Mutagenic and Carcinogenic Significance and the Possible Induction of Lung Cancer by Nitro Aromatic Hydrocarbons in Particulate Pollutants

Hiroshi Tokiwa, Nobuyuki Sera, Akio Nakashima, Koichi Nakashima, Yoichi Nakanishi, and Nobuaki Shigematu

1Fukuoka Institute of Health and Environmental Sciences, Dazaifu, Fukuoka, Japan; 2Department of Internal Medicine, Saiseikai Shimonoseki General Hospital, Shimonoseki, Japan; 3Department of Nutrition, Faculty of Science of Life, Kyushu Women’s University, Kitakyushu, Japan; 4Research Institute for Diseases of the Chest, Faculty of Medicine, Kyushu University, Fukuoka, Japan

Studies of genotoxicity and carcinogenicity of nitro aromatic hydrocarbons focus on their high mutagenicity for bacteria and mammalian cells. Nitrobenzo[a]pyrenes (NBPs) and related nitroazaarenes are also extraordinarily mutagenic. 3-Nitro-6-azabenzo[a]pyrene-N-oxide was found to be a more potent mutagen than 1,8-dinitropyrene. Mutagenicity of NBPs was associated with the position of substitution of the nitro function when nitrogen dioxide (NO₂) was substituted at the third position on the benzo[a]pyrene (BP) structure, as in 3,6-dinitrobenzo[a]pyrene but not in 1,6-dinBP. The NBPs were reduced by a rat liver postmitochondrial fraction to nitroso- and subsequently to amino-derivatives. Therefore, tumoral action in rats was induced at significant levels by subcutaneous injection of 3,6-dinBP, but no tumors were observed in rats given 1,6-dinBP. Carcinogenic nitropyrenes were detected in the resected lung of a patient with lung cancer. It is suggested that the presence of nitropyrenes and the resulting tumor were due to exposure to by-products of combustion of heavy oil. The patient was a nonsmoker and farmer who had bred chickens for 40 years. He used heavy oil for heating the chicken house. Similarly, a group of Chinese people at high risk of developing lung cancer was selected to determine the initiator of lung cancer. Lung cancers were obtained from six Chinese female nonsmokers who were living in Fuyuan County, China. Polycyclic aromatic hydrocarbons were detected in resected lung specimens; they were benzo[k]fluoranthene, BP, benzo[g,h,i]perylen, and pyrene. These cases were associated with exposure to soot from combustion of coal usually used for heating and cooking indoors. — Environ Health Perspect 102:Suppl 4:107–110 (1994).

Key words: nitropyrenes, dinitrobenzo[a]pyrenes, carcinogenicity, mutagenicity, lung cancer

Introduction

Chemical mutagens and carcinogens present in the urban air differ according to the source of the pollutant, and are formed secondarily by reaction of polycyclic aromatic hydrocarbons (PAHs) with gaseous pollutants such as nitrogen dioxide and sulfur dioxide (1). Crude extracts prepared from particulate pollutants normally induce mutagenic activity in Salmonella typhimurium TA98 at a frequency of from 50 to 500 revertants/mg of extracts of particulates (2). This mutagenic potency corresponds to revertants per pmole of dinitropyrenes (dINPs), well known to be extraordinarily mutagenic.

The behavior of nitro aromatic hydrocarbons (nitro-PAHs) in the environment is related to combustion of fossil fuels. It is, therefore, thought that nitro-PAHs are being produced continuously and spread in urban air. This speculation is based on the fact that most nitro-PAHs are detected at substantial levels in airborne and diesel emission particulates (3). On the other hand, it is important that respiratory tract and lung cancer occurs among both smokers and non-smokers. The aim of this study was to clarify whether nitro-PAHs in urban air were involved in the induction of lung cancer.

Materials and Methods

Chemicals

Dinitrobenzo[a]pyrene (DinBPs) and their related compounds were synthesized by Fukuhara et al. (4). DinPs (three isomers) and dinitrofluoranthene (dINFs) (two isomers) were prepared as reported previously (5).

Animals for Carcinogenicity Test

A total of 184 male six-week-old F344/DuCrj rats with body weights of 111 to 125 g were purchased from Charles River Japan Inc. (Atsugi, Japan). The rats were divided into four groups consisting of 84 rats for a 3,6-dinBP-treated group, 44 for a 1,6-dinBP-treated group, 40 for a benzo[a]pyrene (BP)-treated group, and 20 given a mixture of equal volumes of beeswax and tricaprylin. The rats were subcutaneously injected with the chemical dissolved in an equal volume of beeswax and tricaprylin by the method of Stanton et al. (6).

Determination of Mutagen in Lung Specimens

Lung specimens were obtained after surgical resection from one Japanese subject and six Chinese subjects who had lung cancer. Also, 54 other lung specimens from Japanese subjects were examined to determine the values of chemical deposits as the background. To determine the mutagen, the specimens were dried at 20°C for a week in a dark room and weighed. The amounts of material used for analysis were 1517 mg for case 1 (Japanese male); 880 mg for case 2; 1200 mg for case 3; 900 mg for case 4, 1100 mg for case 5; 1300 mg for case 6; and 1200 mg for case 7 (cases 2–7 were all Chinese females).
Table 1. Mutagenicity of nitroarenes and their related compounds.

| Nitroarene                  | Revertants / n mole for |
|----------------------------|-------------------------|
|                            | TA98                    | YG1024                  |
| 3-Nitro-6-azabenzo[α]pyrene-N-oxide | 396,000                 | 9,580,000               |
| 1,8-Dinitropyrene           | 296,000                 | 6,130,000               |
| 3-Nitro-6-cyanozenbenzo[α]pyrene | 174,000                 | 1,230,000               |
| 3,6-Dinitrobenzo[α]pyrene   | 137,000                 | 1,840,000               |
| 1,6-Dinitropyrene           | 126,000                 | 1,420,000               |
| 3,7-Dinitrofluoranthene     | 123,000                 | 6,600,000               |
| 1-Nitro-6-azabenzo[α]pyrene | 105,000                 | 820,000                 |
| 3-Nitro-6-azabenzo[α]pyrene | 104,000                 | 2,230,000               |
| 2,6-Dinitrophenanthrene     | 104,000                 | 1,910,000               |
| 1,3-Dinitropyrene           | 95,900                  | 1,150,000               |
| 1-Nitro-6-cyanozenbenzo[α]pyrene | 43,100                  | 348,000                 |
| 1-Nitro-6-azabenzo[α]pyrene-N-oxide | 36,100                  | 606,000                 |
| 3-Nitrofluoranthene         | 4,670                   | 57,000                  |
| 3,4-Dinitrofluoranthene     | 4,120                   | 52,000                  |
| 4-Nitropyrene               | 2,700                   | 56,400                  |
| 3-Nitrobenzo[α]pyrene       | 1,370                   | 52,000                  |
| 2,6-Dinitrophenanthrene     | 730                     | 1,230                   |
| 1-Nitrobenzo[α]pyrene       | 650                     | 35,000                  |
| 1-Nitropyrene               | 470                     | 2870                    |

Abbreviations: TA 98, Salmonella typhimurium his strain; YG1024, Salmonella typhimurium his carrying a plasmid of the acetyltransferase gene transferred into cells of TA98 (8).

Table 2. Nitro-reduction of nitrobenzo[α]pyrene in the presence of rat liver S-100 fraction.

| Chemical                  | Mutagenicity, rev/μg | Cofactor | Oxygen | Nitroso | Amino |
|---------------------------|----------------------|----------|--------|---------|-------|
| 1-Nitrobenzo[α]pyrene     | 2,200                | NADPH    | –      | 15±1    | 20±1  |
|                           |                      | +        | 5±0    | 5±0     |       |
|                           |                      | –        | 15±6   | 20±8    |       |
|                           |                      | +        | 5±0    | 5±0     |       |
| 3-Nitrobenzo[α]pyrene     | 4,600                | NADPH    | –      | 47±15   | 38±41 |
|                           |                      | +        | 6±1    | 6±1     |       |
|                           |                      | –        | 47±15  | 38±17   |       |
|                           |                      | +        | 6±0    | 6±0     |       |
| 1,6-Dinitrobenzo[α]pyrene | 4,000                | NADPH    | –      | 25±20   | 56±19 |
|                           |                      | +        | 6±0    | 6±0     |       |
|                           |                      | –        | 25±20  | 36±9    |       |
|                           |                      | +        | 6±0    | 6±0     |       |
| 3,6-Dinitrobenzo[α]pyrene | 401,000              | NADPH    | –      | 242±64  | 847±102|
|                           |                      | +        | 89±15  | 112±15  |       |
|                           |                      | –        | 100±11 | 153±11  |       |
|                           |                      | +        | 143±11 | 57±4    |       |

*1-Nitrobenzo[a]pyrene, 3-nitrobenzo[a]pyrene, 1,6-dinitrobenzo[a]pyrene, or 3,6-dinitrobenzo[a]pyrene in 2.5 μM quantities dissolved in 50 mM phosphate buffer, pH 7.4 was exposed to nitrogen gas for 20 sec and reacted with rat liver S-100 at the final concentration of 1 mg of protein per ml in the presence of 50 μM NADPH or 5 μM NADH as a cofactor. The mixtures were incubated at 37°C for 20 min and treated with chloroform and methanol (1:1, vol/vol). *2 Products were developed on a plate of silica gel and analyzed by high-pressure liquid chromatography.

Preparation of Rat Liver Postmitochondrial Fraction

The enzymatic reduction of 1- and 3-nitrobenzo[a]pyrene, and 1,6- and 3,6-diNBP was performed in the presence of rat liver postmitochondrial fraction on the basis of the method of Poirier and Weisburger (7) and Djuric et al. (8).

Mutagenicity Test

The mutagenicity test used was the Salmonella microsome test described previously (9).

Results

Mutagenicity of Nitro-PAHs and Their Related Compounds

The mutagenicity of major nitro-PAHs for strain TA98 is summarized according to the order of their potency (Table 1). In addition to diNPs and diNFs, NBps and their related compounds showed high mutagenicity. 3-Nitro-6-azabenzo[a]pyrene (N-6-ABP), 3-nitro-6-cyanozenbenzo[a]pyrene (N-6-CBP), and 3-nitro-6-azabenzo[a]pyrene (N-6-ABP) as well as diNPs and diNFs were potent mutagens. Nitro derivatives with the substitution at the third position on the BP structure mutated strain TA98 strongly. Therefore, there was a structure–activity relationship between mutagenic potency and the nitro substituent. With the exception of 1-nitropyrene (NP) and 2,6-dinitrophenanthrene, the nitro-PAHs were activated by O-acetyltransferase; the mutagenicity of these chemicals was greatly enhanced in strain YG 1024, which carries a plasmid of the acetyltransferase gene in TA98.

Structure–Activity Relationship of NBPs

The enzymatic reduction of 1- and 3-NBP and 1,6- and 3,6-diNBP was investigated in the presence of rat liver postmitochondrial fraction (S-100). Incubation was under anaerobic conditions. The reduction products were determined by the appearance of 1-nitrosobenzo[a]pyrene and 1-aminoBP for 1-NBP, of 3-nitrosoBP and 3-aminoBP for 3-NBP, of 1-nitroso-6-NBP and 1-amino-6-NBP for 1,6-diNBP, and of 3-nitroso-6-NBP and 3-amino-6-NBP for 3,6-diNBP. In the presence of rat liver S-100, 3-NBP and 3,6-diNBP were reduced to nitroso- and amino-derivatives stronger than 1-NBP and 1,6-diNBP more readily (Table 2). Reduction of 3,6-diNBP yielded 10 times or more of the nitroso- and amino-derivatives compared to reduction of 1,6-diNBP. The ability of the reduction was for diNPs than for mono-NBs. As the first step of the reduction of 3,6-diNBP, 3-nitroso-6-NBP was produced and was further reduced to 3-amino-6-NBP via a 3-hydroxylamino-6-NBP intermediate and finally to 3,6-diaminoBP. Figure 1 is an outline of the pathway of reduction.

Carcinogenicity of 3,6-diNBP in Rats

Table 3 shows the incidence of tumors in rats observed for 100 weeks after injection of chemical. Fourteen rats (70%) given 1000 mg of 3,6-diNBP per rat developed subcutaneous tumors by 35 weeks. The earliest appearance of the tumors was on day 183 in the 3,6-diNBP-treated rats. Similarly, animals given 200, 40, and 5 mg per rat developed tumors at the rate of 38, 24, and 4.7%, respectively, at the local site. The incidence of tumors was dose-dependent. However, no tumors were induced in any rats given 1,6-diNBP. In the rats given 3,6-diNBP and BP as a positive control, more BP-treated rats than 3,6-diNPB-treated rats developed tumors after injection of the minimum dose of 8 μg.
Association between Initiation of Human Lung Cancer and Mutagen in Particulates

We examined human lung cancer tissues for the presence of mutagens or carcinogens and their metabolites and investigated the association between these chemicals and the possible induction of lung cancer. The findings in lung specimens from a Japanese subject and six Chinese subjects are presented. None of the patients were cigarette smokers or had an occupational history of exposure to special pollutants as coke workers.

The Japanese patient was a 64-year-old male farmer. In 1983, he had a chest X-ray because of recurrence of coughing raising much sputum and a slight fever. Consolidation in the lower lobe of the right lung was found. By bronchoscopic brushing and biopsy, squamous cell carcinoma was diagnosed. The tumorous right lung was then resected. Macroscopically, the tumor was grayish white, solid, and hard with irregular foci of hemorrhage. It measured 55 × 66 × 40 mm in size with cavity formation and bronchial invasion. The histological features of the resected lung tumors led to the diagnosis of keratinizing squamous cell carcinoma (10).

The six Chinese patients were also nonsmokers and consisted of a female cook and five female farmers who lived in Fuyuan County, China. Histological features of the resected right or left lung led to the diagnosis of adenocarcinoma for cases 2, 3, and 5; squamous cell carcinoma for cases 4 and 7; and small cell carcinoma for case 6 (Table 4). To determine the mutagens in lung specimens, the materials were extracted with dichloromethane or acetone–hexane mixture. The crude extracts were divided into three fractions, acidic, basic, and neutral. Only the neutral fraction was mutagenic for strain TA98 or TA100, giving 12.2 revertants for strain TA98 without S9 mix for case 1 and 0.01, 1.8, 0.01, 0.2, and 0.01 revertants for TA100 with S9 mix for cases 2, 3, 4, 5, 6, and 7, respectively, per μg of dry lung sample. The active fractions eluted on a high-performance liquid chromatography (HPLC) column and were analyzed by gas chromatography–mass spectrometry.

The major mutagens found and their concentrations are shown in Table 5. 1-NP, 1-nitro-3-hydroxyphenanthrene, BP, benzo[g,h,i]perylene, and pyrene were detected in all lung specimens of cases 2, 3, 4, 5, 6, and 7. Therefore, the lung specimen of case 1 was contaminated substantially with NPs, whereas similar specimens of the other cases were contaminated mostly with PAHs. These results are dependent on the fact that deposition of the chemical in the lung differs according to the source of the environmental pollutant. There was a possibility that the Japanese patient had been exposed to combustion by-products of heavy oil used for air conditioning the chicken house for a period of time over 10 years. In contrast, Chinese specimens were obtained in Fuyuan County,

Figure 1. Reduction of 3,6-dinitrobenzo(a)pyrene.

Table 1. Carcinogenicity of 3,6-dinitrobenzo(a)pyrene compared to benzo(a)pyrene in rats.

| Chemical          | Total dose | No. of rats injected | No. of rats with subcutaneous tumors (%) |
|-------------------|------------|----------------------|------------------------------------------|
| 3,6-dinitroBP     | 1000       | 20                   | 14 (70)                                  |
|                   | 200        | 21                   | 8 (38)                                   |
|                   | 40         | 21                   | 5 (24)                                   |
|                   | 8          | 21                   | 1 (4.7)                                  |
| 1,6-dinitroBP     | 1000       | 11                   | 0                                        |
|                   | 200        | 11                   | 0                                        |
|                   | 40         | 11                   | 0                                        |
|                   | 8          | 11                   | 0                                        |
| BP                | 1000       | 10                   | 10 (100)                                 |
|                   | 200        | 10                   | 6 (60)                                   |
|                   | 40         | 10                   | 5 (50)                                   |
|                   | 8          | 10                   | 2 (18)                                   |
| Control           |            | 20                   | 0                                        |

Abbreviations: 1,6- and 3,6-dinitroBP, 1,6- and 3,6-dinitrobenzo(a)pyrene; BP, benzo(a)pyrene.

Table 4. Lung cancer of Japanese and Chinese subjects; a relationship between carcinogenesis and environmental causes.

| Case no. | Japanese or Chinese | Sex | Age | Occupation       | Lung cancer            | Exposure to combustion products | Status after operation |
|----------|---------------------|-----|-----|------------------|------------------------|-------------------------------|------------------------|
| 1        | Japanese            | Male| 64  | Farmer           | Squamous cell carcinoma| Heavy oil                     | Died after 30 days     |
| 2        | Chinese             | Female| 56  | Farmer           | Adenocarcinoma         | Coal                          | Died after 30 days     |
| 3        | Chinese             | Female| 29  | Farmer           | Adenocarcinoma         | Coal                          | Died after 11 months   |
| 4        | Chinese             | Female| 46  | Farmer           | Squamous cell carcinoma| Coal                          | Died after 15 months   |
| 5        | Chinese             | Female| 51  | Farmer           | Adenocarcinoma         | Coal                          | Not clear              |
| 6        | Chinese             | Female| 53  | Cook             | Small cell carcinoma   | Coal                          | Not clear              |
| 7        | Chinese             | Female| 49  | Farmer           | Squamous cell carcinoma| Coal                          | Not clear              |
Therefore, but no new ones were detected (Table 5).

It is interesting that deposits of large amounts of dust were seen in several areas in the right lung, the bronchus, and the trachea. The patient was a nonsmoker and farmer who had bred many chickens over a 40-year period. It is presumed that the patient might have been exposed to combustion by-products of heavy oil used for air conditioning the chicken house for a long time. Normally, heavy oil as fuel is often used for heating in chicken houses and for drying chicken stooks. It has been found that the mutagenic and carcinogenic NPs detected are inducible from combustion products of fossil fuels (11, 12).

In contrast, all Chinese cases originated in exposure to combustion of coal indoors. This study found that Chinese subjects living in Fuyuan and Xuan Wei Counties, which are next to each other, usually use coal as fuel for heating and cooking indoors. In addition, lung cancer mortality since 1986 has increased to the high level of over 50%. These results are in sharp contrast with those of Japanese case.

Nitro-PAHs are ubiquitous mutagens that are induced in incomplete combustion of fossil fuels or in photochemical reaction of PAH in the environment. Therefore, it is difficult to find a high risk group that is exposed to the chemicals. Microscopically, deposition of dustlike particles was observed in interstitial tissue. These results suggest that the detected mutagens and carcinogens participated in the initiation of the cancer. This hypothesis for the induction of lung cancer might be more valid if DNA adducts for these chemicals are detected in these specimens. Nitro-PAHs are induced in and contaminate urban air pollutants wherever fossil fuels are used.

---

**Table 5. Concentration of mutagen and carcinogen in lung specimen as possible cancer initiation.**

| Mutagen | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Others (54 specimens) |
|---------|---|---|---|---|---|---|---|----------------------|
| 1-Nitropyrene | 0.11 | 0.002 | ND | ND | ND | ND | ND | ND |
| 1-Nitro-3-hydroxyphenanthrene | 0.036 | ND | ND | ND | ND | ND | ND | ND |
| 1,3-Dinitropyrene | 0.095 | ND | ND | ND | ND | ND | ND | ND |
| Chrysene | 0.16 | ND | ND | ND | ND | ND | ND | ND |
| Benzo[k]fluoranthene | ND | 0.11 | 0.32 | 0.06 | 0.14 | 0.17 | 0.1 | ND |
| Benzo[a]pyrene | ND | 0.18 | 0.43 | 0.13 | 0.17 | 0.31 | 0.19 | ND |
| Benzo[g,p,i]perylene | ND | 0.14 | 0.76 | 0.18 | 0.2 | 0.24 | 0.13 | ND |
| Pyrene | ND | 0.29 | ND | 0.16 | 0.16 | ND | ND | ND |

ND, not detected. Lung specimens were extracted with dichloromethane for case 1 and with actone and hexane mixture for cases 2, 3, 4, 5, 6, and 7 by ultrasonification. Crude extracts were separated into three fractions, acidic, basic, and neutral, by liquid-liquid separation. To purify mutagens, the active fraction for strain TA98 or TA100 was partially purified on a column of silica gel for case 1, or, in addition, of ninycol and, subsequently, alumina for cases 2, 3, 4, 5, 6, and 7. Finally, the materials were analyzed by high-pressure liquid chromatography on a Unisil C18 column.

China, and people living in this area usually use coal as fuel for heating and cooking indoors. It is therefore suggested that the Chinese subjects were mostly exposed to soot or tar from combustion of coal indoors. In fact, many deposits of dust were microscopically observed in several areas in all lung tissues.

It is presumed that in human lungs various chemicals are deposited with dust. Therefore, the 54 lung specimens with induced tumors were examined to investigate the distribution of mutagen in background, but none were detected (Table 5).

**Discussion**

It is well known that most dinitro-PAHs are extraordinarily mutagenic for bacteria and mammalian cells (10). Major mutagenic dinitro-PAHs also are carcinogenic in rats and mice (10). Normally, the mutagenic potency is correlated with the carcinogenic potency in rats as demonstrated by dINPs, dINFs, and dINBP. In reference to the NBP structure, the position of the substituted nitro function is important for inducing gene mutation; 3-N-6-ABP, 3-N-6-CBP, and 3,6-dINBP substituted at the third position were classified as the most mutagenic of these dinitro-PAHs, but mutagenicity of 1-N-6-ABP, 1-N-6-CBP, and 1-N-6-ABPO substituted at position 1 and 6 showed a little decrease. The fact that 1,6-dINBP was mutagenic and noncarcinogenic at tested doses may be due to the lack of actual enzymes for reduction in rat liver cytosol or to the lack of products of the hydroxylamino intermediate that bind on DNA in cells.

There is no evidence of an association between mutagenic and carcinogenic nitro-PAHs and the induction of lung cancer. The Japanese case in this study is a case of lung cancer complicated by pulmonary silicosis and pulmonary artery senosis. The histological feature of the lung was that of keratinizing squamous cell carcinoma. It is interesting...

---

**REFERENCES**

1. Tokiwa H, Nakagawa R, Morita K, Ohnishi Y. Mutagenicity of nitro derivatives induced by exposure of aromatic compounds to nitrogen dioxide. Mutat Res 85:195–205 (1981).
2. Tokiwa H, Kitamori S, Horikawa K, Nakagawa R. Some findings on mutagenicity in airborne particulate pollutants. Environ Mutagen 5:87–100 (1983).
3. Newton DL, Erickson MD, Tomer KB, Pellizzari ED, Gentry P. Identification of nitroaromatics in diesel exhaust particulate using gas chromatography/negative ion chemical ionization mass spectrometry and other techniques. Environ Sci Technol 16:206–213 (1981).
4. Fukuwara K, Hakura A, Sera N, Tokiwa H, Miyata N. 1- and 3-Nitro-6-azabenzo[a]pyrene and their N-oxides; highly mutagenic nitroazaaerenes. Chem Res Toxicol 5:149–153 (1992).
5. Nakagawa R, Horikawa K, Sera N, Kodera Y, Tokiwa H. Dinitrofluoranthene; induction, identification, and gene mutation. Mutat Res 191:85–91 (1987).
6. Stanton MF, Miller E, Wrench C, Blackwell, R. Experimental induction of epidermoid carcinoma in the lungs of rats by cigarette smoke condensate. J Natl Cancer Inst 49:867–877 (1972).
7. Poier LA, Weisburger JH. Enzymic reduction of carcinogenic aromatic nitro compounds by rat and mouse liver fractions. Biochem Pharmacol 23:661–669 (1974).
8. Djuric Z, Potter DW, Heffich RH, Beland FA. Aerobic and anaerobic reduction of nitro-pyrenes in vitro. Chem Biol Interact 59:309–324 (1986).
9. Sera N, Kit M, Horikawa K, Fukuwara K, Miyata N, Tokiwa H. Detection of 3,6-dinitrobenzo[a]pyrene in air particulates. Mutat Res 263:27–32 (1991).
10. Tokiwa H, Ohnishi Y. Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment. CRC Crit Rev Toxicol 17:23–60 (1986).
11. Rosenkranz HS, McCoy EE, Sanders DR, Butler M, Kiriazides DK, Mermelstein R. Nitroarenes: Isolation, identification, and reduction of mutagenic impurities in carbon black and toners. Science 209:1039–1043 (1980).
12. Rosenkranz HS, Mermelstein R. Mutagenicity and genotoxicity of nitroarenes; all nitro-containing chemicals were not created equal. Mutat Res 114:217–267 (1983).