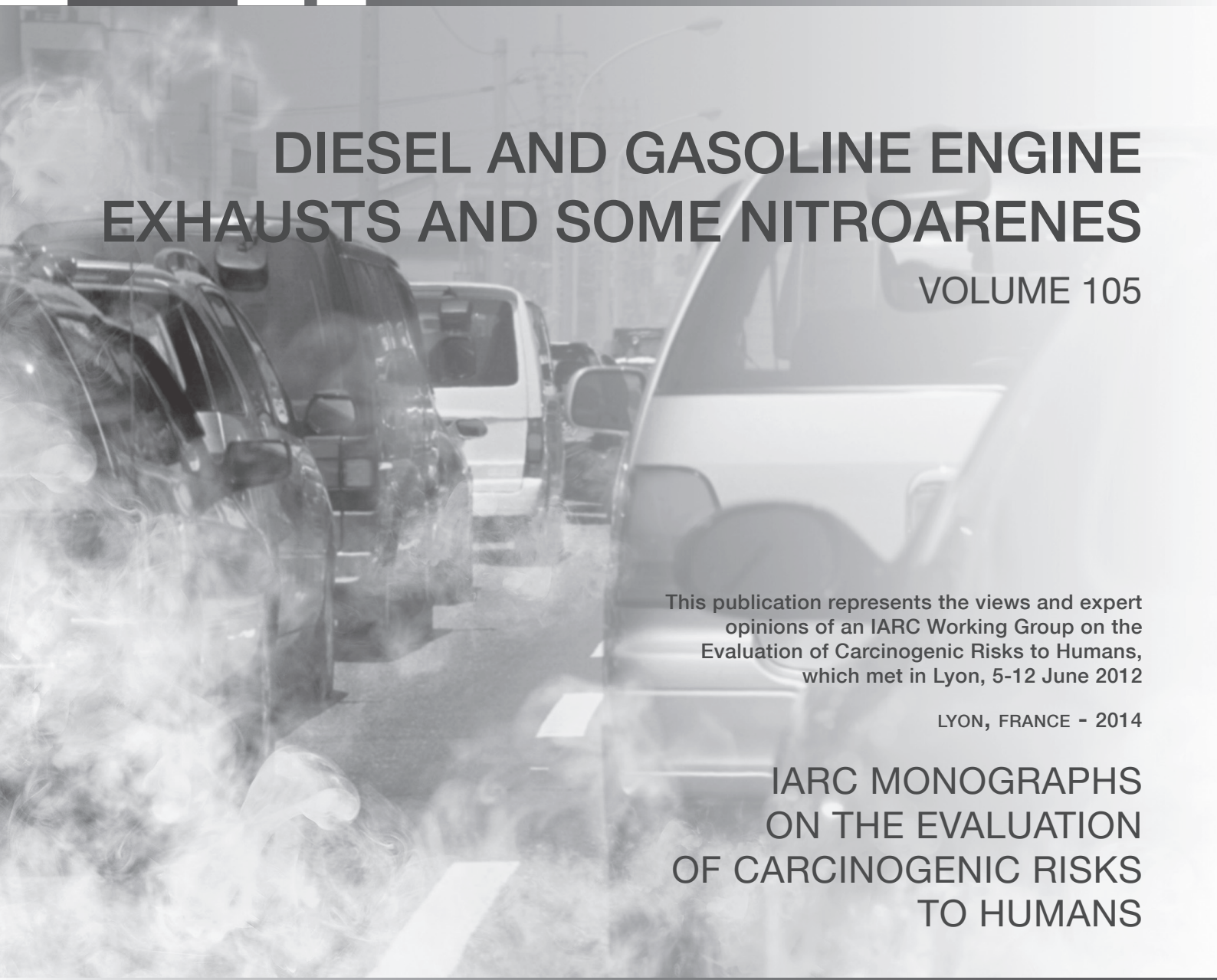
IARC MONOGRAPHS



DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 5-12 June 2012

LYON, FRANCE - 2014

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

3,7-DINITROFLUORANTHENE

3,7-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

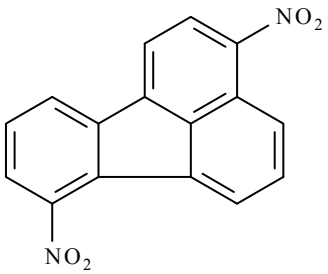
1.1 Chemical and physical data

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 105 735-71-5

Synonym: 3,7-Dinitrofluoroanthene

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)

Melting-point: 203–204 °C (Nakagawa *et al.*, 1987)

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)

Vapour pressure (estimated): 9.1×10^{-10} mm Hg at 25 °C (Lyman, 1985)

Boiling-point: 526.6 °C at 760 mm Hg (Guidechem, 2012)

Flash-point: 268.2 °C (Guidechem, 2012)

Density: 1.574 g/cm^3 (Guidechem, 2012)

Solubility: Practically insoluble in water

1.2 Analysis

For a description of the analytical methods of *N*-polycyclic aromatic hydrocarbons in general, the reader is referred to Section 1.2.2(*d*) of the *Monograph* on Diesel Engine Exhausts.

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

3,7-Dinitrofluoranthene can be synthesized by the nitration of fluoranthene or 3-nitrofluoranthene in the presence of fuming nitric acid, followed by fractionation and purification by recrystallization (Nakagawa *et al.*, 1987; Horikawa *et al.*, 1991; Matsuoka *et al.*, 1993).

No evidence was found that either 3,7- or 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

3,7-Dinitrofluoranthene is not known to occur as a natural product.

1.4.2 Environmental occurrence and exposure

Nitrofluoranthenes can be formed during the combustion of fossil fuels that contain fluoranthene, and from 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).

The use of 3,7-dinitrofluoranthene in laboratory applications may result in its release into the environment through various waste streams.

3,7-Dinitrofluoranthene was detected at a concentration of 0.028 mg/kg in particulates emitted from a diesel engine (Tokiwa *et al.*, 1986). In 1989, 3,7-dinitrofluoranthene was detected in airborne particulates at a level of 0.012 μg/g particulates, corresponding to 0.005 ng/m^3, and in particulate emissions from a kerosene heater at 0.14 μg/g particulates in Sapporo, Hokkaido, Japan (Tokiwa *et al.*, 1990).

Monitoring data indicated that exposure of the general population may occur through the inhalation of ambient air that contains 3,7-dinitrofluoranthene (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

Groups of 21 male Fischer 344/DuCrj rats (age, 6 weeks) received 3,7-dinitrofluoranthene (purity, 99.84%) by subcutaneous injection at a dose of 0 (control) or 0.05 mg dissolved in 0.2 mL of dimethyl sulfoxide twice per week for 10 weeks (total dose, 1 mg/rat). 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 155, and, within 48 weeks after the beginning of treatment, all treated rats had developed tumours at the site of injection; controls did not develop tumours. The incidence of tumours was highly statistically significant [21/21 (100%) versus 0/21; $P < 0.001$ Mann–Whitney U]. Twenty of 21 tumours were described as malignant fibrous histiocytoma and one as a rhabdomyosarcoma. Metastatic foci in the lungs were found in three rats. The mean tumour-induction time was 187 days (Tokiwa *et al.*, 1987).

Table 3.1 Studies of the carcinogenicity of 3,7-dinitrofluoranthene in rats

Strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
F344/DuCrj (M) up to 50 wks Tokiwa *et al.* (1987)	Subcutaneous injection 0, 0.05 mg/rat in DMSO (0.25 mg/mL), twice/wk for 10 wks (total, 1.0 mg or 3.42 μmol) then observed up to 50 wks 21 M/group (age, 6 wk)	Injection site (subcutaneous sarcoma): 0/21, 21/21 (100%) Injection site (malignant histiocytoma): 0/21, 20/21 (95%) Injection site (rhabdomyosarcoma) : 0/21, 1/21 (5%)	[$P < 0.001$, Mann–Whitney U]	Purity, 99.84% by HPLC
F344 (M) up to 100 wks Horikawa *et al.* (1991)	Intrapulmonary implantation 0, 200 μg/rat in 0.05 mL beeswax:tricaprylin, injected directly into the left lower lung after thoracotomy then observed up until 100 wks 20 or 22 M/group (age, 11 wk)	Lung (all tumours): 0/19, 12/22 (54%) Lung (squamous-cell carcinoma): 0/19, 11/22 (50%) Lung (undifferentiated carcinoma): 0/19, 1/22 (5%)	[$P < 0.0002$, Mann–Whitney U]	Purity, > 99.90% by HPLC

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

3.1.2 Intrapulmonary administration

Groups of 20 or 22 male Fischer 344/DuCrj rats (age, 11 weeks) received 3,7-dinitrofluoranthene (purity, > 99.9%) as a single, direct implantation at a dose of 0 (control) 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 was 12 out of 22 (54%; 11 squamous cell carcinomas and one undifferentiated carcinoma) in the treated group and 0 out of 19 in the controls. The incidence of tumours was highly statistically significant [$P < 0.0002$, Mann–Whitney U]. The first tumour was observed at week 51 after the start of the experiment and 12 tumours developed by day 351 after injection (Horikawa *et al.*, 1991).

4. Mechanistic and Other Relevant Data

3,7-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, toxicity or carcinogenicity of this compound in experimental animals or in humans.

4.1 Absorption, distribution, metabolism, excretion

No data were available to the Working Group.

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 the previous *IARC Monograph* (IARC, 1989), 3,7-dinitrofluoranthene was highly active in the induction of reverse mutations in *Salmonella typhimurium* tester strains TA98, TA100, TA1538 and TA1537, but not in TA97, in the absence of a mammalian metabolic-activation system. Of these tester strains, TA98 was the most sensitive to 3,7-dinitrofluoranthene, indicating

that this chemical induces frameshift-type mutations (IARC, 1989; Tokiwa *et al.*, 1993). Several laboratories compared the potencies of various nitroarenes, including 3,7-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 tested, 3,7- and 3,9-dinitrofluoranthenes were highly mutagenic, to an extent that was comparable with that of dinitropyrenes.

The mutagenicity of 3,7-dinitrofluoranthene has also been determined in other strains of *S. typhimurium*, such as TA98/NR and TA98/1,8-DNP6, which are deficient in nitroreductase and *O*-acetyltransferase activities, respectively (IARC, 1989; Yamada *et al.*, 1997). The results showed that 3,7-dinitrofluoranthene was less mutagenic in these strains than in TA98 (Tokiwa *et al.*, 1987, 1993), indicating that it may be activated by nitroreductase to form mutagenic *N*-hydroxyarylamines and then by acetyltransferases to form highly reactive *N*-acetoxy esters, similarly to other potent mutagenic nitroarenes (IARC, 1989; Upadhyaya *et al.*, 1992; Chae *et al.*, 1993; Möller, 1994; Purohit & Basu, 2000).

In the Ames assay, 3,7-dinitrofluoranthene is 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-dinitropyrenes, have also been reported to be inactivated by human and rat cytochrome P450 enzymes to form products that have lost their genotoxic and mutagenic activities in these bacteria (Shimada & Guengerich, 1990; Shane & Winston, 1997).

Oda *et al.* (1992) reported that 3,7-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. Nitroreductase and *O*-acetyltransferase activities were both required for the full stimulation of gene expression in the *umu* tester strains by 3,7-dinitrofluoranthene (Oda *et al.*, 1993). The NM3009 strain, which has both high nitroreductase and *O*-acetylation activity, was most sensitive to the *umu* gene expression induced by 3,7-dinitrofluoranthene and 1,6-dinitropyrene, followed by strains NM2009 and NM1011, which have *O*-acetyltransferase and nitroreductase activity, respectively. As expected, NM1000 and NM2000, which are deficient in nitroreductase and *O*-acetyltransferase activity, respectively, were less sensitive to 3,7-dinitrofluoranthene and 1,6-dinitropyrene (Oda *et al.*, 1993).

3,7-Dinitrofluoranthene also induced chromosomal aberrations in a Chinese hamster cell line in the absence of a rat-liver metabolic system *in vitro* (Matsuoka *et al.*, 1993), and micronucleus formation in mouse bone marrow *in vivo* (Tokiwa *et al.*, 1993).

4.3 Mechanistic considerations

3,7-Dinitrofluoranthene was carcinogenic in experimental animals (IARC, 1989; Horikawa *et al.*, 1991). Assays for genotoxicity and mutagenicity in bacteria indicated that 3,7-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). Although the details of the metabolism of 3,7-dinitrofluoranthene are unknown, this nitroarene may be considered to undergo bioactivation via a similar mechanism as that described for 3,9-dinitrofluoranthene.

5. Summary of Data Reported

5.1 Exposure data

3,7-Dinitrofluoranthene is present in diesel exhaust emissions and emissions from heaters fuelled with liquefied petroleum gas. No evidence was found that it has been produced in commercial quantities or 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 can occur through inhalation of airborne particulate matter in an urban environment, and may also occur from the 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

3,7-Dinitrofluoranthene was tested for carcinogenicity in rats by subcutaneous injection in one study and by intrapulmonary implantation in another study. Subcutaneous injection of 3,7-dinitrofluoranthene caused a significant increase in the incidence of malignant fibrous histiocytoma at the injection site. Implantation of 3,7-dinitrofluoranthene into the lung caused a significant increase in the incidence of carcinoma of the lung.

5.4 Mechanistic and other relevant data

No data were available to the Working Group on the absorption, distribution, metabolism or excretion of 3,7-dinitrofluoranthene in humans, experimental animals or *in vitro*, or on the genetic and related effects of 3,7-dinitrofluoranthene in humans. The compound was strongly mutagenic in bacteria, mainly as a frameshift mutagen. The mutagenicity was weaker in bacterial strains that are deficient in nitroreductase or in *O*-acetyltransferase, indicating that 3,7-dinitrofluoranthene is activated through nitroreduction and acetyltransfer. 3,7-Dinitrofluoranthene was genotoxic in the *umu* gene-expression assay, and induced chromosomal aberrations in Chinese hamster cells and micronucleus formation in the bone marrow of mice *in vivo*. It induced tumours in rats at the sites of subcutaneous injection and intrapulmonary implantation. The evidence suggests that this chemical produces mutagenic metabolites that could play a role in its carcinogenicity.

Overall, these data provide *weak mechanistic evidence* to support the carcinogenicity of 3,7-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 3,7-dinitrofluoranthene.

6.3 Overall evaluation

3,7-Dinitrofluoranthene is *possibly carcinogenic to humans (Group 2B)*.

References

Chae YH, Yun CH, Guengerich FP *et al.* (1993). Roles of human hepatic and pulmonary cytochrome P450 enzymes in the metabolism of the environmental carcinogen 6-nitrochrysene. *Cancer Res*, 53: 2028–2034. PMID:8481905

Guidechem (2012). Available at: http://www.guidechem.com/products/105735-71-5.html. Accessed 29 May 2013

Horikawa K, Otofuji T, Nakagawa R *et al.* (1987). Mutagenicity of dinitrofluoranthenes (DNFs) and their carcinogenicity in F344 rats (Abstract no. 11). *Mutat Res*, 182: 360–361. doi:10.1016/0165-1161(87)90087-2

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). *3,7-Dinitrofluoranthene*. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+7089. 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. 1. Neely WB, Blau GE, editors. Boca Raton, FL: CRC Press.

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

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 Mutagen*, 21: 357–364. doi:10.1002/em.2850210407 PMID:8491215

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

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, Otofuji T, Horikawa K *et al.* (1987). Induction of subcutaneous tumors in rats by 3,7- and 3,9-dinitrofluoranthene. *Carcinogenesis*, 8: 1919–1922. 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 of Complex Mixtures*. Waters MD, Daniel FB, Lewtas J *et al.*, editors. New York, USA: Plenum Press, pp. 165–172.

Upadhyaya P, Roy AK, Fu PP, el-Bayoumy K (1992). Metabolism and DNA binding of 2-nitropyrene in the rat. *Cancer Res*, 52: 1176–1181. PMID:1737377

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