assess the role of DNA repair by examining the mutational spectrum in *E. coli* strains that differ in nucleotide excision repair (+*uvr*) and *mucA*/*B*-mediated error-prone repair (+pKM101) (*156*). In addition to −1 frameshifts, GC-to-TA transversions are the major type of point mutations induced by 1-nitroso-8-nitropyrene. However, the GC-to-TA transversions neither are influenced by the local DNA sequence context nor exhibit a strand preference. The presence of the pKM101 plasmid increases the frequency of the GC-to-TA transversions 30−60-fold, whereas the

frequency of −(GC) frameshifts is increased only 2−4-fold. With pKM101, however, G exists primarily on the transcribed strand. Nucleotide excision repair has a strong effect on the frequency of all types of mutations but does not affect either the distribution of different types of mutations or the strand specificity of these events.

2-NF produces positive results in the *E. coli rec* A and *rec* B assay (*157*−*159*). 2-NF did not cause tryptophan reversion in *E. coli* WP2; however, it did induce mutations in CM891 (*160*, *161*). In both strains, a forward mutation to L-azetidine-2-carboxylic acid (*A2C*^{r}) was induced. The adducts derived from oral administration of 2-NF to rats include dG-C8-AF and dG-C8-AAF (*106*). These adducts have also been isolated after incubation of the reductive metabolites of 2-NF with calf thymus DNA. An extensive review of the literature of dG-C8-AF and dG-C8-AAF bacterial mutagenicity has been presented by Heflich and Neft (*103*).

In *E. coli*, the mutagenic activity of a series of nitrofurans paralleled toxicity. The *uvrA* strain exhibited 6−7-fold more mutations with AF2 and nitrofurazone than the excision repair-competent strain, which suggests that the mutagenic adducts are subject to repair by the excision repair system (*162*). McCalla and co-workers have shown that many nitrofurans can induce the error-prone SOS system. They attempted to distinguish between mutants that arose from mispairing and those which occurred following induction of SOS (*134*). The mutational mechanism of AF2 is almost entirely dependent on the SOS repair system (*163*). The frameshift mutagenicity of AF2 was examined in several *E. coli* strains. AF2 induced a variety of frameshifts, including deletions of A and G, addition of A, and CpG dinucleotide deletions. Except for the CpG deletions, all other frameshifts were enhanced by the presence of the *mucAB* plasmid. In contrast, introduction of the *umuDC* plasmid enhanced only the +A and −A events, albeit to a lesser extent than the *mucAB* plasmid (*164*).

Many nitroimidazoles have been found to be mutagenic in bacteria. Metronidazole exhibits mutagenic activity in *E. coli* strains K12, K12 343/113, and WP2 *uvrA*− (*165*−*168*). It also induces an SOS response in the K12 SOS chromotest as well as in strain PQ37 with and without metabolic activation with S9 (*169*, *170*). The effect of nucleotide excision repair on the mutagenesis of misonidazole has been examined in two *E. coli* strains that differ in their ability to carry out excision repair. The toxicity of the drug is slightly higher in the excision repair-deficient strain, but more noteworthy is the result that the mutagenicity is considerably higher in this strain than in the wild type *E. coli*. Therefore, the DNA lesions formed by misonidazole are subject to excision repair in *E. coli* (*171*).

4. Mutagenesis in Mammalian Systems

Compared to the studies in bacteria, relatively few mutagenesis/genotoxicity studies of these compounds have been performed in mammalian cells. 4-NQO induces chromosome aberrations and mutagenesis in Chinese hamster ovary (CHO) cells as determined by the hypoxanthine guanine phosphoribosyl transferase (*hprt*) assay. It also induces sister chromatid exchange in bone marrow cells of Chinese hamsters and unscheduled DNA synthesis in rat hepatocytes (*172*). A dose-dependent increase in the number of mutations in the L5178K+/− mouse lymphoma forward mutation assay by 4-NQO was also shown (*173*).

Although 1-NP is a potent mutagen in bacteria, it is only weakly mutagenic in mammalian cells. For example, studies with CHO cells produced a relatively low mutation frequency in the *hprt* gene locus upon incubation with 1-NP. Increasing mutation frequency was shown, however, with increasing amounts of S9 homogenate (*174*). Heflich et al. (*175*) proposed two possible reasons for this observation. 1-NP is a weak mutagen in CHO cells because these cells are deficient in enzymes necessary for metabolic activation. Alternatively, the mammalian assays that are used are insensitive to the mutations induced by 1-NP. A comparison of mutagenesis by 1-NP and 1-NOP showed that the latter induced a much higher frequency of mutations in CHO cells (*90*), supporting the hypothesis that CHO cells lack the ability to reduce 1-NP to its active metabolite. The major adduct formed by 1-NOP is dG-C8-AP. An analysis of the mutations induced in the *hprt* gene of CHO cells show predominantly GC-to-TA transversions. The second most common base pair substitution is the GC-to-AT transition. In contrast to the results in bacteria, only 15% of mutations are frameshifts in this study (*176*).

While 1-NP is not detectably mutagenic in CHO cells, both 1,6- and 1,8-DNP are weakly mutagenic (*90*). The observation that the nitrosonitro derivatives of the DNPs are more mutagenic reiterates the fact that CHO cells cannot adequately metabolize these compounds. However, in contrast to the observation that the DNPs are much more mutagenic than 1-NP in bacteria, the mutagenicities of the nitroso forms of 1-NP and the DNPs are of similar magnitude in CHO cells. This suggests that esterification may not be involved in the activation mechanism in CHO cells, because the reduced derivatives of DNPs have been shown to exhibit significantly high mutagenicity in the presence of esterification enzymes. Other studies of 1,8-DNP in mammalian cells include demonstration of a positive mutagenic response in L5178Y mouse lymphoma cells following prolonged incubation. Although 1,8-DNP is a potent frameshift mutagen in bacteria, in L5178Y cells it induced ouabain mutants which usually result from base pair substitutions (*177*).

Unlike the CHO cells, HepG2 cells, derived from a human hepatoma cell line, have been shown to metabolize carcinogens through ring oxidation and nitroreduction. In this cell line, a dose-dependent enhancement of mutagenesis by 1-NP was observed in the *hprt* locus as determined by 6-thioguanine resistance (*178*). However, 1,8-DNP is not mutagenic and does not induce unscheduled DNA synthesis in these cells. This discrepancy was suggested to be due to the lack of arylhydroxylamine *O*-esterification enzyme in HepG2 cells (*179*).

The cytotoxicity and mutagenicity of 1-NP and 1-NOP were studied in diploid human fibroblasts (*180*). Normal cells were compared with repair-deficient xeroderma pigmentosum (XP) cells and cells from a patient with hereditary cutaneous malignant melanoma (HCMM). In all cell lines, the amount of 1-NP necessary to attain the same level of toxicity is much greater than the amount of 1-NOP. The XP and HCMM cells exhibited increased sensitivity to 1-NP compared to the normal cells. The same level of killing in XP cells was achieved by a 7-fold lower number of bound adducts, underscoring the importance of nucleotide excision repair in removing these lesions.

Incubation of 1-NOP with human T-lymphocytes resulted in mutations in the *hprt* gene (*181*). The major class of mutations (63%) is base substitution, and most are at GC base pairs. Nine of the 35 mutants are missing exons 2 and 3, which was suggested to be a result of an error in V(D)J recombinase activity. This finding may be linked to the etiology of lymphoid cancer.

In a site-specific study, dG-C8-AP, the major DNA adduct formed by 1-NP, was inserted into a bacteriophage M13 DNA containing the simian virus 40 origin of replication (*182*). The constructed DNA template was replicated in vitro using extracts from normal human fibroblasts. The adduct was not removed from the progeny DNA following bidirectional semiconservative replication, which suggests that it had been bypassed, rather than repaired, by the cell extract. When newly replicated bacteriophage was evaluated for mutations in the region of the modified G, most contained a G at the adduct site, indicating error free replication. A small number of mutants ($\sim 2 \times 10^{-3}$) were detected, all of which contained a targeted GC base pair deletion. This study unequivocally demonstrates that the major DNA adduct formed by 1-NP can induce frameshift mutagenesis in mammalian cells.

The mutational spectrum in the *supF* gene was determined using a shuttle vector, pS189, that was incubated with 1-nitroso-6-nitropyrene followed by replication in human kidney cells (*183*). Mutations are mostly base substitutions, although single-base deletions occurred at a significant frequency. Predominantly GC-to-TA transversions occurred. Most of the −1 deletions are located in a unique run of five G bases in the gene. Examination of lung tumors induced by 1,6-DNP in F344 rats shows mutations in both the *p53* and *K-ras* genes. *K-ras* mutations were at codon 12 and included four GGT-to-TGT transversions and one GGT-to-GAT transition. Eight of the nine mutations detected in *p53* were GC base pair substitutions (four G-to-T transversions and four G-to-A transitions), and one was a single GC deletion. The types of mutations detected are consistent with the mutations induced by 1,6-DNP (*184*).

As described earlier, 6-NC can be activated by two different pathways: nitroreduction to *N*-hydroxy-1-aminochrysene and a combination of ring oxidation and nitroreduction to form 6-AC-1,2-dihydrodiol. The latter activated derivative is mutagenic in the *hprt* gene of CHO-K_1 cells (*185*). Predominant mutations are GC-to-TA transversions (60%), although GC-to-CG transversions and −1 deletions also occur at a lower frequency. All GC base pair substitutions had the mutated G in the nontranscribed strand, and 86% of these contained a purine 3′ to the mutated G. It is interesting to note that following injection of 6-NC in CD-1 mice the primary mutations were at codon 61 of the *H-ras* gene, inducing C-to-A mutations in 90% of the tumors. Incubation of 6-NC with human lymphoblastoid cells, however, failed to induce mutations in the *hprt* locus (*186*). 6-NC-induced lung tumors in transgenic mice with a human hybrid c-*Ha-ras* gene, encoding a prototype *p21* gene product, were examined (*187*). The primary mutation at codon 61 of *Ha-ras* exon 2 was CAG (Gln) to CTG (Leu).

2-NF induces sister chromatid exchange in Chinese hamster bone marrow after oral administration, though the same result was not observed following ip injection (*188*). 2-NF was also found to induce unscheduled DNA synthesis in hepatocytes isolated from rat liver following oral administration (*188*, *189*). 2-NF is positive in the mouse lymphoma L5178Y thymidine kinase in vitro assay without a metabolizing system (*173*). In the same study, 2-AF produced negative results whereas 2-acetylaminofluorene (2-AAF) produced positive results. In a related study, 2-NF was found to induce 6-thioguanine resistance in Chinese hamster V79 cells only in the presence of isolated hepatocytes, indicating the need for exogenous metabolic activation in this cell line (*191*). As discussed earlier, dG-C8-AF, the major adduct formed by 2-NF, is identical to those isolated from incubation with 2-AF. For a discussion of this adduct, readers are referred to an extensive review by Helfich and Neft (*103*).

3-NBA induces micronuclei in mouse peripheral blood reticulocytes following ip injection, which indicates that 3-NBA is capable of inducing chromosomal aberrations in mammalian cells (*38*). To investigate the mutagenicity of 3-NBA, a shuttle vector was transfected in normal and repair-deficient human cells following adduction with the acetoxyacetyl derivative of 3-NBA. The presence of dG-*N*-Ac-ABA was confirmed in human HepG2 cells treated with 3-NBA (Scheme 4) (*192*), and was also detected in DNA treated with the acetoxyacetyl derivative. Repair-deficient XP cells exhibited higher mutagenicity and toxicity than normal cells. In these mammalian cells, 3-NBA produces mostly base substitution mutations at GC sites with GC-to-TA transversions being the predominant base change. A polymerase stop assay indicated that the activated derivative bound primarily to guanine residues. It is important to point out that the metabolism of 3-NBA is not well-characterized, and this study probably reflects a subset of the types of damage induced by 3-NBA (*192*).

4-NBP has been reported to induce sister chromatid exchange in Chinese hamster bone marrow cells (*172*). However, in the L5178Y TK+/− mouse lymphoma forward mutation assay, 4-NBP produced negative results (*173*). The effect of nitrobenzenes in mammalian cells has not been actively investigated; however, in an in vitro chromosomal aberration test with human peripheral lymphocytes, dinitrobenzenes have been listed as more genotoxic than the mononitrobenzene derivatives (*193*).

Several nitrofurans are mutagenic in mammalian cells. Nitrofurantoin exhibited a linear dose−response relationship in K1-BH4 and UV5 strains of CHO cells that are normal and deficient in DNA excision repair, respectively. In the same study, furazolidone was found to be a potent mutagen in these strains. Increased mutagenicity in cells deficient in excision repair suggests that large bulky adduct formation may be the cause of the mutagenic response. This result is supported by the fact that AF2 exhibits a greater mutagenic and toxic response in excision repair-deficient human fibroblasts from XP patients (*194*, *121*).

Due to their use as therapeutic agents in humans, nitroimidazoles have been extensively studied in mammalian systems. These compounds continue to be used in humans despite their demonstrated mutagenicity in bacteria, because their activity in mammalian aerobic cells is significantly different [see reviews by Voogd (*50*) and Dobias et al. (*135*)]. Several studies showed that metronidazole is not mutagenic in mice and rats. In studies with L5178Y mouse lymphoma cells, induction of mutants deficient in the *hprt* gene was not observed following incubation with metronidazole (*50*). However, nitroimidazoles induce chromosome aberrations in mam-

malian cell lines under anaerobic conditions (*195*). It is noteworthy that mutagenic activity in Chinese hamster V79 lung cells could not be detected in a study of four 5-nitroimidazole derivatives used in human therapy, even though a strong positive response in *Salmonella* strain TA100 was observed (*196*). Also, DNA strand breaks were not detected in peripheral lymphocytes following administration of metronidazole to humans (*197*). Finally, a study following 771 women treated with metronidazole for *T. vaginalis* infections found no significant increase in tumor incidence 6 years after administration of the drug. Followup studies with those same patients showed a statistically significant increase in lung cancer incidence for the 15−25-year followup study, which was not confirmed in the 11−15-year followup (*198, 199*).

5. Structure−Activity Relationship

Analysis of the metabolism and mutagenicity data of the nitroaromatic compounds may provide the underlying factors that contribute to the mutagenesis of this group. Several attempts have been made to develop a model that can assess the genotoxicity and/or mutagenicity of these compounds. At least two different groups of variables must be considered. First, there are factors that contribute to the covalent binding of the compound to DNA. These include the hydrophobicity, metabolism, ability to intercalate, orientation prior to binding, and DNA sequence specificity of binding. Second, there are also factors that influence the mutagenicity of the adduct or lesion induced by the activated compounds. These include the DNA-bound conformation, interactions with DNA polymerases and repair proteins, and effect of DNA sequence.

Since nitroreduction plays a crucial role in activating many of these nitroaromatics, the energy required to reduce the nitro group has been examined. The half-wave potentials of 10 nitroaromatics were compared with their reversion potency in TA1538 and TA98, and a good correlation was observed ($r^2 = 0.944$ for TA1538) (*200*). Likewise, Hückel calculation of LUMO energies of these compounds showed a good correlation with reversion values in TA98 ($r^2 = 0.917$).

It was pointed out by Fu et al. (*83*) that despite the success of the relationship described above, it is not sufficient to predict the activities of all nitroaromatic compounds. For example, 6-nitrobenzo[*a*]pyrene (LUMO energy of -0.208β) would be predicted to be more mutagenic than 1-NP (LUMO energy of -0.263β), although the latter is actually a much more potent mutagen than the former in several tester strains. This study indicated that of the 24 nitroaromatic compounds that were analyzed, those compounds in which the nitro group is oriented nearly perpendicular to the aromatic plane have little or no direct-acting bacterial mutagenic activity. However, when a nitropolycyclic of this type has a low first half-wave reduction potential, it exhibits substantial direct acting mutagenicity (*201*). It is unclear what this relation between the orientation of the nitro group and the mutagenicity of these compounds implies. It is possible that the perpendicular orientation of the nitro group makes the compound unfit to occupy the active site of the nitroreductase. Alternatively, the reduced derivatives may undergo redox cycling to regenerate the parent nitro compound. A third possibility is that the perpendicular orientation may not allow the formation of stable *N*-hydroxyamine intermediates of these compounds. It was shown that while 1-, 2-, and 3-nitrobenzo[*a*]pyrenes are potent mutagens in *Salmonella*, 6-nitrobenzo[*a*]pyrene is a weak mutagen (*63*), and it is noteworthy that only when the nitro group is at the 6 position of nitrobenzo[*a*]pyrene is it oriented perpendicular to the aromatic system.

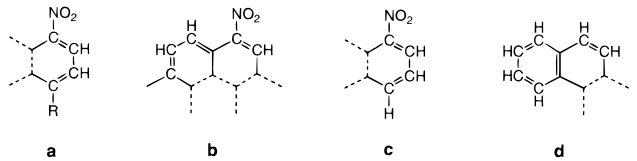


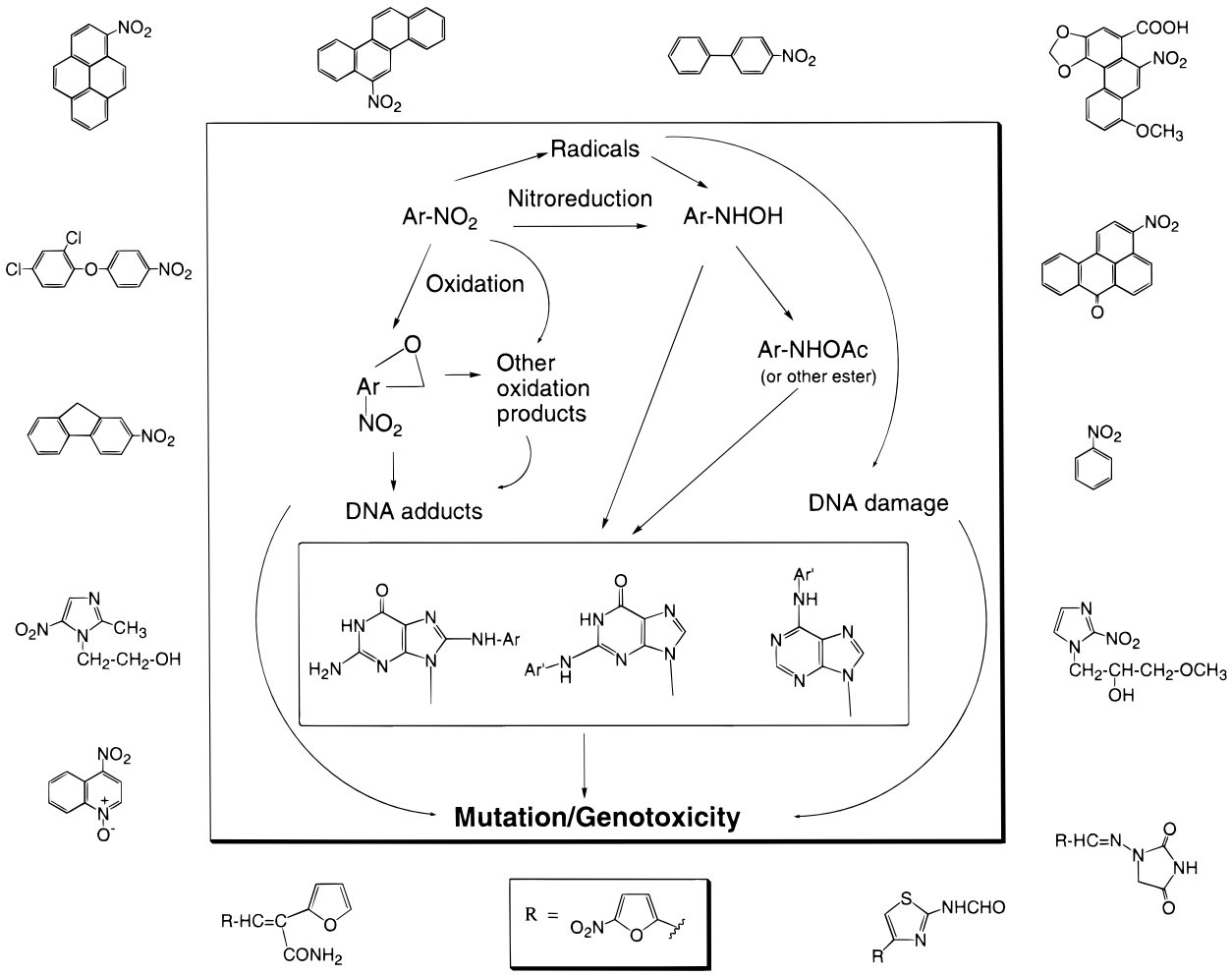
Figure 6. Fragments responsible for the mutagenicity of nitroarenes per Klopman et al. (*200*). Fragments **a** and **b** are required for activity, while fragments **c** and **d** are deactivating. Please note that **a** differs from **c** in that C4 is not bonded to a hydrogen. For fragment **d**, the nitro group is located outside the fragment that is shown. The deactivating action of fragment **d** was postulated to be associated with detoxification taking place due to the availability of the exposed benzene ring or to the presence of an activated K-type region (i.e., the carbon double bond attached to the benzene ring system in fragment **d**).

To further assess the effect of nitro group orientation, Fu and co-workers (*202*) recently studied the tumorigenicity of dibenz[*a*,*h*]anthracene (DB[*a*,*h*]A) and its 7-nitro derivative (7-NDB[*a*,*h*]A). X-ray crystallographic analysis established that the nitro group in the latter resides in a plane nearly perpendicular to the aromatic plane. As predicted on the basis of the orientation of the nitro group, 7-NDB[*a*,*h*]A induces a much lower incidence of hepatocellular adenoma and carcinoma and lung adenoma in B6C3F_1 neonatal mice than the parent compound DB[*a*,*h*]A. Also, no nitroreduction occurs in the anaerobic liver microsomal metabolism of 7-NDB[*a*,*h*]A in vitro. Likewise, 7-nitrobenz[*a*]anthracene, in which the nitro group is in a perpendicular orientation, is a weaker tumorigen than its parent compound. These data, taken together, support the hypothesis that the perpendicular orientation of a nitro group results in a drastic reduction in mutagenicity and tumorigenicity.

In another approach, the structures of 53 nitroaromatic compounds were analyzed by a Computer Assisted Structure Evaluation (CASE) program for their ability to induce reversion in TA98 (*203*). Structural analysis identified four chemical fragments, two associated with mutagenic activity and two possessing deactivating properties. These fragments are shown in Figure 6. For example, the investigators noted that substitution at the carbon para to the nitro group is required for mutagenicity, while the presence of a hydrogen at that position prevents activity. It was also noted that the coexistence of active and deactivating fragments on the same molecule results in a nitroaromatic compound with weak or no mutagenic activity. The activity of 47 of 53 nitroarenes was correctly predicted by this program. Although the significance of these fragments is unclear, it was hypothesized that deactivating fragments represent part of the structure where detoxification may take place. Activating fragments, on the other hand, may consist of regions that bind to enzymes responsible for metabolic activation through nitroreduction.

Substituents other than the nitro group also may influence the mutagenicity and carcinogenicity of the nitroaromatics. For example, methyl substitution of nitrobiphenyls and nitronaphthalenes decreases mutagenicity, although the effect is less pronounced in the

Scheme 6. An Overview of Metabolism, DNA Damage, and Mutagenesis Induced by the Nitroaromatic Compounds



presence of the S9 fraction (*204*). Also, 11-methyl-6-nitrochrysene is more mutagenic and 5-methyl-6-nitrochrysene less mutagenic than 6-nitrochrysene in TA98 and TA100 (*29*). In the latter case, this pattern is valid both in the presence and in the absence of the rat liver 9000*g* supernatant. Vance and co-workers have examined several disubstituted derivatives of amino-, nitroso-, and nitrofluorenes to determine the physicochemical basis for their mutagenic activity in *Salmonella* (*205*). For 2-NF, a reasonable correlation between the inductive and resonance effects of an electron-withdrawing substituent at C7 and the mutagenicity of these derivatives in TA98 was observed. Likewise, electron-donating substituents at C7 decreased the number of revertants in TA98 for 2-AF, 2-nitrosofluorene, and 2-NF. Acetylation of a hydroxy or an amino group at C7 increased the mutagenic potency of 2-NF, which was suspected to be due to a decrease in the electron-donating ability of the hydroxy or amino functionality. These types of structure−activity relationships can sometimes be good predictors for mutagenicity under a given set of conditions, but this approach must always be used with caution because frequently there are exceptions to the rule.

Derivation of a quantitative structure−activity relationship (QSAR) for the reversion in TA100 of 117 aromatic and heteroaromatic nitro compounds has been attempted by Debnath et al. (*206*). The authors conclude that relative mutagenicity is bilinearly dependent on hydrophobicity, and is linearly dependent on the LUMO energy of the nitroaromatic compound. While such a simplified approach to rationalizing a biological effect may be subject to criticism, this study could be the basis for more thorough QSAR studies.

6. Concluding Comments

Scheme 6 provides an overview of the pathways that lead to mutagenesis and genotoxicity by the nitroaromatics. Despite the ubiquitous presence of these compounds, only a small number of nitroaromatic mutagens received much attention. Nevertheless, it is evident that one or more nitro groups profoundly influence the DNA binding and mutagenic profile of an aromatic compound. The location of the nitro group on the aromatic system is crucial since it affects metabolism and DNA binding properties. For most compounds, nitroreduction plays a major role in mutagenesis, while the role of ring oxidation is still unclear. The major DNA adducts formed by many nitroarenes have been isolated and characterized. However, their rate of formation, the sites where they are preferentially introduced, and their mutagenic effects appear to be very different. Like several other groups of chemicals, each nitroaromatic mutagen leaves a characteristic "signature" of mutagenic specificity. On the basis of the data from many research groups, we now have better insight into the metabolic products and DNA damage induced by the more common nitroaromatics and

whether they are mutagenic. Such accomplishments notwithstanding, relatively few investigations have been performed on the persistence and repair of these types of DNA damage. Other areas in which our understanding is limited include the regulation of expression of the nitroaromatic metabolizing enzymes and the biological effects of nitroarenes in a mixture. Since these compounds continue to pose a threat to human health, future studies should employ specific metabolites and DNA adducts as markers for risk assessments, which will provide useful data for epidemiological studies. The scientific methods for more sensitive detection of the DNA adduct markers and development of statistical models for measuring exposure to these compounds in complex mixtures such as polluted air, tobacco smoke, and diesel exhaust are likely to be the areas of great interest in the future. Also important is development of collaborative ties among scientists, regulatory agencies, and industry so that the knowledge base can be shared, and appropriate measures can be taken to reduce uncertainties in each step of data collection. Recent advances in molecular biology now allow us to measure alterations not only in cells but also in genes with remarkable accuracy. Therefore, one can anticipate that soon we shall have a battery of highly sophisticated tools for focusing on risk assessment from exposure to nitroaromatic compounds in a susceptible population. Evidently, such studies are also intimately linked to mutagenicity and genotoxicity and the kinetics of DNA repair. Therefore, more studies on the mechanism of mutagenesis, especially in mammalian systems, are needed. The kinetics of formation and removal of DNA adducts in specific sites in *p53* and *ras* genes and their relationship with the mutational hot spots, for example, may provide insight into the human health risks. The link between DNA methylation and hot spots for DNA damage should also be investigated. Complementary information may come from identification of the genes involved in repair of the DNA damages and from a better understanding of the mechanisms of DNA repair.

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