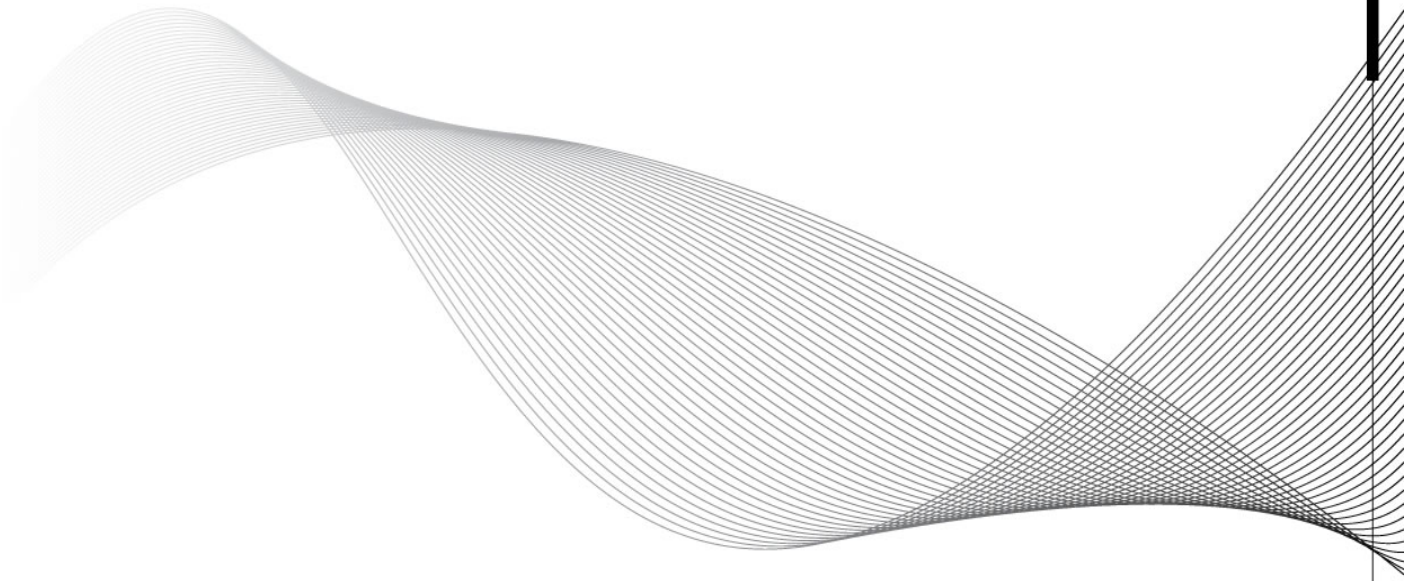
Social Europe



Recommendation from the Scientific Committee on Occupational Exposure Limits for for o-anisidine

SCOEL/SUM/144
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8 hour TWA:	not assigned
STEL (15 min):	not assigned
Additional classification:	"skin" notation
[by analogy to structurally related aromatic amines]	
SCOEL carcinogen group:	B (genotoxic carcinogen, for which a threshold is not sufficiently supported)
BLV:	not assigned

Substance identification

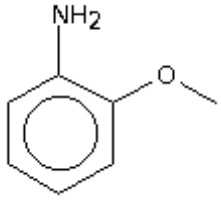
o-Anisidine

Synonyms: 1-amino-2-methoxy-benzene, 2-methoxyaniline, 2-aminoanisole, 2-aminomethoxybenzene, 2-methoxy-1-aminobenzene, 2-methoxybenzenamine, o-aminoanisole, o-aminomethoxybenzene, o-methoxyaniline, o-methoxyphanylamine

EINECS No. 201-963-1
EU-Classification

Carc. 1B	H350	May cause cancer
Muta. 2	H341	Suspected of causing genetic defects
Acute Tox. 3 *	H331	Toxic if inhaled
Acute Tox. 3 *	H311	Toxic in contact with skin
Acute Tox. 3 *	H301	Toxic if swallowed

CAS No. 90-04-0
Molecular formula C_7H_9NO



Structural formula
MWt 123.16 g/mol
Conversion factor: At 25°C 1 ppm = 5.037 mgm^{-3}; 1 mgm^{-3} = 0.199 ppm
This document is based on the EU-RAR (2002) and the references based therein.

Physico-chemical properties

At 20 °C and 1013 hPa, o-anisidine is a light red to yellow liquid with a faintly aromatic odour. It is soluble in water (15gL^{-1} at 20°C). o-Anisidine acid is flammable and combustible. The melting temperature is 5->7 °C and the boiling temperature is 224-225°C. The vapour pressure is 0.02/0.05 hPa at 20 °C.

1. Occurrence/Use

o-Anisidine is produced from *o*-nitroanisol (2-methoxy-nitrobenzene) by catalytic reduction with hydrogen under pressure in an inert liquid medium. It is estimated that less than 1,000 tonnes are produced annually within the EU and it is thought that most of the o-anisidine produced is used in the production of dyes. *o*-Anisidine is an intermediate for a number of direct yellow, red and blue azo dyes and pigments and some acid dyes. The main primary product derived from *o*-anisidine in the EU is acetoacet-*o*-anidide used in the production of yellow azo pigments. Much smaller quantities of *o*-anisidine are used in the production of naphthol (red azo) pigments and dyes or azo dyes. About 90% of the dyes produced from *o*-anisidine are used in textiles whereas the pigments are used mainly for printing paper and cardboard. In the past o-anisidine was also used as a bactericide in metal working fluids. The quantity of o-anisidine used in the EU has declined during the last 10-15 years. It is estimated that less than 850 tonnes were used in the EU in 1997. There is, however, some current interest in the development of *o*-Anisidine polymers for high tech applications.

The production of *o*-anisidine and its subsequent processing to form dyes and pigments occurs within closed systems. Small amounts of *o*-anisidine have been detected as a residue in pigments. Occupational exposure to *o*-anisidine is possible during primary production or secondary processing to form dyes and pigments during cleaning, inspection and sampling of what are essentially closed production systems. Low-level occupational exposure is also possible during the handling of dyes, pigments and inks that may contain small quantities of residual *o*-anisidine. *o*-Anisidine is also found in cigarette smoke.

1.1. Methods of exposure monitoring and analysis

1.1.1. Concentrations of o-anisidine in air

The US National Institute for Occupational Health and Safety (NIOSH) published an appropriate method (NIOSH Method 2514, Issue 2). The method involves collection onto a sorbent tube (XAD-2) at a flow rate of 0.5 to 1 $Lmin^{-1}$ and analysis by HPLC using UV detection. The method has been validated for concentrations from 0.06 to 0.8 mgm^{-3} in 200 litre air samples.

1.1.2. Biological monitoring

There are single reports on possibilities of biological monitoring.
Weiss et al. (2002) described a method for determination of the urinary excretion of some aromatic amines, including o-anisidine, based on gas chromatography-mass spectrometry. With a limit of detection of 0.05 microg/liter, a median and 95-percentile of excretion ion the normal population in Germany was reported of 0.22 and 0.68 microg/liter, respectively.

Richter et al. (2001) described the determination of haemoglobin adduct levels of o-anisidine and other aromatic amines in children of different areas in Southern Germany. After base-catalysed hydrolysis of the adducts and derivatisation of the released amines with pentafluoropropionic acid the separation was performed by gas chromatography-mass spectrometry (Kutzer et al. 1997). Means were 284, 242 and 254, with S.D. of 73, 129 and 179 (pg amine released per g haemoglobin) for unit, Augsburg and Eichstätt, respectively. The origin of the adduct is not known (Richter et al. 2001).

2. Health Effects

2.1. Toxicokinetics

There is no specific information about the absorption of o-anisidine following exposure by inhalation, skin contact or ingestion, although the evidence of toxicity by all these routes indicates that some level of absorption occurs. Following intraperitoneal administration of radio-labelled o-anisidine in rats, the highest concentrations of the radio-label were found in the liver, kidney and muscle tissue (Sapota *et al*, 2003).

The metabolism and mode of action of o-anisidine is not well established. Other aromatic amines are oxidised to a N-hyroxy derivative that interacts with the haem group of haemoglobin to form
methaemoglobin (McLean et al. 1969, Uehleke 1971, EU RAR 2002). Significantly elevated methaemoglobin levels were found in mice and rats at 3 to 48 hours following exposure by gavage to doses of 690 or 1380 mg/kg (Ashby *et al*, 1991). In cats a single intravenous injection of 7.7 $mgkg^{-1}$ resulted in significantly elevated methaemoglobin levels in samples taken 1 to 5 hour after administration (McLean *et al*, 1969). Cats are regarded as having a similar capacity to form methaemoglobin as humans (EU RAR, 2002).

Following intraperitoneal administration of radio-labelled o-anisidine, about 70% of the administered dose was eliminated in urine after 72 hours (Sapota *et al*, 2003). The main urinary metabolites identified were N-acetyl-2-methoxyaniline and N-ethyl-4-hydroxy-2-methoxyaniline.

2.2. Acute toxicity

There are no reports of acute o-anisidine poisoning in humans (EU RAR, 2002).

In an unpublished OECD compliant inhalation study, the LC_{50} for rats was found to exceed 3,800 mgm^{-3} (as an aerosol). No mortality was observed. Animals showed impaired movement, respiration and reflexes as well as bloody nasal discharge and cyanosis. All signs of toxicity had resolved within 8 days of exposure (Hoechst AG, 1989a). The potential formation of methaemoglobin which would affect the oxygen-carrying capacity of the blood and its role in toxic effects following inhalation has not been investigated.

No mortalities were observed in rats exposed to 2,000 $mgkg^{-1}$ by dermal application. Animals showed ataxia, lacrimation, eyelid constriction and orange urine. All signs of toxicity resolved within two days (Hoechst AG, 1989c).

The oral LD_{50} for rats reported in an OECD compliant study was 1,890 mg/kg. Effects included squatting, staggering gait, reduced spontaneous activity, dizziness and respiratory depression. At doses close to the LD_{50}, animals showed negative righting reflex, orange urine and pale skin. Most of the surviving animals showed no signs of toxicity after 4 days (Hoechst AG, 1989b).

In other oral studies that were not OECD compliant LD_{50} values of 2,020, 1,410 and 870 mg/kg have been reported for rats, mice and rabbits respectively. Reported adverse effects include haematological changes, anaemia and nephrotoxicity (EU RAR, 2002).

2.3. Irritation and Corrositivity

There are few human data for irritation from o-anisidine. The US Department of Health and Human Services Hazardous Substances Data Bank (http://toxnet.nlm.nih.gov/) indicates that o-anisidine is a skin irritant on the basis of information from the Merck Index.

Negative results were obtained in rabbit skin and eye irritation studies that were compliant with OECD guidelines (EU RAR, 2002). Negative results were also obtained for corrosion in rabbit skin and eye tests (EU RAR, 2002).

The EU RAR did not consider o-anisidine to be irritant or corrosive.

2.4. Sensitization

There are no human data related to the potential of o-anisidine to cause sensitization. An increased frequency of atopic dermatitis was seen in children exposed to a mixture of over twenty substances including o-anisidine following a chemical accident, but these effects could not be directly attributed to o-anisidine (EU RAR, 2002).

The animal data on sensitization are limited. In a poorly documented study, Ilichkina (1985) reported that o-anisidine was a weak sensitizer in the guinea pig following intra- and epi-cutaneous application of 0.5 or 3.5 $mgkg^{-1}$. A possible metabolite of o-anisidine, o-aminophenol, has been shown to give a positive result in two separate test systems for skin sensitisation (EU RAR, 2002). Some compounds that are structurally similar to o-anisidine (o-aminophenol, p-toluidine and aniline) have also shown sensitizing properties in animal tests whereas the structural analogue o-phenetidine yielded a negative result in a well-validated test system (EU RAR, 2002).

Overall, it is uncertain whether o-anisidine can cause skin sensitization.

2.5. Repeated dose toxicity

2.5.1. Human data

No human data are available concerning the toxicity of o-anisidine following repeated exposure. Workers exposed to *o*-anisidine by inhalation to a concentration of 2 mgm^{-3} for 3.5 hours/day for 6 months developed headaches, vertigo, and effects on the blood (increased methaemoglobin; Pascari *et al*, 1958 – cited by the American Conference of Governmental Hygienists). Workplace personal exposure concentrations of airborne o-ansidine in Europe are thought to be less than 0.1 mgm^{-3}, and so the absence of reported effects might reflect the relatively low levels of exposure and also the relatively small number of exposed workers.

2.5.2. Animal data

No data are available for toxicity from exposure via inhalation or the skin.

In an OECD compliant 28 day study, rats were exposed to 0, 16, 80 or 400 mg/kg/day o-anisidine by gavage. No effects were observed at 16 mg/kg/day, but at doses of 80 mg/kg/day or greater, yellow urine and haemolytic anaemia were observed. Morphological changes in the spleen (i.e. haemeosiderosis, hyperaemia and increased haematopoiesis) were also found and the 400 mg/kg group showed salivation, squatting and inflated abdomens. Males also showed a reduction in body weight and an increase in relative liver and kidney weight whereas females showed increased glutamic pyruvic transaminase levels. Both sexes showed increased drinking water consumption, increased bilirubin and urea-nitrogen levels in blood and increased relative spleen weights. The NOEL was derived as 16 mg/kg/day and the LOEL as 80 mg/kg/day (EU RAR, 2002; reference to original study not provided).

In a seven week feeding study undertaken by the National Cancer Institute (NCI, 1978), o-anisidine hydrochloride (anticipated to be toxicologically similar to o-anisidine) was

administered to rats and mice at doses equivalent to 0, 75, 225, 750 or 2,250 mg/kg/day in rats (as o-anisidine) and 0, 150, 450, 1,500 or 4,500 mg/kg/day in mice (as o-anisidine). Doses of 750 mg/kg/day or greater in rats resulted in dose-dependent reductions in weight and moderate enlargement of the spleens which were black and granular. At 75 mg/kg and 225 mg/kg, spleens of male rats became granular. In mice, doses 450 mg/kg or greater caused dose-dependent reduction in weight and at doses of 1,500 mg/kg/day or greater, the spleens were black and enlarged.

In a two year feeding study (NCI, 1978), *o*-anisidine hydrochloride was administered to rats at doses equivalent to 256 or 512 mg *o*-anisidine/kg/day in males and 385 or 770 mg *o*-anisidine /kg/day in females. Mice received doses equivalent 164 or 328 mg/kg/day for males and 192 or 384 mg/kg/day for females. In both species there was a dose-related reduction in body weight. No haematological or biochemical investigations were undertaken.

2.6. Mutagenicity

Genotoxicity data for o-anisidine were reviewed by Ashby et al. (1991). Regarding the *S. typhimurium* assay and mutagenicity in *E. coli WP2 uvrA*, both positive and negative results were reported in the literature. The EU RAR (2002) concluded that there is sufficient evidence to classify *o*-anisidine as a Category 3 mutagen. This was based on there being sufficient evidence of mutagenicity in short term *in vitro* tests. *In vivo* tests gave mostly negative results (Ashby 1991, EU RAR 2002).

2.7. Carcinogenicity

There are no human data relating to the carcinogenicity of *o*-anisidine or data on carcinogenicity from animal inhalation experiments.

In the two year NCI (1978) feeding study described above, transitional-cell carcinomas or papillomas of the urinary bladder were observed in male and female rats in the mid and high dose groups, and in the high dose group of mice. Transitional-cell carcinomas of the pelvis of the kidney and follicular-cell tumours of the thyroid were observed in the male rats only. It has been suggested that the thyroid tumours were caused by thyroid-pituitary imbalance leading to the growth and proliferation of certain thyroid cell types in order to increase hormone production (Thomas & Williams, 1991; Andrae & Greim, 1992).

o-Anisidine has also been shown to have tumour promoting activity (Ono *et al*, 1992). A much greater proportion of animals that were exposed to a combination of N-butyl-N-hydroxybutylnitrosamine (BBN) in drinking water and *o*-anisidine in food developed bladder tumours than those treated with BBN alone and no tumours were observed in animals exposed to o-anisidine in the absence of BBN.

Although *o*-anisidine is generally considered as being genotoxic, the mutagenicity data are inconsistent (*v.s.*). Indirect mechanism of carcinogenicity must therefore be considered (EU RAR, 2002). Results of a recent mechanistic study by Stiborova *et al* (2005), suggest similarities in the metabolism of *o*-anisidine in humans and rats that could be consistent with o-anisidine being a human carcinogen.

2.8. Reproductive toxicity

There are no human data relating to the reproductive toxicity of *o*-anisidine.

The developmental toxicity and teratogenicity of o-anisidine have not been investigated in animals. No adverse effects on reproductive organs were observed in a two year

carcinogenicity study (NCI, 1978). There is limited evidence that structurally similar compound o-aminophenol is teratogenic in animals and much stronger evidence that the slightly less similar compound, p-aminophenol, is both a developmental toxicant and teratogen (EU RAR, 2002). It is possible, therefore, that *o*-anisidine may also display teratogenic properties.

Recommendation

There is no specific information about the absorption of *o*-anisidine following exposure by inhalation, skin contact or ingestion, and its metabolism and mode of action are not well established. Other aromatic amines are oxidised to a N-hydroxy derivative that interacts with haem group of haemoglobin to form methaemoglobin that reduces the oxygen carrying capacity of blood. The methaemoglobin-inducing potency of o-anisidine is higher compared to the structurally related compounds 2-methoxy-5-methyl-aniline and 2,4-dimethoxy-aniline (Ashby et al. 1991). Workers exposed to 2 mgm^{-3} over a six month period developed headaches and vertigo and methaemoglobinaemia (Pascari *et al*, 1958, as cited by ACGIH).

There is relatively little animal toxicity data available. In an acute inhalation study, no deaths occurred in rats following exposure to 3,800 mgm^{-3} (as an aerosol). Animals showed impaired movement, respiration and reflexes as well as bloody nasal discharge and cyanosis. All signs of toxicity had resolved within 8 days of exposure. In a 28 day study, oral exposure of rats gave rise to effects on liver and kidney function and on blood chemistry. A NOEL was derived as 16 mg/kg/day and the LOEL as 80 mg/kg/day (EU RAR, 2002; reference to original study not provided). A NCI study found a LOEL of 75 mg/kg/day for effects on the spleen.

In 2 year feeding studies, o-anisidine caused bladder cancer in rats and mice, and thyroid tumours in male rats (NCI, 1978). IARC has classified it in 1999 as a carcinogen category 2B (possibly carcinogenic for humans). It is generally considered to be genotoxic, but the mutagenicity data is inconsistent and it cannot be excluded that it causes cancer in animals by an indirect mechanism. This is experimentally supported by a synergism of o-anisidine with the induction of bladder tumours in rats by the bladder-specific nitrosamine *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (Ono et al. 1992). As the most relevant target of carcinogenicity of aromatic amines in humans is the urothelium, the clear finding of o-anisidine being a rodent bladder carcinogen must be taken very serious. Although there are some indications that epigenetic mechanisms may largely be involved (Ashby et al. 1991), the mode of action is only poorly understood. Under these circumstances the compound is categorized by SCOEL as a group B carcinogen, for which the existence of a threshold cannot be sufficiently supported at present. The setting of an health-based OEL is therefore not possible at the present time. There are no data to perform a risk assessment exercise.

Single publications have described applications of methods for biological monitoring (see chapter 4.2), including analysis of o-anisidine in urine and analysis of the haemoglobin adduct. By use of these methods, it is possible to compare measured values with those of the general population.

Other structurally related aromatic amines are known to penetrate through the skin easily, but there are no specific data on skin absorption of o-anisidine. Based on the high potential for skin resorption of other related monocyclic aromatic amines, a "skin" notation is recommended.

The EU RAR did not consider *o*-anisidine to be an irritant or corrosive. It is uncertain whether o-anisidine can cause respiratory or skin sensitisation. The developmental toxicity and teratogenicity of *o*-anisidine have not been investigated.

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