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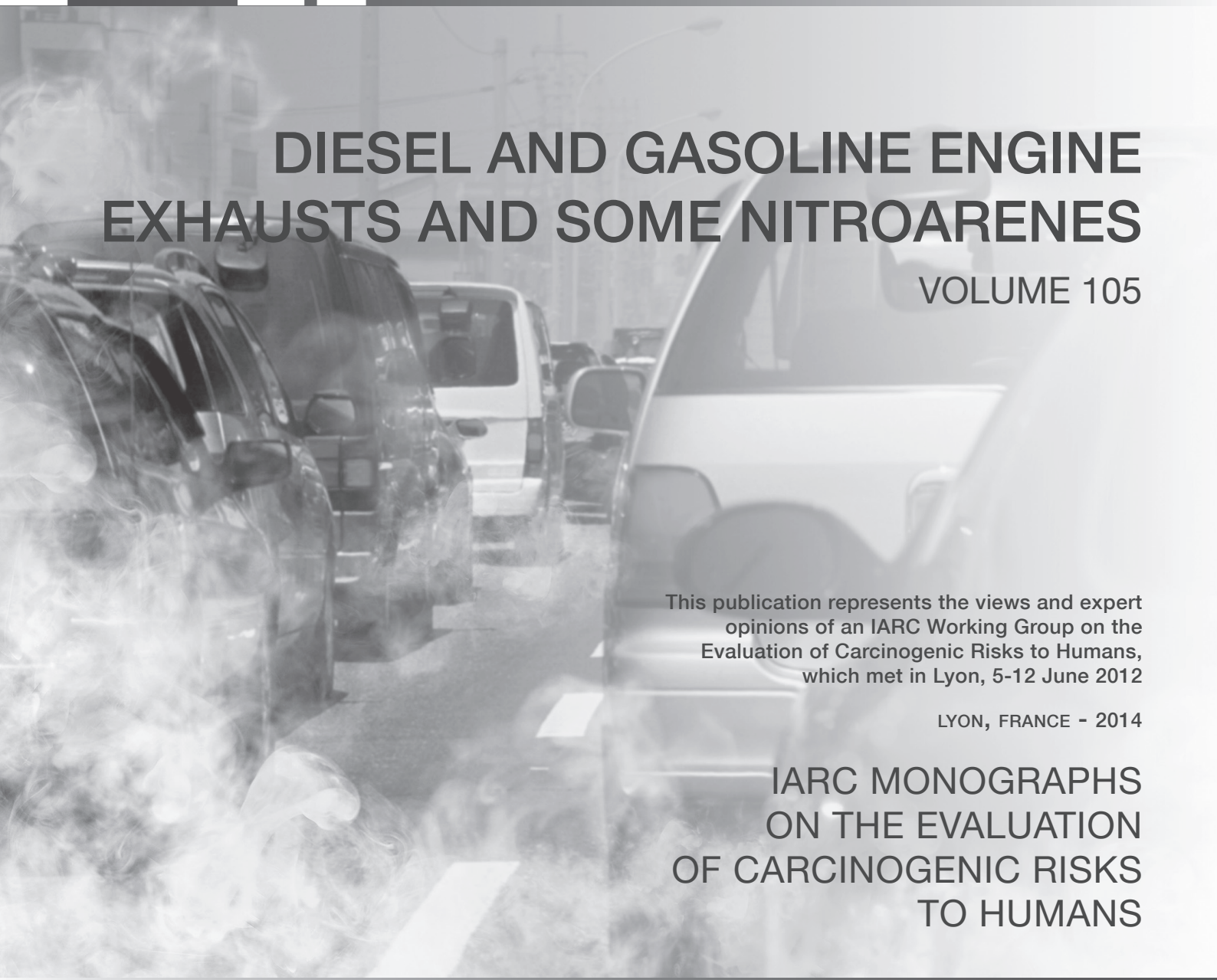

DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

3,9-DINITROFLUORANTHENE

3,9-Dinitrofluoranthene was evaluated by previous IARC Working Groups in 1988 and 1995 (IARC, 1989, 1996). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

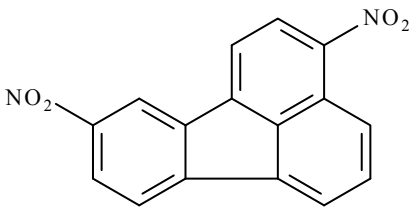
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 22506-53-2

Synonym: 3,9-Dinitrofluoroanthene; 4,12-dinitrofluoranthene

1.1.2 Structural and molecular formulae and relative molecular mass



$C_{16}H_8N_2O_4$

Relative molecular mass: 292.26 g/mol

1.1.3 Chemical and physical properties of the pure substance

Description: Yellow needles (Nakagawa *et al.*, 1987); yellow-orange crystals (Charlesworth & Lithown, 1969)

Melting-point: 222–224 °C (Nakagawa *et al.*, 1987); 275–276 °C (Charlesworth & Lithown, 1969)

Spectroscopy data: Nuclear magnetic resonance and ultraviolet spectral data have been reported (Ramdahl *et al.*, 1988)

Octanol/water partition coefficient: log K_{ow} = 4.44 (Lyman, 1985)

1.2 Analysis

Tokiwa *et al.* (1990) reported a method to separate and identify dinitrofluoranthenes in airborne particulates. The particulate matter was collected on a silica fibre filter and extracted with dichloromethane. The crude extracts were applied to a column filled with silica gel and were eluted step by step with hexane, hexane:benzene (1:1, v/v), benzene, benzene:methanol (1:1, v/v) and methanol. The components were fractionated and identified by high-performance liquid chromatography and gas chromatography with mass spectrometry.

1.3 Production and use

3,9-Dinitrofluoranthene can be synthesized by the nitration of fluoranthene or 3-nitrofluoranthene in the presence of fuming nitric acid, with subsequent fractionation and purification by recrystallization (Nakagawa *et al.*, 1987; Horikawa *et al.*, 1991; Matsuoka *et al.*, 1993).

No evidence was found that 3,9-dinitrofluoranthene has been produced in commercial quantities or used for any purpose other than laboratory applications.

1.4 Occurrence and exposure

1.4.1 Natural occurrence

3,9-Dinitrofluoranthene is not known to occur as a natural product.

1.4.2 Environmental exposure

Nitrofluoranthenes can be formed during the combustion of fossil fuels that contain fluoranthene, and through atmospheric reactions of fluoranthene with nitrogen oxides. Sources of nitrofluoranthenes include diesel emissions, combustion emissions from kerosene heaters, gas fuel, liquefied petroleum, airborne particles, coal fly ash and food (Horikawa *et al.*, 1987).

3,9-Dinitrofluoranthene was detected at a concentration of 0.013 mg/kg in particulates emitted from a diesel engine (Tokiwa *et al.*, 1986). In 1989, 3,9-dinitrofluoranthene was detected in airborne particulates at a level of 0.009 μg/g particulates, corresponding to 0.004 ng/m^3, in Sapporo, Hokkaido, Japan (Tokiwa *et al.*, 1990).

Monitoring data indicate that exposure of the general population to 3,9-dinitrofluoranthene may occur through its inhalation in the ambient air (HSDB, 2013).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

3.1 Rat

See Table 3.1

3.1.1 Subcutaneous administration

A group of 11 male Fischer 344/DuCrj rats (age, 6 weeks) received 3,9-dinitrofluoranthene (purity, 99.8%) by subcutaneous injection at a dose of 0.05 mg dissolved in 0.2 mL of dimethyl sulfoxide twice per week for 10 weeks (total dose, 1 mg/rat). A group of 21 males was injected similarly with the solvent alone. Animals were observed for 50 weeks; those with tumours at the site of injection were killed when moribund. The first subcutaneous tumour was observed in the treated group on day 88, and 10 out of 11 (91%) treated rats had developed tumours at the site of injection by 48 weeks after the beginning of treatment; controls did not develop tumours. The incidence of tumours was highly statistically significant [10/11 versus 0/21; $P < 0.001$]. Seven of 10 tumours were described as malignant fibrous histiocytoma and three as rhabdomyosarcoma. No metastasis was found. Mean tumour-induction time was 107 days (Tokiwa *et al.* 1987).

3.1.2 Intrapulmonary administration

Groups of 10–21 male Fischer 344/DuCrj rats (age, 11 weeks) received 3,9-dinitrofluoranthene (purity, > 99.98%) as a single implantation at a dose of 0, 50, 100 or 200 μg in 0.05 mL of beeswax:tricaprylin vehicle into the left lung by thoracotomy under ketamine anaesthesia, and were then observed for up to 100 weeks. The incidence of tumours of the lung in the groups at 50, 100 and 200 μg was 1 out of 10 (10%), 7 out of 10 (70%) and 19 out of 21 (90%), respectively. No tumours were found in the vehicle-control group (0 out of 19). In rats at 200 μg, the first tumour was observed on day 257 after injection. The incidence of tumours in the groups at 100 μg and

Table 3.1 Studies of the carcinogenicity of 3,9-dinitrofluoranthene in rats

Strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M) up to 50 wks Tokiwa *et al.* (1987)	Subcutaneous injection 0, 0.05 mg/rat in 0.25 mg/mL DMSO, twice/wk for 10 wks (total, 1.0 mg or 3.42 µmol) then observed up to 50 wk 11 or 21 M/group (age, 6 wk)	Injection site (subcutaneous sarcoma): 0/21, 10/11 (91%) Injection site (malignant histiocytoma): 0/21, 7/11 (64%) Injection site (rhabdomyosarcoma): 0/21, 3/11 (27%)	[$P < 0.001$, Mann–Whitney U]	Purity, 99.8% by HPLC
F344 (M) up to 100 wks Horikawa *et al.* (1991)	Intrapulmonary implantation 0, 50, 100, 200 µg/rat in 0.05 mL beeswax:tricaprylin, injected directly into the left lower lung after thoracotomy then observed up until 100 wk 10–21 M/group (age, 11 wk)	Lung (squamous cell and adenosquamous carcinoma combined): 0/19, 1/10 (10%), 7/10 (70%), 19/21 (90%) Lung (squamous-cell carcinoma): 0/19, 18/21 (86%) Lung (adenosquamous carcinoma): 0/19, 1/21 (5%)	[$P < 0.001$, mid- and high-dose; Mann–Whitney U]	Purity, > 99.9% by HPLC

DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; M, male, wk, week

200 µg was statistically significantly increased in comparison with the control group [$P < 0.001$, Mann–Whitney U]. The tumours in the group at 100 µg were described as five squamous cell carcinomas, one adenosquamous carcinoma and one undifferentiated carcinoma, and those in the group at 200 µg as 18 squamous-cell carcinomas and one adenosquamous carcinoma (Horikawa *et al.* 1991).

4. Mechanistic and Other Relevant Data

3,9-Dinitrofluoranthene was considered by previous IARC Working Groups in 1988 and 1995 (IARC, 1989, 1996). Since that time, no new data have become available on the biological fate, metabolism or toxicity of this compound in experimental animals or humans.

4.1 Absorption, distribution, metabolism, excretion

4.1.1 Humans

No data were available to the Working Group.

4.1.2 Experimental systems

Mitchell *et al.* (1993) reported the metabolism of 3,9-dinitrofluoranthene under anaerobic conditions *in vitro* in subcellular fractions of the lung, the target organ for carcinogenicity in rats (Tokiwa *et al.*, 1987; Horikawa *et al.*, 1991). The rate of metabolism of 3,9-dinitrofluoranthene, the most carcinogenic compound of the various nitrofluoranthene derivatives, was compared with that of 2-, 3- and 8-nitrofluoranthenes, which have been reported to be relatively weak or inactive in the induction of tumours in experimental animals (Ohgaki *et al.*, 1982; Tokiwa & Ohnishi, 1986; Tokiwa *et al.*, 1986, 1993; Horikawa *et al.*, 1991). Both the cytosolic and microsomal fractions from rat lung anaerobically converted 3,9-dinitrofluoranthene and 2-, 3- or 8-monofluoranthene into their amino derivatives, i.e. 3-amino-9-nitrofluoranthene and 2-, 3- or 8-aminofluoranthene, respectively. The extent of formation of the amino derivative of 3,9-dinitrofluoranthene was found to be much greater than that of the three mononitrofluoranthenes (Mitchell *et al.*, 1993). Because the formation of amino derivatives of carcinogenic nitroarenes, such as 1,3-, 1,6- and 1,8-dinitropyrenes, has been reported to be a key step in the metabolic activation of nitroarenes to DNA-binding products (Rosenkranz & Mermelstein, 1983; Tokiwa *et al.*, 1986; Wislocki *et al.*, 1986; Möller, 1994; Purohit & Basu, 2000), 3,9-dinitrofluoranthene may also be classified as potentially carcinogenic in view of this metabolic conversion (Mitchell *et al.*, 1993; Möller, 1994).

Mitchell *et al.* (1993) also reported that 3-nitrofluoranthene was oxidized by microsomes from rat lung to form at least three metabolites, the most important of which was identified as 3-nitrofluoranthene-8-ol.

4.2 Genetic and related effects

4.2.1 Humans

No data were available to the Working Group.

4.2.2 Experimental systems

As reported in a previous *IARC Monograph* (IARC, 1989), 3,9-dinitrofluoranthene was highly active in the induction of reverse mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1538 and TA1537, but not TA97, in the absence of a mammalian metabolic-activation system. Of these tester strains, TA98 was the most sensitive to 3,9-dinitrofluoranthene, indicating that this chemical induces frameshift-type mutations (IARC, 1989; Tokiwa *et al.*, 1993). Several laboratories compared the potencies of various nitroarenes, including

3,9-dinitrofluoranthene, to induce reverse mutations in *S. typhimurium* TA98 in the absence of metabolic activation (IARC, 1989; Tokiwa *et al.*, 1993; Reifferscheid & Heil, 1996). Among several mono-, di- and trinitrofluoranthene derivatives, 3,7- and 3,9-dinitrofluoranthene were highly mutagenic, to an extent that was comparable with that of the dinitropyrenes.

The mutagenicity of 3,9-dinitrofluoranthene was also determined in other strains, such as *S. typhimurium* TA98/NR and TA98/1,8-DNP6, that are deficient in nitroreductase and *O*-acetyltransferase activities, respectively (IARC, 1989; Yamada *et al.*, 1997). The results showed that 3,9-dinitrofluoranthene was less mutagenic in these strains than in TA98 (Tokiwa *et al.*, 1987, 1993), indicating that this chemical may be activated to mutagenic products by nitroreductase (IARC, 1989).

In the Ames assay, 3,9-dinitrofluoranthene was less mutagenic in the presence than in the absence of metabolic activation, which suggests that xenobiotic-metabolizing enzymes convert this compound to a less mutagenic product. It should be noted that several other mutagenic nitroarenes, such as 1,3-, 1,6- and 1,8-dinitropyrene, have also been reported to be inactivated by human and rat cytochrome P450 enzymes to form products that have lost their genotoxic and mutagenic activity in bacteria (Shimada & Guengerich, 1990; Shane & Winston, 1997).

Oda *et al.* (1992) reported that 3,9-dinitrofluoranthene was genotoxic and induced *umu* gene expression (measured as β-galactosidase activity) more strongly in *S. typhimurium* NM1011, which has high nitrofurazone-reductase activity, than in the parent strain, *S. typhimurium* TA1535/pSK1002. The strains deficient in nitroreductase and/or *O*-acetyltransferase were less sensitive to the induction of *umu* gene expression by 3,9-dinitrofluoranthene and other nitroarenes, such as 3,7-dinitrofluoranthene, 3-nitrofluoranthene, and 1,3-, 1,6- and 1,8-dinitropyrene, which indicates that these two enzyme activities are required for the activation process (Oda *et al.*, 1993). As expected, *S. typhimurium* NM3009, which expresses both high nitroreductase and high *O*-acetyltransferase activity, showed sensitivity for *umu* gene expression induced by 3,9-dinitrofluoranthene, followed by strains NM2009 and NM1011, which have *O*-acetyltransferase and nitroreductase activity, respectively.

3,9-Dinitrofluoranthene also induced chromosomal aberrations in a Chinese hamster cell line in the absence of rat-liver metabolic activation *in vitro* (Matsuoka *et al.*, 1993) and micronucleus formation in mouse bone marrow *in vivo* (Tokiwa *et al.*, 1993).

4.3 Mechanistic considerations

(See also the corresponding Section in the *Monograph* on 3,7-Dinitrofluoranthene in this Volume.)

Assays for genotoxicity and mutagenicity in bacteria indicated that 3,9-dinitrofluoranthene requires metabolic activation by xenobiotic-metabolizing enzymes to form active metabolites (Nakagawa *et al.*, 1987; Oda *et al.*, 1992, 1993; Tokiwa *et al.*, 1993).

3,9-Dinitrofluoranthene was metabolized to its amino derivative by rat lung cytosolic and microsomal nitroreductases under anaerobic conditions *in vitro* (Mitchell *et al.*, 1993), and was hypothesized to be activated primarily to a reactive intermediate by such nitroreductases (Tokiwa *et al.*, 1986; Möller, 1994; Purohit & Basu, 2000).

Although 3,9-dinitrofluoranthene has been postulated to be activated to reactive metabolites by bacterial and mammalian enzyme systems, the detoxification pathways of this chemical by various xenobiotic-metabolizing enzymes are not fully understood. Further studies are required to understand the underlying mechanisms of the genotoxicity, mutagenicity and carcinogenicity of 3,9-dinitrofluoranthene.

5. Summary of Data Reported

5.1 Exposure data

3,9-Dinitrofluoranthene is present in diesel exhaust emissions and emissions from heaters fuelled by liquefied petroleum gas. No evidence was found that it has been produced in commercial quantities or used for purposes other than laboratory applications. Due to its low vapour pressure, this substance is associated with the particulate phase of these combustion emissions. Exposure of the general population to 3,9-dinitrofluoranthene occurs through inhalation of airborne particulate matter in urban environments. Exposure may also occur from domestic use of burners of liquefied petroleum gas. No data on occupational exposure were available to the Working Group.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

3,9-Dinitrofluoranthene was tested for carcinogenicity in rats by subcutaneous injection in one study and by direct implantation into the lung in another study. Subcutaneous injection of 3,9-dinitrofluoranthene induced a significant increase in the incidence of subcutaneous sarcomas, and intrapulmonary implantation produced a significant increase in the incidence of carcinomas of the lung.

5.4 Mechanistic and other relevant data

No data were available on the absorption, distribution, metabolism and excretion, or genetic and related effects of 3,9-dinitrofluoranthene in humans. Cytosolic and microsomal fractions from rat lung anaerobically converted 3,9-dinitrofluoranthene into 3-amino-9-nitrofluoranthene. The compound was strongly mutagenic in bacteria in the absence of metabolic activation, acting mainly as a frameshift mutagen. The mutagenicity was weaker in bacterial strains that are deficient in nitroreductase or *O*-acetyltransferase, indicating that the activation of 3,9-dinitrofluoranthene occurs through its nitroreduction and acetyltransfer. It was genotoxic in the *umu* gene-expression assay, and induced chromosomal aberrations in Chinese hamster cells *in vitro* and micronucleus formation in the bone marrow of mice *in vivo*. 3,9-Dinitrofluoranthene induced tumours in rats at the site of exposure, while its intrapulmonary implantation produced lung tumours. This evidence suggests that the mutagenic metabolites formed from this agent could play a role in its carcinogenicity.

Overall, these data provide *weak mechanistic evidence* to support the carcinogenicity of 3,9-dinitrofluoranthene.

6. Evaluation

6.1 Cancer in humans

No data were available to the Working Group.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 3,9-dinitrofluoranthene.

6.3 Overall evaluation

3,9-Dinitrofluoranthene is *possibly carcinogenic to humans (Group 2B)*.

References

Charlesworth EH & Lithown CU (1969). Fluoranthene studies. IV. Nitration of 2-nitro-, 3-nitro- and 2-acetamido-fluoranthene. *Can J Chem*, 47: 1595–1599. doi:10.1139/v69-262

Horikawa K, Otofuji T, Nakagawa R *et al.* (1987). Mutagenicity of dinitrofiuoranthenes (DNFs) and their carcinogenicity in F344 rats (Abstract no. 11). *Mutat Res*, 182: 360–361.

Horikawa K, Sera N, Otofuji T *et al.* (1991). Pulmonary carcinogenicity of 3,9- and 3,7-dinitrofluoranthene, 3-nitrofluoranthene and benzo[a]pyrene in F344 rats. *Carcinogenesis*, 12: 1003–1007. doi:10.1093/carcin/12.6.1003 PMID:2044179

HSDB; Hazardous Substances Data Bank (2013). *3,9-Dinitrofluoranthene*. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. Accessed 13 November 2013.

IARC (1989). Diesel and gasoline engine exhausts and some nitroarenes. *IARC Monogr Eval Carcinog Risks Hum*, 46: 1–458. PMID:2483415

IARC (1996). Printing processes and printing inks, carbon black and some nitro compounds. *IARC Monogr Eval Carcinog Risks Hum*, 65: 1–578.

Lyman WJ (1985). *Environmental Exposure from Chemicals*, Vol. I. Neely WB, Blau GE, editors. Boca Raton, FL, USA: CRC Press: p. 31

Matsuoka A, Horikawa K, Yamazaki N *et al.* (1993). Chromosomal aberrations induced in vitro by 3,7- and 3,9-dinitrofluoranthene. *Mutat Res*, 298: 255–259. doi:10.1016/0165-1218(93)90004-W PMID:7678161

Mitchell CE, Bechtold WE, Belinsky SA (1993). Metabolism of nitrofluoranthenes by rat lung subcellular fractions. *Carcinogenesis*, 14: 1161–1166. doi:10.1093/carcin/14.6.1161 PMID:8508503

Möller L (1994). In vivo metabolism and genotoxic effects of nitrated polycyclic aromatic hydrocarbons. *Environ Health Perspect*, 102: Suppl 4: 139–146. PMID:7821288

Nakagawa R, Horikawa K, Sera N *et al.* (1987). Dinitrofluoranthene: induction, identification and gene mutation. *Mutat Res*, 191: 85–91. doi:10.1016/0165-7992(87)90134-5 PMID:3110614

Oda Y, Shimada T, Watanabe M *et al.* (1992). A sensitive umu test system for the detection of mutagenic nitroarenes in Salmonella typhimurium NM1011 having a high nitroreductase activity. *Mutat Res*, 272: 91–99. PMID:1383753

Oda Y, Yamazaki H, Watanabe M *et al.* (1993). Highly sensitive umu test system for the detection of mutagenic nitroarenes in Salmonella typhimurium NM3009 having high O-acetyltransferase and nitroreductase activities. Environ. mol. *Mutag.*, 21: 357–364. doi:10.1002/em.2850210407

Ohgaki H, Matsukura N, Morino K *et al.* (1982). Carcinogenicity in rats of the mutagenic compounds 1-nitropyrene and 3-nitrofluoranthene. *Cancer Lett*, 15: 1–7. doi:10.1016/0304-3835(82)90068-4 PMID:6277471

Purohit V & Basu AK (2000). Mutagenicity of nitroaromatic compounds. *Chem Res Toxicol*, 13: 673–692. doi:10.1021/tx000002x PMID:10956054

Ramdahl T, Zielinska B, Arey J, Kondrat RW (1988). The electron impact mass spectra of di- and trinitrofluoranthenes. *Biomed Environ Mass Spectrom*, 17: 55–62. doi:10.1002/bms.1200170111 PMID:3203149

Reifferscheid G & Heil J (1996). Validation of the SOS/umu test using test results of 486 chemicals and comparison with the Ames test and carcinogenicity data. *Mutat Res*, 369: 129–145. doi:10.1016/S0165-1218(96)90021-X PMID:8792833

Rosenkranz HS & Mermelstein R (1983). Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal. *Mutat Res*, 114: 217–267. PMID:6300670

Shane BS & Winston GW (1997). Activation and detoxification of dinitropyrenes by cytosol and microsomes from Aroclor-pretreated rats in the Ames and umu assays. *Environ Mol Mutagen*, 30: 303–311. doi:10.1002/(SICI)1098-2280(1997)30:3<303::AID-EM9>3.0.CO;2-L PMID:9366909

Shimada T & Guengerich FP (1990). Inactivation of 1,3-, 1,6-, and 1,8-dinitropyrene by cytochrome P-450 enzymes in human and rat liver microsomes. *Cancer Res*, 50: 2036–2043. PMID:2180560

Tokiwa H, Horikawa K, Ohnishi Y (1993). Genetic toxicology and carcinogenicity of mono- and dinitrofluoranthenes. *Mutat Res*, 297: 181–195. PMID:7687324

Tokiwa H & Ohnishi Y (1986). Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment. *Crit Rev Toxicol*, 17: 23–60. doi:10.3109/10408448609037070 PMID:2427276

Tokiwa H, Otofuji T, Horikawa K *et al.* (1987). Induction of subcutaneous tumors in rats by 3,7- and 3,9-dinitrofluoranthene. *Carcinogenesis*, 8: 1919–1922. doi:10.1093/carcin/8.12.1919 PMID:2824085

Tokiwa H, Otofuji T, Nakagawa R *et al.* (1986). Dinitro derivatives of pyrene and fluoranthene in diesel emission particulates and their tumorigenicity in mice and rats. *Dev Toxicol Environ Sci*, 13: 253–270. PMID:2435491

Tokiwa H, Sera N, Kai M *et al.* (1990). *The Role of Nitroarenes in the Mutagenicity of Airborne Particles Indoors and Outdoors*. In: *Genetic Toxicology af Complex Mixtures*. Waters MD, Daniel FB, Lewtas J *et al.*, editors. New York, USA: Plenum Press, pp. 165–172.

Wislocki PG, Bagan ES, Lu AYH *et al.* (1986). Tumorigenicity of nitrated derivatives of pyrene, benz[a]anthracene, chrysene and benzo[a]pyrene in the newborn mouse assay. *Carcinogenesis*, 7: 1317–1322. doi:10.1093/carcin/7.8.1317 PMID:3731386

Yamada M, Espinosa-Aguirre JJ, Watanabe M *et al.* (1997). Targeted disruption of the gene encoding the classical nitroreductase enzyme in Salmonella typhimurium Ames test strains TA1535 and TA1538. *Mutat Res*, 375: 9–17. doi:10.1016/S0027-5107(96)00243-6 PMID:9129675