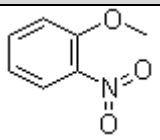
2-NITROANISOLE

$C_7H_7NO_3$

Chemical structure



Chemical formula (ChemIDplus, 2011).

2-nitroanisole (N° CAS: 91-23-6) also known as o-Nitroanisole's is a nitrogen Compound.

Synonyms: 1-Methoxy-2-nitrobenzene; 1-Nitro-2-methoxybenzene; 2-Methoxy-1-nitrobenzene; 2-Methoxynitrobenzene; 2-Nitroanisole; Anisole, o-nitro-; Benzene, 1-methoxy-2-nitro-; o-Nitrophenyl methyl ether; ortho-Nitrobenzene methyl ether; Nitros, Aromatic o-Nitroanisole's **(**ChemIDplus, 2011).

Contamination source

Aromatic nitro-compounds and amines are widely distributed environmental pollutants found in workplaces (e.g. in chemical industry), in emissions from diesel and gasoline engines and on the surface of ambient air particulate matter (NTP, 1993), where they add to local and regional pollution (car exhausts, technical spills) (Stiborovã et al., 2009).
2-Nitroanisole is used primarily as precursor in the synthesis of o-anisidine (2-methoxyaniline), an intermediate in the manufacture of many azo dyes (NTP, 1993). 2-Nitroanisole has also been used as an intermediate for various pharmaceuticals (IARC, 1996).
Hydrogenation of *o*-nitroanisole to *o*-anisidine is one of the most common reactions in the fine chemicals and pharmaceutical industries (Naiman et al., 2011).
In western Europe, in 1983, production of 2-nitroanisole was approximately 7,200 tons per year, including approximately 4,000 tons per year in Germany (IARC, 1996). 2-Nitroanisole is known to be produced by five companies in Japan, four in China and three in India and by one company each in Brazil, Germany and the Ukraine (Chemical Information Services, 1994).

The routes of potential human exposure to o-nitroanisole are dermal contact, ingestion, and inhalation (NTP, 2011). o-Nitroanisole's production and use in organic synthesis, and in the manufacture of dyes and pharmaceuticals may result in its release to the environment through various waste streams (HSDB, 2011). o-Nitroanisole has been detected in drinking water.
If released to soil, o-nitroanisole will have moderate mobility. Volatilization of o-nitroanisole will not be important from moist soil surfaces based on the estimated Henry's Law constant of $4.3X10^{-7}$. Volatilization of o-nitroanisole is not important from dry soil surfaces either based on an estimated vapor pressure of $3.6X10^{-3}$ mm Hg. Biodegradation of o-nitroanisole will not be an important fate process in soil based on two biodegradation studies.
If released to water, o-nitroanisole may adsorb to suspended solids and sediment. o-Nitroanisole may volatilize very slowly from water surfaces with estimated half-lives for a model river and model lake of about 105 days and 772 days, respectively. Bioconcentration and biodegradation of o-nitroanisole will not be important fate processes in water (NTP, 2011).
If released to the atmosphere, o-nitroanisole will exist in the vapor phase in the ambient atmosphere. Vapor-phase o-nitroanisole is degraded in the atmosphere by reaction with photochemically produced hydroxyl radicals with an estimated half-life of about 109 hours (NTP, 2011). It is not expected to bioaccumulate in aquatic organisms.
Occupational exposure is associated with the widespread use of *o*-nitroanisole in the manufacture of azo dyes (NTP 1993); however, no estimates of occupational exposure to *o*-nitroanisole were found (NTP, 2011).

On 22 February 1993, approximately 10 tones of vapour containing 2-nitroanisole and other halogenated aromatics were accidentally released from a chemical plant (Hoechst) in Grieshem, Germany (IARC, 1996) leading to a large-scale leakage of 2-nitroanisole and subsequent local and regional contamination (Svobodová et al., 2008).
2-Nitroanisole has been detected in water samples in Japan (0.7µg/l) and in the Netherlands (0.3-

1.0 µg/l). Also, nitroanisole of unspecified isomerism has been detected in the Netherlands (0.3-1.0 µg/l) and Germany (0.1-0.9 µg/l) ((IARC, 1996).
2-Nitroanisole has been detected 10 sediment samples taken in Japan (0.01 µg/l) (IARC, 1996).

Analytical method

2-Nitroanisole in environmental samples can be analyzed by gas chromatography with electron capture detection.

Toxicity

Carcinogenicity

Humans

o-Nitroanisole is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals (NTP, 2011; NTP, 2005).
According to Naiman et al. (2011), in contrast to the urinary bladder, the liver is most probably not the major target organ for the carcinogenicity of o-anisidine and o-nitroanisole in humans.

Cancer Studies in Experimental Animals

Oral exposure to *o*-nitroanisole caused tumors in two rodent species and at several different tissue sites. In rats of both sexes, dietary administration of *o*-nitroanisole caused mononuclear-cell leukemia and increased the combined incidences of benign and malignant tumors of the urinary bladder, kidney, and large intestine (NTP, 1993; IARC, 1996). In mice, it caused benign and malignant liver tumors (hepatocellular adenoma and carcinoma and hepatoblastoma) in males and benign liver tumors (hepatocellullar adenoma) in females (NTP, 1993).

Studies on Mechanisms of Carcinogenesis

An aromatic amine, *o*-anisidine (2-methoxyaniline) and its oxidative counterpart, 2-nitroanisole (2-methoxynitrobenzene), are the industrial and environmental pollutants causing tumors of the urinary bladder in rats and mice (Stiborovã et al., 2009). Both carcinogens are activated to the same proximate carcinogenic metabolite, *N*-(2-methoxyphenyl)hydroxylamine, which spontaneously decomposes to nitrenium and/or carbenium ions responsible for formation of deoxyguanosine adducts in DNA *in vitro* and *in vivo*. In other words, generation of *N*-(2-methoxyphenyl)hydroxylamine is responsible for the genotoxic mechanisms of the o-anisidine and 2-nitroanisole carcinogenicity. Analogous enzymes of human and rat livers are capable of activating these carcinogens. Namely, human and rat cytochorme P450 2E1 is the major enzyme oxidizing *o*-anisidine to *N*-(2-methoxyphenyl)hydroxylamine, while xanthine oxidase of both species reduces 2-nitroanisole to this metabolite. Likewise, *O*-demethylation of 2-nitroanisole, which is the detoxication pathway of its metabolism, is also catalyzed by the same human and rat enzyme, cytochorme P450 2E1. The results suggest that both compounds are potential carcinogens also for humans (Stiborovã et al., 2009).

2-Nitroanisole (2-NA) is a potent carcinogen for rodents. 2-NA is oxidized by rat hepatic microsomes to 2-nitrophenol (2-NP) as the major metabolite, and to 2,6-dihydroxynitrobenzene (2,6-DNB) and 2,5-dihydroxynitrobenzene (2,5-DNB) as the minor products. All these metabolites are suggested as detoxication products. Using hepatic microsomes of rats pre-treated with specific CYP inducers and microsomes from Baculovirus transfected insect cells expressing recombinant rat and human CYP enzymes Svobodová et al. (2008) found that rat recombinant CYP2E1, 2D2, 2B2, 2C6 and 1A1, as well as orthologous human CYP enzymes are the most efficient enzymes metabolizing 2-NA. However, human CYP1A1 oxidize 2-NA with a higher efficiency than the enzyme of rats. The results show the participation of orthologous CYPs in 2-NA oxidation by both species and underline the suitability of rat species as a model to evaluate human susceptibility to 2-NA (Svobodová et al., 2008).

Stiborová et al. (2004) have shown that hepatic cytosol activates the carcinogen 2-NA. The results of the study demonstrate that cytosolic enzyme from human liver catalyze nitro-reduction, which is considered to be an activation pathway for aromatic nitro compounds in rodents, and are capable of activating this carcinogen to species binding to DNA.

Orally administered *o*-nitroanisole is metabolized predominantly to *o*-nitro phenol, which is conjugated to sulfate or glucuronide and eliminated in the urine. Less than 1% of *o*-nitroanisole is metabolized to *o*-anisidine, which is listed in the Report on Carcinogens as *reasonably anticipated to*

be a human carcinogen. Dietary administration of *o*-anisidine hydrochloride caused tumors of the urinary bladder (transitional-cell neoplasia) in mice and rats and the kidney (transitional- cell carcinoma of the renal pelvis) in rats. *o*-Nitroanisole causes genetic damage in a wide variety of bacterial and *in vitro* mammalian test systems (NTP 1993, IARC 1996). Since *o*-nitroanisole was listed in the *Eighth Report on Carcinogens,* additional studies relevant to mechanisms of carcinogenesis have been identified. *In vitro, o*-nitroanisole is metabolized by O-demethy lation to 2-nitrophenol, which is oxidized to 2,5-dihydroxynitrobenzene and 2,6-dihydroxynitrobenzene (Miksanova *et al.* 2004a,b, Stiborova *et al.* 2004, Dracinska *et al.* 2006). *o*-Nitroanisole is also metabolized by nitroreduction to the DNA-reactive products 2-anisidine and *N*-(2-methoxyphenyl)hydroxylamine. DNA adducts similar to those found *in vitro* were found in the urinary bladder, liver, kidney, and spleen of male rats following intraperitoneal injection with *o*-nitroanisole. There is no evidence to suggest that mechanisms by which *o*-nitroanisole causes tumors in experimental animals would not also operate in humans (NTP, 2011).

Oral administration of 2-nitroanisole for up to two years increased the incidence of tumours in the urinary bladder (rats), large intestines (rats), the kidneys (rats), and the liver (mice) compared to no treated animals. These findings give sufficient evidence that oral exposure to 2-nitroanisole results in tumour development (Gezondheid raad, 2008).

There is inadequate evidence in humans for the carcinogenicity of 2-nitroanisole. **There is sufficient evidence in experimental animals for the carcinogenicity of 2-nitroanisole**. **Overall evaluation 2-Nitroanisole is possibly carcinogenic to humans (Group 2B) (IARC, 1996).**

Genetic and related effects

It has not been determined exactly whether 2-nitroanisole is a genotoxic or epigenetic carcinogen. 2-Nitoanisole was positive in the *rec* assay in *Bacillus subtilis* strains H17 and M45 (IARC, 1996).
2-Nitoanisole was tested in several laboratories for the induction of gene mutations in Salmonella typhimurium. Positive responses were obtained consistently with the train TA 100. Variable response were obtained with some other strains.
According to Chiu et al. (1978), 2-Nitroanisole was mutagenic for both strains of salmonella typhimurium TA98 & TA100.
In single studies with cultured mammalian cells, 2-nitroanisole induced sister chromatid exchange and chromosomal aberrations in Chines hamster ovary (CHO) cells and mutation at the *tk* locus of mouse mymphoma L5178Y cells. The clastogenic activity was weak and observed only in the presence of S9, whereas sister chromatid exchange and *tk* mutations were induced in the absence of S9 (IARC, 1996).

2-Nitroanisole is mutagenic in bacteria. In single studies, it induced mutations, sister chromatid exchange and a low frequency of chromosomal aberrations in cultured mammalian cells (IARC, 1996).

The number of studies on the mutagenic and genotoxic potential of the agent is limited. Overall, 2-nitroanisole induced mutations in bacteria and in mammalian cells, and showed to be clastogenic *in vitro*. It was also able to transform normal peritoneal macrophages into immortal cells. These immortal cells had altered expression of proto-oncogenes and, furthermore, formed tumours at the injection site. Based on these mutagenicity and genotoxicity data, the committee of Health Council of the Nertherlands considers 2-nitroanisole as a genotoxic carcinogen that acts by a stochastic mechanism and is of the opinion that 2-nitroanisole should be considered as carcinogenic to humans (Gezondheid raad, 2008).

Toxic effects

The oral LD_{50} of 2-nitroanisole is 740 mg/kg body weight in rats and 1300 mg/kg body weight in mice (United States National Institute for Occupational Safety and Health, 1994).

Metabolism and other effects

No human data were available on the metabolism of 2-nitroanisole. In rats, 2-nitroanisole is absorbed after oral administration, and the major route of its rapid elimination is the urine. The predominant metabolic pathway involves the formation of 2-nitrophenol, with its subsequent conjugation with sulfate and glucuronic acid (IARC, 1996).
2-Nitroanisole causes methaemoglobinaemia following dietary administration of high doses to rats

and mice. Pathological lesions observed in rats occurred in the urinary bladder, spleen, kidney and liver. In mice, 2-nitroanisole causes hypertrophy in the liver (IARC, 1996).
2-Nitroanisole is also a toxic compound, causing anemia. The anemia is characterized by increased levels of methemoglobin and accelerated destruction of erythtocytes (NTP, 1993).

Establishment of Health Based Reference Values

TD_{50}, is the daily dose rate in mg/kg body weight/day to induce tumors in half of test animals that would have remained tumor-free at zero dose. Whenever there is more than one positive experiment in a species, the reported TD_{50} value is a Harmonic Mean calculated using the TD_{50} value from the most potent target site in each positive experiment.
Superscripts: m = There is more than one positive experiment in the species, and TD_{50} values from each positive experiment are used in the calculation of the reported Harmonic mean of TD_{50}. v = Variation is greater than ten-fold among statistically significant (two-tailed $p<0.1$) TD50 values from different positive experiments.
TD50 (mg/kg/day) Rat $15.6^{m,v}$, mouse: 178^{m} (http://potency.berkeley.edu/chempages/o-NITROANISOLE.html).

Occurrence in food

o-Nitroanisole may be released into the environment by dye and pharmaceutical manufacturing facilities through various waste streams (HSDB, 2011). When released to air, *o*-nitroanisole will remain in the vapor phase and will be degraded by reactions with photochemically produced hydroxyl radicals, with an estimated half-life of 109 hours. When released to water, it may adsorb to sediments and suspended solids. Volatilization is very slow, with a half-life of 105 days in a model river and 772 days in a model pond. When released to soil, *o*-nitroanisole has moderate mobility. It is not expected to bioaccumulate in aquatic organisms. *o*-Nitroanisole has been identified in drinking water, but no concentrations have been reported (NTP, 2011).

Dietary exposure assessment

It is not possible to make a dietary exposure assessment due to lack of data in food and drinking water.

Risk characterization

It is not possible to characterize the risk linked to a dietary exposure.

Legislation

The former USSR has set a short-term exposure limit for 2-nitroanisole of 1 mg/m^3, with skin absorption noted as a potentially significant route of exposure (effective date, 1989) (IARC, 1996).

Recommendations

Data on the occurrence of 2-nitroanisole in food and drinking water are needed for estimation of the dietary exposure and characterization of the risk.
It is recommended to analyze 2-nitroanisole in drinking water.

References

ChemIDplus. 2011. ChemIDplus Advanced. National Library of Medicine. http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp and search on CAS number.
Chiu CW et al. 1978. Mutat Res, 58, 11 http://scorecard.goodguide.com/health-effects/references.tcl?short_hazard_name=cancer
Chemical information services. 1994. Directory of World chemical producers 1995/96 Editions, Dallas, TX, p.524.
Dracinska H, Miksanova M, Svobodova M, Smrcek S, Frei E, Schmeiser HH, Stiborova M. 2006. Oxidative detoxication of carcinogenic 2-nitroanisole by human, rat and rabbit cytochrome P450. Neuro Endocrinol Lett 27(Suppl 2): 9-13.
Gezondheidsraad, 2008. Evaluation of the carcinogenicity and genotoxicity of 2-Nitroanisole. Health Council of the Nertherlands. No. 2008/02OSH, The Hague, April 1, 2008.
HSDB (Hazardous Substances Data Bank). 2011. 2-Nitroanisole. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~UrCoO7:1. Last Revision Date: 20110616.

IARC. 1996. 2-Nitroanisole. In Printing Processes and Printing Inks, Carbon Black and Some Nitro Compounds. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol 65. Lyon, France: International Agency for Research on Cancer. pp. 369-380. Available at: http://monographs.iarc.fr/index.php

Miksanova M, Novak P, Frei E, Stiborova M. 2004a. Metabolism of carcinogenic 2‑nitroanisole in rat, rabbit, porcine and human hepatic cytosol. Collec Czech Chem Commun 69(3): 589-602.

Miksanova M, Sulc M, Rydlova H, Schmeiser HH, Frei E, Stiborova M. 2004b. Enzymes involved in the metabolism of the carcinogen 2-nitroanisole: evidence for its oxidative detoxication by human cytochromes P450. Chem Res Toxicol 17(5): 663-671.

Mitchell J. Jr & Deveraux HD. 1978. Determination of traces of organic compounds in the atmosphere: role of detectors in gas chromatography. Anal. Chim. Acta, 100,45-52.

Naiman K., Martínková M., Schmeiser H.H., Frei E., Stiborová M. 2011. Human cytochrome –P450 enzymes metabolize N-(2-methoxyphenyl)hydroxylamine, a metabolite of the carcinogens *o*-anisidine and *o*-nitroanisole, thereby dictating its genotoxicity. Mutation research 776, 160-168.

NTP (National Toxicology Program). 1993. Toxicology and Carcinogenesis Studies of o-Nitroanisole (CAS No. 91-23-6) in F344 Rats and B6C3F1 Mice (Feed Studies). NTP Technical Report Series no. 416, NIH Publication no. 93-3147. Research Triangle Park, NC: National Toxicology Program. 482 pp.

NTP (National Toxicology Program). 2005; Eleventh Report on Carcinogens: o-Nitroanisole (91-23-6) (January 2005). Available at: http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s120onit.pdf

NTP(National Toxicology Program), 2011. Report on Carcinogens. Twelfth Edition U.S. Department of Health and Human Services Public Health Service.

SRI. 2009. Directory of Chemical Producers. Menlo Park, CA: SRI Consulting. Database edition.

Stiborova M, Miksanova M, Smrcek S, Bieler CA, Breuer A, Klokow KA, Schmeiser HH, Frei E. 2004. Identification of a genotoxic mechanism for 2-nitroanisole carcinogenicity and of its carcinogenic potential for humans. Carcinogenesis 25(5): 833-840.

Stiborová M., Naiman K., Martinková M., Martínek V., Svobodová M., Schmeiser H. H., Frei E. 2009. Genotoxic mechanisms for the carcinogenicity of the environmental pollutants and carcinogens o-anisidine and 2-nitroanisole follow from adducts generated by their metabolite N-(2-methoxyphenyl)-hydroxylamine with deoxyguanosine in DNA. Interdisc Toxicol. 2009; Vol. 2(1): 24–27.

Svobodová M., Draćinská H., Martínkova M., Hudećek J., Hodek P., Frei E., Stiborová M. 2008. Oxidation of carcinogenic 2-nitroanisole by rat cytochromes P450 – similarity between human and rat enzymes. Interdisc Toxicol.,1(2):182-185.