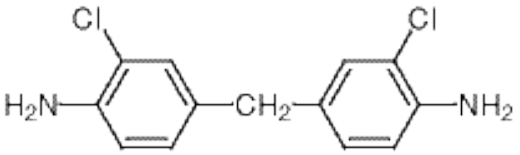
SIDS INITIAL ASSESSMENT PROFILE

CAS No.	101-14-4
Chemical Names	4,4'-Methylenebis(2-chloroaniline)
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

To avoid duplication of assessment work, only summary information of genotoxicity and carcinogenicity in IARC monographs[1] and Toxicological Profiles by ATSDR[2] and State of the Science Reports by Health Canada[3] is described, as secondary information, in this SIAP. Robust study summaries for new genotoxicity studies were generated for the purpose of the OECD Cooperative Chemicals Assessment Programme.

Physical-chemical properties

Pure 4,4'-methylenebis(2-chloroaniline) is a colourless and crystalline solid, but the commonly used forms (industry grade) of this chemical are tan-coloured pellets or flakes with a faint, amine-like odour. Melting point is 110 °C. Boiling point cannot be obtained because this substance decomposes on heating above 277 °C. Density is 1.44 g/cm^3. Measured value of vapour pressure is 5.19×10^{-7} Pa at 25 °C. Measured value of water solubility is 0.509 mg/L at 20 °C. Measured value of partition coefficient between octanol and water (log K_{ow}) is 3.66 at 25 °C. 4,4'-Methylenebis(2-chloroaniline) exists in the neutral form between pH 3 and pH 9 in water.

Human Health

Toxicokinetics

Absorption: 4,4'-methylenebis(2-chloroaniline) seems to be easily absorbed via the oral route based on the similar urinary excretion profile for i.p. and oral administration in rats. It was estimated that 2.4 - 10% or 11.5 - 21.9% of the dose was absorbed through the skin in 24 h in dogs or in 72 h in rats. Less substance was absorbed from the skin if the skin was washed within 8 h after application in dogs.

Distribution: The distribution of 4,4'-methylenebis(2-chloroaniline) in rats and dogs is relatively similar after oral and dermal exposures, and 4,4'-methylenebis(2-chloroaniline) was detected in the various tissues such as the liver (mainly), kidney, fat, lung, spleen, urinary bladder, and/or testes.

Metabolism: 4,4'-methylenebis(2-chloroaniline) metabolism can proceed via several pathways: N-acetylation, N-hydroxylation, which may be followed by N-oxidation, and ring hydroxylation. Some of these processes may be followed by conjugation. In workers occupationally exposed to 4,4'-methylenebis(2-chloroaniline), N-acetyl-4,4'-methylenebis(2-chloroaniline) and N,N′-diacetyl-4,4'-methylenebis(2-chloroaniline) were observed in urine

[1] IARC (1993). *Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines.* IARC Monogr Eval Carcinog Risks Hum, 57, 271–303. IARC (2010). *Some aromatic amines, organic dyes, and related exposures.* IARC Monogr Eval Carcinog Risks Hum, 99, 325. IARC (2012). *Chemical agents and related occupations.* IARC Monogr Eval Carcinog Risks Hum, 100F, 73–82.

[2] ATSDER (1994). *Toxicological Profile for 4,4'-Methylenebis(2-chloroaniline),* Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services.

[3] Health Canada (2005). *State of the Science Report for a Screening Health Assessment: 4,4'-Methylenebis(2-chlorobenzenamine) [MBOCA]*; CAS No. 101-14-4. 14 pp.

samples.

Adducts formation: 4,4'-methylenebis(2-chloroaniline) forms N-(deoxyadenosin-8-yl)-4-amino-3-chlorobenzyl alcohol. This adduct was found in urothelial cells that were exfoliated into urine of a worker who was accidentally sprayed with 4,4'-methylenebis(2-chloroaniline) on his upper body. The same DNA adduct was found in rats. Adduct formations with DNA were found in the liver, lung and kidney of rats and in the liver and bladder of dogs. Hemoglobin adduct was also found in workers exposed to 4,4'-methylenebis(2-chloroaniline) and in rats.

Excretion: the major route of elimination was faeces and urine in rats and dogs. The rate of excretion of radiolabelled 4,4'-methylenebis(2-chloroaniline) in urine and faeces was very high in the first 24 h (68.3%) but decreased rapidly (2.07%) by the 3rd day in rats treated by 4,4'-methylenebis(2-chloroaniline) by gavage. A significant amount of the substance was recovered from faeces after dermal and intravenous administration. It is considered that most of absorbed 4,4'-methylenebis(2-chloroaniline) is excreted in bile. 4,4'-Methylenebis(2-chloroaniline) was also found in urine samples of occupationally exposed workers.

Acute toxicity

The dermal LD_{50} of 4,4'-methylenebis(2-chloroaniline) in rats was reported to be greater than 2000 mg/kg bw (OECD TG 402). Neither substance related mortality nor clinical signs of toxicity were observed. The oral LD_{50} value was greater than 2000 mg/kg bw for female rats following a study conducted according to OECD guideline 423. The substance caused unkempt fur and deep breathing and temporary dark red discoloration of the ear auricles and limbs. Necropsy of the dead animal revealed white foci in the liver, dark red adrenals, dark red foci in the forestomach and glandular stomach, and dark red contents in the intestine from the jejunum to ileum.

Irritation

4,4'-Methylenebis(2-chloroaniline) was not corrosive to isolated bovine eyes (OECD TG 437). 4,4'-Methylenebis(2-chloroaniline) was not irritating to human epidermis (OECD TG 439). According to the limited human information, signs of toxicity from exposure of workers to molten or hot 4,4'-methylenebis(2-chloroaniline) that was sprayed accidentally on the upper body or face included slight erythema in affected skin areas (from upper body exposure), burning sensation in affected skin areas (from upper body and face exposure), burning sensation and conjunctivitis in both eyes (from face exposure), and upset stomach (from face exposure), even after attempts at decontamination in the workers. However, it is difficult to say if the effects were caused by the chemical or the temperature of the chemical.

Sensitization

4,4'-Methylenebis(2-chloroaniline) was not sensitising in a local lymph node assay (OECD TG 429).

Repeated dose toxicity

In a repeated dose oral toxicity study in rats following OECD TG 422, the substance was administered via gavage to 12 animals/sex/dose at 0 (vehicle, olive oil), 0.4, 2, 10, and 50 mg/kg bw/day, for 42 days (males) or from 14 days before mating to the 4th day of lactation. As recovery groups, 5/12 males at 0 and 50 mg/kg bw/day were observed for 14 days after the administration period. An additional five females at 0 and 50 mg/kg bw/day were treated for 42 days without mating and observed for 14 days as satellite groups. No death was observed in either sex. Treatment-related effects such as salivation in both sexes and significant decrease in female's body weight in later stages of pregnancy were observed at 50 mg/kg bw/day. Significant decreases in the levels of total protein and albumin in the 50 mg/kg bw/day groups in both males and females and in the 10 mg/kg bw/day groups only in females were observed. There were increases in absolute and/or relative weights of the liver in both sexes, and absolute and relative weights of the spleen and relative weights of the thyroid in females at 50 mg/kg bw/day. Increases of relative weights of the kidney were observed in females at $\geq$ 10 mg/kg bw/day. Histopathology revealed centrilobular swelling and mid-zonal fatty degeneration of hepatocytes in both sexes at 50 mg/kg bw/day. Increase in basophilic tubules in the kidney was dose dependant in males starting at 10 mg/kg bw/day. In the spleen, hemosiderin deposits were significantly higher in males at $\geq$ 10 mg/kg bw/day and females at 50 mg/kg bw/day. Furthermore, increased extramedullary hematopoiesis was observed in females in the 50 mg/kg bw/day group. In recovery animals that had been dosed at 50 mg/kg bw/day, significantly higher relative weights

of the liver and kidneys in females, and significantly lower changes in mean corpuscular hemoglobin concentration and hematocrit in males and in methemoglobin in females were also observed. Based on the changes in clinical chemistry in females (decreased albumin and total protein) and histopathological findings in the kidney, liver and spleen, the NOAEL for systemic toxicity in this OECD TG 422 study in rats (exposure duration = 42 - 55 days) was considered to be 2 mg/kg bw/day.

There are four supporting chronic studies in rats, mice, or dogs, designed as carcinogenicity studies, and providing only limited information.

4,4'-Methylenebis(2-chloroaniline) treatment in the diet for 18 months shortened survival times and decreased body weight in rats and mice.

In a study conducted in rats (25 – 50/sex/dose), hepatomegaly, fatty change, necrosis, fibrosis, and bile duct proliferation in the liver was observed in both sexes at 50 mg/kg bw/day (only dose tested).

Similar changes were seen in 6 female dogs given 4,4'-methylenebis(2-chloroaniline) in a gelatine capsule at ca. 7.6 - 11.8 mg/kg bw/day for 9 years. Histopathology revealed nodular hepatic hyperplasia and disruption of liver architecture. A statistically significant increase in serum glutamic-pyruvic transaminase was also observed. Based on the occurrence of hepatic effects in dogs exposed to 4,4'-methylenebis(2-chloroaniline), the chronic LOAEL was determined to be 7.6 mg/kg bw/day.

Genotoxicity

In a bacterial reverse mutation assay performed according to OECD TG 471, 4,4'-methylenebis(2-chloroaniline) was positive in *Salmonella typhimurium* TA100 and TA98 with metabolic activation. An *in vitro* chromosomal aberration test (OECD TG 473) in cultured Chinese hamster lung fibroblasts (CHL/IU) cells was positive without metabolic activation.

The following is other reliable information in IARC monographs, Toxicological Profiles and Health Canada State of the Science Reports:

4,4'-Methylenebis(2-chloroaniline) was shown to cause prophage induction in *Escherichia coli* and differential toxicity in *Bacillus subtilis* rec-deficient strains. It was mutagenic to *S. typhimurium, E. coli*, and at the Tk locus in mouse lymphoma L5178Y cells, but not to *Saccharomyces cerevisiae*. 4,4'-Methylenebis(2-chloroaniline) caused aneuploidy in *S. cerevisiae* but demonstrated equivocal results with regard to gene conversion and did not induce mitotic crossing-over in the same organism. It induced unscheduled DNA synthesis in primary cultures of hepatocytes from mice, rats, and Syrian hamsters. Sister chromatid exchange, but not chromosomal aberration, was induced in Chinese hamster ovary cells. 4,4'-Methylenebis(2-chloroaniline) induced cell transformation in mammalian (hamster/rat/mouse) cells and inhibited gap-junctional intercellular communication in cultured rat liver cells. Positive results were observed in *in vitro* micronuclei assays.

An *in vivo* assay, sister chromatid exchange in lymphocytes of rats, was positive. *In vivo* studies in bone marrow and peripheral blood of rats showed no evidence of micronuclei induction, but a positive result was observed in mice at a high dose in a two-phase micronucleus assay. 4,4'-Methylenebis(2-chloroaniline) induced mutation in *Drosophila melanogaster*. DNA damage was observed in the liver, urinary bladder and brain of mice by a comet assay.

Based on these results, 4,4'-methylenebis(2-chloroaniline) is considered to be genotoxic *in vitro* and *in vivo*.

Carcinogenicity

Summary information for carcinogenicity in IARC monographs (classified in group 1) is as follows: Oral administration of 4,4'-methylenebis(2-chloroaniline) increased the incidence of liver tumours in female mice. In a series of experiments in which rats were fed either standard or low protein diets, it induced liver cell tumours and malignant lung tumours in males and females in one study, a few liver cell tumours in male rats in a second study, lung adenocarcinomas and hepatocellular tumours in males and females in a third study, and malignant lung tumours, mammary gland adenocarcinomas, Zymbal gland carcinomas, and hepatocellular carcinomas in a fourth study. Oral administration of 4,4'-methylenebis(2-chloroaniline) to female beagle dogs produced

transitional cell carcinomas of the urinary bladder and urethra. Subcutaneous administration to rats produced hepatocellular carcinomas and malignant lung tumours.

Bladder cytology surveys identified bladder cancer cases in workers exposed to 4,4'-methylenebis(2-chloroaniline) in Michigan, USA, New Jersey, USA, and Taiwan, China. A cohort of 308 male 4,4'-methylenebis(2-chloroaniline) production workers in the United Kingdom found one bladder cancer death during 1979 – 2007, with 0.18 deaths expected (SMR 5.6; 95%CI: 0.14 – 31.2), based on the United Kingdom mortality rates. However, no adequate epidemiological studies were available to evaluate an association between 4,4'-methylenebis(2-chloroaniline) and bladder cancer risk.

Other relevant data

The provable carcinogenic mechanism of 4,4'-methylenebis(2-chloroaniline) includes, metabolic activation and formation of DNA adducts. DNA adducts were considered to be produced after N-oxidation of 4,4'-methylenebis(2-chloroaniline) to N-hydroxy-4,4'-methylenebis(2-chloroaniline) (see toxicokinetics section for further information for the DNA adducts). On the other hand, a cross-sectional survey at 4,4'-methylenebis(2-chloroaniline) producing factories showed that neither the 4,4'-methylenebis(2-chloroaniline)-exposed workers nor the high urinary 4,4'-methylenebis(2-chloroaniline) workers had a significant increase in the mean plasma 8-OHdG level after adjustment for potential confounders. This result suggested that oxidative DNA damage does not play an important role in the carcinogenic processes of 4,4'-methylenebis(2-chloroaniline).

Overall, 4,4'-methylenebis(2-chloroaniline) is considered to be carcinogenic based on the tumours observed in a range of tissues in a number of animal studies.

Reproductive toxicity

The reproductive toxicity of 4,4'-methylenebis(2-chloroaniline) was investigated in a reproductive and developmental toxicity screening test in rats OECD TG 422. In this study, 4,4'-methylenebis(2-chloroaniline) was administered via gavage to 12 animals/sex/dose at 0 (vehicle, olive oil), 0.4, 2, 10, and 50 mg/kg bw/day, for 42 days (males) or from 14 days before mating to the 4th day of lactation (42 - 52 days). No death was observed in either sex. A significant decrease in body weight was observed in dams in the late pregnancy period at 50 mg/kg bw/day, but it was considered to be general toxicity rather than reproductive toxicity. No effects were observed in the reproductive organ weights and histopathological examination of the reproductive organs. During the mating period, pseudopregnancy (no copulation) was observed in 1 and 2 females at only 2 mg/kg bw/day and 10 mg/kg bw/day, respectively. However, this effect was not statistically significant and was not dose-dependent. The prolonged pairing days until copulation was significantly observed at 50 mg/kg bw/day. No other adverse effects on reproductive/developmental parameters (such as copulation index, implantation index, fertility index, gestation index, maternal behaviour; numbers of offspring, or live offspring at birth or day 4, sex ratios of offspring, live birth index, viability index, body weight at birth or on day 4) were observed. Based on increased paring days until copulation at 50 mg/kg bw/day, the NOAEL for reproductive toxicity was considered to be 10 mg/kg bw/day. The NOAEL for developmental toxicity was considered to be 50 mg/kg bw/day (highest dose tested) based on no toxicological effects.

4,4'-Methylenebis(2-chloroaniline) possesses properties indicating a hazard for human health (repeated dose toxicity (anemia, methemoglobinemia, effects on kidney, liver and spleen), genotoxicity, carcinogenicity (tumours of liver, lung, mammary gland, Zymbal gland, bladder, urethra) and reproductive toxicity). Adequate screening level data are available to characterize the human health hazard for the purposes of the OECD Cooperative Chemicals Assessment Programme.

Environment

4,4'-Methylenebis(2-chloroaniline) in the atmosphere is expected to be degraded by hydroxyl radicals. Using AOPWIN (ver. 1.92a), a calculated half-life time of 0.14 days and a rate constant of 77.5×10^{-12} cm^3/molecule-sec are obtained for the indirect photo-oxidation of 4,4'-methylenebis(2-chloroaniline) by reaction with hydroxyl radicals in air. For the purposes of the AOPWIN, it is assumed that the concentration of hydroxyl radicals in air is 1.5×10^6 OH/cm^3 and that the hydroxyl radicals are available to react with 4,4'- methylenebis(2-chloroaniline) for 12 hours/day.

It is thought that 4,4'-methylenebis(2-chloroaniline) is not hydrolyzed due to the lack of hydrolysable functional groups in its structure. A ready biodegradation test on 4,4'-methylenebis(2-chloroaniline) based on a protocol equivalent to OECD TG 301C was conducted with activated sludge. The concentration of the test substance was 100 mg/L and the concentration of the activated sludge was 30 mg/L as suspended solid matter with a cultivation period of four weeks. The test result showed 0 % degradation by BOD. BIOWIN (ver. 4.10) prediction shows no biodegradability of 4,4'-methylenebis(2-chloroaniline). According to these results, 4,4'-methylenebis(2-chloroaniline) is considered to be not readily biodegradable.

A study on 4,4'-methylenebis(2-chloroaniline) according to a protocol equivalent to OECD TG 305 with carp was performed. Bioconcentration factors of 130 - 398 and 114 - 232 were obtained for the concentration of 50 µg/L and of 5 µg/L, respectively, for the 8-week exposure period. Using an octanol-water partition coefficient (log K_{ow}) of 3.66, a bioconcentration factor of 121 was calculated with BCFBAFWIN, version 3.01. This chemical has a low potential for bioaccumulation.

Fugacity level III calculations show that 4,4'-methylenebis(2-chloroaniline) is mainly distributed in soil (84.7 %) and water (12.0 %) compartments if equally and continuously released to the air, soil and water. A Henry's law constant of 2.72×10^{-4} Pa.m^3/mole at 20 - 25 °C suggests that 4,4'-methylenebis(2-chloroaniline) is non-volatile from water. A soil adsorption coefficient of log K_{oc} = 3.56 indicates that 4,4'-methylenebis(2-chloroaniline) has adsorption potential to soil and sediment.

The following acute toxicity test results have been determined for aquatic species:

Fish [*Oryzias latipes*]: 96 h LC_{50} = 0.61 - 0.66 mg/L (nominal, semistatic), OECD-TG 203

Daphnid [*Daphnia magna*]: 48 h EC_{50} = 0.25 - 0.92 mg/L (measured, static), OECD-TG 202

Algae [*Pseudokirchneriella subcapitata*]: 72 h E_rC_{50} > 0.85 mg/L (measured, growth rate, static), OECD-TG 201

The following chronic toxicity test results have been determined for aquatic species:

Daphnid [*Daphnia magna*]: 21 d EC_{50} = 0.052 mg/L (nominal, semistatic) OECD-TG 211

21 d LOEC = 0.03 mg/L (nominal, semistatic), OECD-TG 211

21 d NOEC = 0.0095 mg/L (nominal, semistatic), OECD-TG 211

21 d LOEC = 0.075 mg/L (nominal, semistatic), OECD-TG 202, part 2

21 d NOEC = 0.0375 mg/L (nominal, semistatic), OECD-TG 202, part 2

Algae[*Pseudokirchneriella subcapitata*]: 72 h NOE_rC = 0.54 mg/L (measured, growth rate, static), OECD-TG 201

The following chronic sediment toxicity test result has been determined for aquatic species:

Chironomid [*Chironomus yoshimatsui*] 27 d EC_{50} = 150 mg/Kg dry sediment

27 d LOEC = 180 mg/Kg dry sediment

27 d NOEC = 84 mg/Kg dry sediment (measured, emergence rate) OECD-TG 218

4,4'-Methylenebis(2-chloroaniline) possesses properties indicating a hazard for the environment (acute aquatic toxicity values less than 1 mg/L for fish and invertebrates, chronic aquatic toxicity values less than 1 mg/L for algae and less than 0.01mg/L for invertebrates). This chemical is not readily biodegradable and has a low bioaccumulation potential. Adequate screening-level data are available to characterize the hazard to the environment for the purposes of the OECD Cooperative Chemicals Assessment Programme.

Exposure

Total amounts of production and import of 4,4'-methylenebis(2-chloroaniline) in Japan (sponsor country) were reported to be 2,751 tonnes (fiscal year 2010) and 3,013 tonnes (fiscal year 2011). In the United States, total

amounts of production and/or import were reported to be between 500,000 - 1 million pounds (between 227 to 454 tonnes) in 2006 according to the inventory updating rule. Total volume of production and import in the European Union is in a range of 1000 - 10,000 tonnes/year. Production volume in the world is not available. 4,4'-Methylenebis(2-chloroaniline) is manufactured based on the reaction of formaldehyde with 2-chloroaniline.

4,4'-Methylenebis(2-chloroaniline) is used as a curing agent for polyurethanes and epoxy resins which are used in the manufacture of specialized products, particularly integral-skin polyurethane semi-rigid foam and solid urethane rubber moulding such as gear blanks and industrial tires. 4,4'-Methylenebis(2-chloroaniline) is added to vary the hardness, flexibility, and impact strength of these products.

4,4'-Methylenebis(2-chloroaniline) is also used as a coating in chemical reactions to set glues, plastics and adhesives. A use of 4,4'-methylenebis(2-chloroaniline) in Japan is as a curing agent in water-proofing materials, flooring materials and pavement materials.

Based on the Japanese Pollutant Release and Transfer Register system which is equivalent to the TRI system, 171 kg of 4,4'-methylenebis(2-chloroaniline) was released into the atmosphere and no releases were estimated into surface water, lands and landfills in fiscal year 2009. Based on the TRI in the United States, a total of 617 kg (1,362 pounds) of 4,4'-methylenebis(2-chloroaniline) was released into the atmosphere from manufacturing and processing facilities in 1991. No discharges into surface water and land were reported.

During the environmental survey and monitoring of chemicals conducted by the Japanese Ministry of Environment, 4,4'-methylenebis(2-chloroaniline) was not detected in surface water in any of 6 different localities (detection limit of 30 ng/L), although 4,4'-methylenebis(2-chloroaniline) was detected in sediments in three places out of 7 localities in fiscal year 2005. Concentrations detected in sediments were 19 - 32 ng/g-dry wt, 8 - 9 ng/g-dry wt and 9 ng/g-dry wt. 4,4'-Methylenebis(2-chloroaniline) was not detected in surface water nor in sediments in any of 36 different localities (detection limit of 0.17 µg/L for surface water and detection limit of 0.031 µg/g-dry wt for sediment) in fiscal year 1999.

As mentioned above, release to the atmosphere is limited and releases to surface water and soil are negligible. It is thought that environmental release of 4,4'-methylenebis(2-chloroaniline) is not high.

As the vapour pressure is low, inhalation of vapour is not expected except when 4,4'-methylenebis(2-chloroaniline) is melted for manufacture of polyurethanes. Inhalation of dust may be a main exposure route where workers handle this chemical directly during emptying bags of 4,4'-methylenebis(2-chloroaniline) pellets. It is indicated by the toxicokinetics investigations that dermal intake is possible. In order to prevent exposure to inhaled dust and dermal intakes, proper engineering control (enclosure, local exhaust ventilation) and/or personal protective equipment are necessary at manufacturing and processing sites. A time weight average threshold limit value of 4,4'-methylenebis(2-chloroaniline) is decided to be 0.01 ppm (0.11 mg/m^3) by the American Conference of Industrial Hygienists.

Although trace amounts of unreacted 4,4'-methylenebis(2-chloroaniline) may be present in consumer products manufactured from polyurethane resins, no data have been identified on potential concentrations.