In Vivo Metabolism and Genotoxic Effects of Nitrated Polycyclic Aromatic Hydrocarbons

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During incomplete combustion of organic matter, nitro-polycyclic aromatic hydrocarbons (nitro-PAHs), are formed in a reaction that is catalyzed by a low pH. 2-Nitrofluorene (NF), a marker for nitro-PAHs, is metabolized *in vivo* by two different routes. After inhalation, potent mutagenic metabolites, hydroxylated nitrofluorenes (OH-NFs), are formed. The metabolites are distributed by systemic circulation. After oral administration, NF is reduced to the corresponding amine, a reaction mediated by the intestinal microflora. This metabolite is acetylated to 2-acetylaminofluorene (AAF), a potent carcinogen. Further ring-hydroxylation of AAF leads to detoxification and excretion. Induction of cytochrome P450s affects the metabolism, and more OH-NFs are formed. As a consequence, more mutagenic metabolites are found in the circulation. OH-NFs are excreted in the bile as, in terms of mutagenicity, totally harmless glucuronide conjugates. When these conjugates are excreted via the bile, intestinal β-glucuronidase can liberate direct-acting mutagens in the intestine. Thus, inhalation of NF can lead to formation of potent mutagens in the intestine. NF is a direct-acting mutagen in bacterial assays and an initiator and promoter of the carcinogenic process, and gives rise to DNA adduct formation in laboratory animals. — Environ Health Perspect 102(Suppl 4):139–146 (1994).

Key words: genotoxicity, intestinal microflora, metabolism, 2-nitrofluorene, nitro-PAH

Introduction

Incomplete combustion is a major problem in terms of pollution. Examples include emissions from energy production, vehicles, smoking, and industrial processes. The biological effects induced by compounds formed by incomplete combustion can be divided into effects on human health and the ecosystem. Both effects can be acute or long-term. The different biological responses can be related to each other because the same substance in the emissions can give rise to several reactions in the organism and the ecosystem. One example of this is the nitrated polycyclic aromatic hydrocarbons (nitro-PAHs). For the formation of nitro-PAHs, incompletely combusted organic material (PAH) and oxidized nitrogen (NO_x) are necessary. A low pH (SO_2, NO_x) catalyzes the reaction. Because the formation of nitro-PAHs is catalyzed by a low pH, NO_x, catalyzes its own reaction with PAHs to form nitro-PAHs. NO_x is one important combustion product responsible for acidification of the environment, acute health effects (*1*) as well as formation of nitro-PAHs (*2*). These compounds are strong genotoxic agents in mammalian systems (*3–7*).

Nitro-PAHs are found in emissions from diesel (*8*) as well as petrol (*9*) vehicles, the exhaust from kerosene heaters (*10*), urban air (*11–14*), river sediments (*15*), and certain food products (*16,17*), (Tables 1–3). Nitro-PAHs can be formed during the process of combustion or as a result of photochemical reactions of PAHs (*18*) or amino-PAHs (*19*) (Table 4). Nitro-PAH formation reportedly has occurred in the water phase with nitrite as a donor of the nitro-group (*20*).

Nitro-PAHs are a group of at least 200 different substances. Many of them are mutagens (*21–24*), and the most potent mutagenic substances known of today,

Table 1. 2-Nitrofluorene in diesel exhaust.

Level	Driving cycle	Comment	Reference
0.13 to 0.94 μg/km	Bus		(*49*)
0.11 to 1.5 μg/km	Bus terminal		(*49*)
4.1 μg/g		LDD	(*48*)
15 ± 1 μg/g		SRM 1650 [a]	(*48*)
0.63 μg/g		100% load, moderated speed, HDD	(*48*)
8.8 μg/g		75% load, high speed, HDD	(*48*)
5.52 μg/g		In muffler	(*9*)
+		New engine	(*29*)
+		Dilution tunnel	(*29*)
+		One cylinder engine, 75% load	(*106*)
+	FTP	LDD, Oldsmobile 5.7 liter, V8 engine	(*106*)
+		Diesel exhaust	(*105*)
+		Diesel exhaust	(*32*)
+		Diesel exhaust	(*107*)
+		Diesel exhaust	(*30*)
+		LDD	(*108*)
+		Diesel exhaust	(*28*)
84 ppm [b]		HDD, idle	(*51*)
62 ppm [b]		HDD, high speed, zero load	(*51*)
1.9 ppm [b]		HDD, high speed, full load	(*51*)
+		Diesel particles	(*19*)
0.11 ppm [b]		LDD	(*109*)
90 μg/mile	FTP	LDD gas phase (summary 1980–1985)	(*35*)
97 μg/mile	FTP	LDD particle phase (summary 1980–1985)	(*35*)
+		LDD	(*8*)

Abbreviations: HDD, heavy-duty diesel; LDD, light-duty diesel; +, identified, not quantified; FTP, U.S. federal test procedure. [a] SRM, The National Bureau of Standards reference diesel particulate. [b] Concentration in particles.

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Table 2. Formation of 2-nitrofluorene in exhaust other than diesel exhaust.

Concentration	Source	Reference
0.16 µg/g soot	In muffler from gasoline vehicle	(9)
568 ng/m^3	Exhaust from kerosene heater	(116)
19.8 ng/m^3	Close to kerosene heater exhaust	(116)
+	Fuel aromatics + nitrogen dioxide	(29)
++	Airplane engine emissions	(120)

Abbreviations: +, identified, not quantified; ++, identified, high concentrations.

Table 3. 2-Nitrofluorene in the environment.

Concentration	City/country	Comments	Reference
24–71 pg/m^3	Tokyo and Kawasaki, Japan	Three samples from urban environments	(74)
50–700 pg/m^3	Beijing, China	38 samples, higher levels in urban areas	(74)
310–5220 pg/m^3	Berlin, Germany	24 samples, higher levels during winter and in areas dominated by heating	(116,118)
+	Berlin, Germany		(117)
+	Tokyo, Japan		(119)
1.5 µg/kg	Suimon River, Japan	River sediments	(15)

+, identified, not quantified.

Table 4. Formation of 2-nitrofluorene by photochemical reactions.

Reaction	Reference
F + nitrite + UV light = increased mutagenicity [a]	(20)
F + nitrogen dioxide = 2NF	(2)
2AF + sunlight = 2NF	(110)
2AF + fluorescent light = formation of mutagens [a]	(111)
2AF + UV = 2NF	(19,110,112–115)

Abbreviations: 2NF, 2-nitrofluorene; 2AF, 2-aminofluorene; F, fluorene; UV, ultraviolet light. [a] No identification of reaction products.

Table 5. The International Agency for Research on Cancer's evaluation on the human cancer risk of vehicle emissions and some nitro-PAHs (33).

Classification	1	2A	2B	3
Diesel engine exhaust		X		
Gasoline engine exhaust			X	
3,7-Dinitrofluoranthene				X
3,9-Dinitrofluoranthene				X
1,3-Dinitropyrene				X
1,6-Dinitropyrene			X	
1,8-Dinitropyrene			X	
7-Nitrobenz[*a*]anthracene				X
6-Nitrobenz[*a*]pyrene				X
6-Nitrochrysene			X	
2-Nitrofluorene			X	
1-Nitronaphthalene				X
2-Nitronaphthalene				X
3-Nitroperylene				X
1-Nitropyrene			X	
2-Nitropyrene				X
4-Nitropyrene			X	

Abbreviations: 1, carcinogenic to humans; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, is not classifiable as to its carcinogenicity to humans.

dinitropyrenes, are found in this group (25). A number of the nitro-PAHs are also carcinogenic to laboratory animals (26,27).

2-Nitrofluorene (NF) is one of the more common nitro-PAHs and is found in the environment (28–32) with 1-nitropyrene (NP). Normally NP is the dominating species (29), although that is not always the case (30). NF may be a model substance for nitro-PAHs in the gas and particle phase, while NP is regarded to be a model substance for nitro-PAHs in the particle phase (35). NF is a mutagen (24) as well as a carcinogen (27) in laboratory animals.

NF has been studied in detail in our laboratory from an analytical point of view (36) and with regard to metabolism (37–39), lung effects (40,41), and genotoxic effects (42–45). The International Agency for Research on Cancer (IARC) has evaluated the data on NF and some other nitro-PAHs in terms of human cancer risk (33). The evaluation on diesel exhaust (probably the major source for NF and other nitro-PAHs) and other nitro-PAHs are shown in Table 5.

Metabolism of 2-Nitrofluorene

Humans can be exposed to NF, and nitro-PAHs mainly via two routes. The direct exposure is via inhalation with NF present in the gas phase or absorbed on the surface of particles. Large particles will enter into the gastrointestinal tract after deposition in the upper part of the inhalational system or as a consequence of ciliary transport up from the lungs.

The indirect exposure will occurs when deposition of particles occurs on vegetables or other products of agricultural origin for human consumption. With the addition of what can be added during food processing, this way of contamination results in an exposure to the gastrointestinal tract. There exist other, possibly minor routes, such as by contaminated water and by water-living organisms used as food. However, in this case the gastrointestinal tract is the end point. Therefore, it is important to study the effect of NF following oral and intratracheal administration.

Liquid Chromatography and Mass Spectrometry Analyses of Metabolites

Liquid chromatography and mass spectrometry analysis (LC/MS) with a nebulizer and a moving belt was used to characterize NF and its metabolites (36). The properties and fragmentation patterns of 18 different fluorene derivatives were examined first. Without prior derivation, all substances yielded interpretable mass spectra. The LC/MS system had the capacity to distinguish between seven different hydroxylated isomers of hydroxylated acetylaminofluorene (OH-AAF). In combination with the UV-analyses in the high pressure liquid chromatography (HPLC) system, an identification could be performed based on the retention time from the total or single ion current chromatograms, differences in mass spectral intensities, and specific losses of fragments. In addition, HPLC analysis

coupled with the radioactivity detector on line indicated peaks with metabolites originating from the radiolabeled NF. The UV-detector gave a signal that was linear and parallel with the signal from the ion source, demonstrating that although the two detectors measure different parameters, they do so at a constant ratio. Thus, the described system was considered to be well suited for the studies on the metabolism of NF.

Metabolism after Oral Administration of Nitrofluorene

Although NF (and nitro-PAH in general) is a chemically stable molecule, it is metabolized extensively in the organism. After oral administration of NF, the major part of the dose is excreted within 48-hr (*37,39*). After 4 hr, approximately 1.5% of the dose has been metabolized (in several steps) in the liver, distributed in the circulation, filtered by the kidneys, and excreted in the urine. The excretion of metabolites is accompanied by excretion of mutagenicity. Typically, direct-acting mutagenicity (-S9) dominated over mutagenicity in the presence of S9, both in urine and feces (*37,39*).

The *in vivo* formation (*37*) of the potent carcinogen 2-acetylaminofluorene (AAF) (*46*) is indicated. After an oral dose of NF to conventional rats, NF is reduced to 2-aminofluorene (AF) by the intestinal microflora and acetylated and further hydroxylated in the liver, which results in OH-AAFs. These can be excreted as such or in conjugated form. This metabolic route is quantitatively most important. AAF has been a model compound for chemical carcinogenesis since Wilson's discovery of its carcinogenic potential in 1941 (*47*). AAF is not found in the environment, and occupational exposure can occur only when AAF is used in research. Thus, it is of concern when an environmental pollutant (NF) commonly found in diesel exhaust (*9,48,49,51*) is metabolized to this potent carcinogen (AAF) *in vivo*. Although the biological significance of these metabolites is not known, other nitro-PAHs have been shown to form acetylated metabolites (*52,53*).

After oral administration of NF, an alternative metabolic route that results in the formation of OH-NFs in the conventional animal forms (*37*). While OH-AAFs are considered to be detoxification products (*54*), they have a low mutagenic potency (*55*). OH-NFs, on the other hand, are more mutagenic (TA 98-S9) than NF alone (*39*). In conventional rats (*37*) treated with β-naphtoflavone prior to administration of NF, the metabolic pattern shifted towards excretion of a larger proportion of OH-NFs in comparison with uninduced rats, and the mutagenicity of urine increased simultaneously. Presently the carcinogenic potential of OH-NFs has not been investigated in detail, but it can not be denied that they are carcinogenic. Therefore, they may be involved in the induction of forestomach tumors seen after oral dosing of NF. In contrast, no forestomach tumors are seen following administration of AF or AAF. OH-NFs may also play a role in the formation of subcutaneous tumors after skin application of NF to rats (*56,27*).

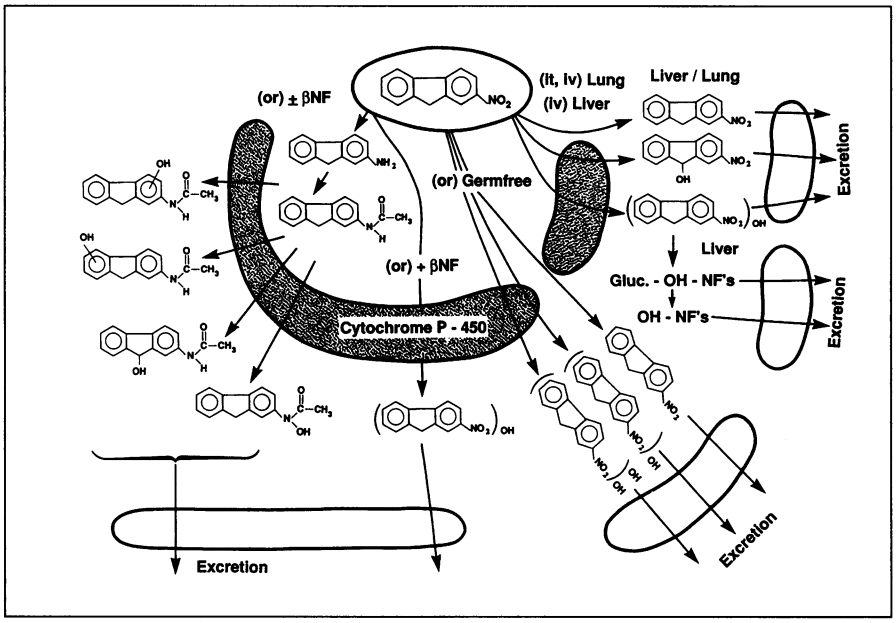

Figure 1. A summary of the metabolism of 2-nitrofluorene (NF) in laboratory animals. or, oral; it, intratracheal; iv, intravascular (organ perfusions) administration of NF; βNF, induction with β-naphthoflavone; germ-free, germ-free animals (otherwise conventional animals).

The involvement of the intestinal microflora in the metabolism of NF was studied using germ-free and conventional rats (*39*). The mutagenicity of urine from germ-free animals exceeded the conventional urine in direct-acting mutagenicity by a factor of approximately six. The same observation was made in feces (*39*). The LC/MS analyses of urine and feces from germ free animals confirmed the presence of OH-NFs and the absence of OH-AAFs. NF was excreted, to a small extent, in the urine on 1 day following administration, indicating the absorption of unreduced NF from the gastrointestinal tract. The major metabolic route in germ-free animals was the formation of OH-NFs, which also were responsible for the excreted direct-acting mutagenicity. In the urine from germ free animals a di-OH-NF was detected as the major metabolite in terms of radioactivity (34%), although it was only of minor importance in terms of mutagenicity (2%).

The mutagenicity of NF was increased significantly after monohydroxylation (*39*). Further hydroxylation appeared to decrease the mutagenicity to levels below NF. The formation of OH-NFs, their potency in genotoxic assays, and possible carcinogenic character indicate the need for carcinogenicity studies on this class of compounds. The metabolism of NF is summarized in Figure 1.

Oral administration of NP to rats resulted in the formation of reduced, acetylated, and hydroxylated metabolites, but ring-hydroxylated NPs were reported to be responsible for a higher, direct-acting mutagenicity in urine of rats treated with phenobarbital (*57*), indicating the importance of enzyme induction in the metabolism of nitro-PAHs to mutagenic compounds. Other studies have shown that pretreatment of rats with β-naphtoflavone increased the amount of ring-OH-AAFs in milk after intraperitoneal administration of AAF (*58*). This fact raises the question whether the OH-NFs can also be excreted in milk following the mothers inhalation of urban air and/or diesel exhaust, which would expose infants to a genotoxic risk.

Hydroxylated metabolites of NP undergo nitroreduction and subsequent DNA binding much more readily than NP (*59*), leading to the conclusion of Beland et al (*60*) that tumorigenicity assays should be

conducted not only with the parent compound and their reduced derivatives but also with their ring-hydroxylated metabolites to assess human health risks from nitro-PAHs.

Metabolism after Inhalation of Nitrofluorene

In the isolated perfused rat lung there is a rapid metabolism to direct acting mutagens when NF is administered intratracheally and intravascularly. The metabolites formed are unconjugated ring OH-NFs. Unmetabolized NF given intratracheally can also pass through the lung into the circulation together with metabolites (OH-NFs). Thus, it is likely that inhalation might result in whole-body exposure to circulating mutagens and carcinogens (NF and OH-NFs). In the study performed on the isolated perfused liver (*38*), the purpose was to examine the type of metabolism occurring when NF was administered intravascularly, as the liver can be exposed to after inhalation. The liver metabolized NF to OH-NFs but excreted them, in terms of mutagenicity, in a harmless form as glucuronides. Treatment of the bile with β-glucuronidase liberated the direct-acting mutagens, the OH-Ns.

β-glucuronidase is an intestinal enzyme, and its activity is high in individuals on an "Western diet" (high in fat and protein) (*70*). Thus, the results indicate a chain of events. Inhaled NF is metabolized by the lung to OH-NFs or transported to the liver as NF and then is ring-hydroxylated. The liver conjugates the OH-NFs and excretes them via the bile. In the intestine, the OH-NFs may be liberated, exposing the intestine to a genotoxic risk. In other words, air pollutants such as nitro-PAHs could be found in the colon or other organs.

The results presented (*38*) are in accordance with lung metabolic data on NP. Lung microsomes from rats, rabbits, and hamsters metabolize NP to mutagenic products that were ring-hydroxylated (*71*). Interestingly, nasal mucosa also metabolizes NP to OH-NPs, a metabolic route that represented more than 90% of the metabolites (*72*). Isolated perfused lung metabolized NP in the same way as the nasal mucosa. The rate of metabolism of NP in the lung increased (i.e., the probability increased for production of genotoxic metabolites, following exposure to diesel exhaust) (*72*).

Comparison between Animals and Man

One can always argue about whether data on animal metabolism is relevant to humans, but in the case of nitro-PAHs, there are a number of arguments to indicate that the animal studies are relevant to the human situation. *a*) Reduction of nitro-PAHs to amino-PAHs can be performed by anaerobic fecal bacterial suspension from humans as well as rats (*61–63*). *b*) Human liver S9 bioactivated AF and AAF to mutagens (*64*). *c*) Human hepatoma cell lines can perform nitroreduction as well as ring-hydroxylation of NP (*65*). *d*) Liver microsomal metabolism of AAF is similar in rats and humans (*66*). *e*) Human lymphocytes metabolize AAF to ring- and N-hydroxy derivatives of AAF (*67*). *f*) AAF metabolism is similar in cultures of epithelial cells from human and rat bladder (*68*). *g*) It has been shown that the carcinogen AAF given orally to humans results in the same urinary metabolites as in the rat (*69*).

Risk Identification of 2-Nitrofluorene

Acute Toxicity

The acute toxicity of NF is low, indicated by the low 24-hr LD_{50}, which has been shown to be 1.6 g/kg body weight (bw) in male Swiss-Webster rats (*80*). The major risk with exposure to NF is genotoxic effects.

Genotoxic Effects

NF commonly is used as a positive control in bacterial mutagenicity assays. Typically NF has a direct-acting mutagenic effect (does not require a metabolic system) in a number of the *Salmonella typhimurium* strains (*95–101*). NF is not among the most potent bacterial mutagens that can be found in the family of nitro-PAHs (Table 6).

In a liver model for chemical carcinogenesis, NF has been shown to be a potent initiator. The statistically significant dose response curve was approximately 10 times the background at the highest dose (*44*). When NF was characterized as a promoter, the basic concept for the liver model was used, but the dietary AAF-promotion regimen was replaced by six intragastric administrations of NF. At the lower dose similar to the doses used in the NF metabolic studies, NF and AAF were both weak promoters. At high doses, AAF was a very potent promoter, while NF remained at a low but statistically significant level of promoting activity (*44*).

NF given to laboratory animals under different conditions gave rise to DNA adduct formation. The major DNA adduct characterized in the liver was C8-guanine-2-aminofluorene, indicating the importance of the intestinal microflora in the reduction of the nitro function to an amine (*73*).

Table 6. Mutagenicity (plate incorporation) of nitrofluorene and related substances.

Nitro-PAH	TA98-S9, revertants/nmole	Reference
1-Nitronaphthalene	0.05	(*89*)
2-Nitrochrysene	0.6	(*103*)
5-Nitroacenaphtene	2.5	(*89*)
1-Nitrocoronene	2.8	(*103*)
2-Nitrofluorene	18	(*89*)
1-Nitrofluoranthene	74	(*103*)
2-Nitrophenanthrene	128	(*46*)
2-Nitrophenanthrene	453	(*89*)
2-Nitropyrene	2225	(*103*)
3-Nitrofluoranthene	3735	(*89*)
1,3-Dinitropyrene	144,700	(*89*)
1,6-Dinitropyrene	183,600	(*89*)
1,8-Dinitropyrene	254,000	(*89*)

Table 7. Sites of tumors after administration of nitrofluorene to rats.

Organ	Route: Oral [a,b]	Skin [b]	Oral [c]
Mammary gland	+	+	
Ear duct	+		*d*
Pituitary gland		+	*d*
Adrenal gland		+	
Lung	+	+	*d*
Salivary gland		+	
Forestomach	+		+
Liver	+		+
Intestine	+		*d*
Subcutaneous		+	*d*
Kidney			+
Number of animals	18 [a] 9 [b]	10	80

[a] Data from Miller et al. (*27*). [b] Data from Morris et al. (*56*). [c] Ongoing study 1991 to 1993 (*75*). [d] Possible tumors.

The DNA adducts formed by NF and derivatives were characterized by the ^{32}P-postlabeling method (thin-layer chromatography analyses) as well as by HPLC analyses of ^{32}P-labeled DNA adducts. The development of a HPLC method to characterize ^{32}P-labeled DNA adducts opens new perspectives in terms of separation and characterization DNA adducts in general (*73*).

There are three studies on the carcinogenicity of NF. Two were performed in the early 1950s by Morris et al. (*56*) and Miller et al. (*27*) on small groups of animals with only one dose studied. The third is an ongoing study by Möller et al. (*75*).

These incomplete studies indicate that NF is a potent carcinogen and induces tumors in many different organs and glands (e.g., liver, forestomach, intestine, kidneys, lung, mammary gland, subcutaneous, pituitary gland, ear duct, adrenal gland, and salivary gland (Table 7) (*56,27,75*). In the

Table 8. A summary of nitrofluorene's biological effects.

Assay	Effect	Reference
Sister chromatid exchange	+	(*3,82,83*)
Chromosomal aberrations	+	(*104*)
Initiator	+	(*44*)
Promoter	+	(*44*)
Carcinogenicity	+	(*27,56,75*)
Formation of DNA adducts	+	(*84*)
Micronuclei assays	–	(*6,86,87,88*)
Mutation assay, mammalian cells	+	(*85*)
Bacterial mutagenicity (*Salmonella*)	+	(*89,90,91*)
Bacterial mutagenicity (*Escherichia coli*)	+	(*92*)
Mutagenicity, nematode assay	+	(*81*)
Mutagenicity, mouse lymphoma assay	+	(*3,85,93*)
Induction of unscheduled DNA synthesis	+	(*43*)

Abbreviations: +, positive effect; –, no effect.

ongoing study with NF administration in the food at ppm levels, there was a dramatic tumor formation in the high dose group of 500 ppm after 10 months of feeding. In approximately 4 weeks, all animals developed very large liver tumors and multiple forestomach tumors. The tumors in the liver were up to 55 mm in diameter. In many cases there were three to five tumors with diameters above 20 mm. These preliminary tumor data are in accordance with the formation of DNA adducts in the liver (*73,75*). The genotoxic effects of NF are summarized in Table 8. A more extensive review of NF and its biological effects has been published by Beije and Möller (*34*).

Additional Factors in the Risk Assessment of Genotoxic Effects

In addition to the initiation of tumors, additional risks of NF as a marker for nitro-PAHs, should be taken into consideration in the risk assessment process. These risk factors might involve promotion of tumor development and cocarcinogenic effects. NF has been shown to have tumor promoting capacity (*44*). Nitro-PAHs and PAHs always occur together. Recent data indicate that NP and benzo[*a*]pyrene are potent cocarcinogens (*94*). Humans on a "Western diet" have higher levels of intestinal β-glucuronidase (*70*), which could result in an increased liberation of genotoxic NF-metabolites in the colon (*44*). Induction of cytochrome P450s (possibly by other environmental contaminants) results in the formation of potent, direct-acting mutagens and possible carcinogens (*37*). Certain food components can affect intestinal nitro-reductases dramatically. Because the nitroreduction is a metabolic step this could be of great importance (L. Möller, unpublished data). The possible effects of alcohol consumption on the metabolism of nitro-PAHs is also a risk factor. Liver microsomes from rats with prior dosing of ethanol metabolize NP in a different manner (*77*), and hepatic microsomes from ethanol-fed hamsters bioactivated AF more effectively (*78*).

There also could be effects on reproduction considering that metabolites of nitro-PAH have been reported to cause malformations in laboratory animals (*76*). Nitrocompounds have also been reported to cause infertility and reduced sperm counts in rats (*50*). There is a risk that human fertility could be affected since nitro-derivatives negatively affect human sperm motility (L. Möller, unpublished data).

If a risk assessment is performed on nitro-PAHs, there are certain groups of individuals that can be considered high risk groups if the data from animal models are relevant for humans. The high risk groups could be defined by exposures and eating habits. One group would include those exposed to other genotoxic substances (smokers, certain occupational environments and urban populations) that can initiate tumor formation. Nitro-PAHs then could function as cocarcinogens, promoters, or both. Another group would include those with certain food habits, like people on a "Western diet." Also in this group would be people who eat food containing inducers of the cytochrome P450 system or food prepared over an open fire.

Consumption of alcohol is another possible risk factor. Food or components in food have an influence on risk mainly via effects on different enzyme systems, but food processing under certain conditions could be the major source for nitro-PAHs. For example, a popular Japanese chicken dish can contain as much nitropyrene as 3.5 years of breathing on the streets of Tokyo if all inhaled nitropyrene is retained in the lung (*74,17*).

Conclusion

NF is a result of incomplete combustion and consequently can be found where combustion takes place. It has been found in emissions from vehicles with diesel-driven diesel engines as the dominating source. Other sources of combustion are gasoline vehicles and kerosene heaters. NF can also be found in different environments like river sediments and urban air. The sources producing it in urban air can be energy generation, especially the combustion of coal, as well as vehicles. An additional source of NF in the environment is the photochemical formation.

NF and derivatives may represent a large portion of present nitro-PAHs in the environment. In one sample of airborne particles from Japan, NF and two isomers of dinitro-NF represented 55% of the amount of 19 analyzed nitro-PAHs (*79*). Humans are exposed to NF, nitro-PAHs, or both wherever combustion if found.

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