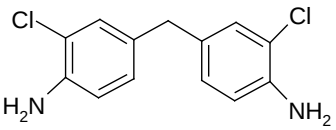
SCREENING-LEVEL HAZARD CHARACTERIZATION

4,4’-Methylenebis(2-chlorobenzenamine) (MBOCA; CASRN 101-14-4)

Chemical Abstract Service Registry Number (CASRN)	**101-14-4**
Chemical Abstract Index Name	**Benzenamine, 4,4'-methylenebis[2-chloro-**
Structural Formula	 SMILES: Nc1c(cc(cc1)Cc1ccc(N)c(c1)Cl)Cl

Summary

4,4'-Methylenebis(2-chlorobenzenamine) (MBOCA) is a white, crystalline powder with low vapor pressure and moderate water solubility. It is expected to possess low mobility in soil. Volatilization is low based on the Henry’s Law constant. The rate of hydrolysis is negligible. The rate of atmospheric photooxidation is rapid. MBOCA is expected to have moderate persistence (P2) and low bioaccumulation potential (B1).

Acute oral (rats and mice) and dermal (rabbits) toxicity of MBOCA is low. In an oral gavage combined repeated-dose/reproductive/developmental toxicity study in rats, effects on the spleen, liver and kidneys were observed at 10 mg/kg-day; the NOAEL for systemic toxicity is 2 mg/kg-day. No adverse treatment-related effects were observed on reproductive and developmental parameters; the NOAEL for reproductive and developmental toxicity is 50 mg/kg-day (highest dose tested) and for parental systemic toxicity is 2 mg/kg-day. MBOCA was mutagenic in bacteria and mammalian cells *in vitro* and did not induce chromosomal aberrations *in vitro* but did induce sister chromatid exchanges in Chinese hamster ovary cells *in vitro*.

For MBOCA, the 96-h LC_{50} for fish ranges from 0.61 to 0.66 mg/L, the 48-h EC_{50} for aquatic invertebrates ranges from 0.25 to 0.92 mg/L, and the 72-h EC_{50} to aquatic plants is 0.85 mg/L, which is based on growth rate. The chronic 21-d NOEC to aquatic invertebrates is 0.01 mg/L, and the 21-d LOEC is 0.03 mg/L, for reproduction and mortality.

No data gaps were identified under the HPV Challenge Program.

The sponsor, the MBOCA Consortium, submitted a test plan and robust summaries to EPA for 4,4'-methylenebis(2-chlorobenzenamine) (CASRN 101-14-4; CA Index name: benzenamine, 4,4'-methylenebis[2-chloro-) on December 29, 2005. EPA posted the submission on the ChemRTK HPV Challenge website on February 7, 2006 (http://www.epa.gov/oppt/chemrtk/pubs/summaries/44methyl/c16124tc.htm). EPA comments on the original submission and the revised submission were posted to the website on September 24, 2008. Public comments were also received and posted to the website. The sponsor submitted updated/revised documents on July 3, 2007, which were posted to the ChemRTK website on August 30, 2007.

MBOCA was recently evaluated in the OECD HPV program (CoCAM 5; 2013) and the data can be viewed at: http://webnet.oecd.org/HPV/UI/handler.axd?id=3c245da6-4669-4341-bf18-9182b9972ec8

1. Chemical Identity

1.1 Identification and Purity

4,4'-Methylenebis(2-chlorobenzenamine) (MBOCA) is a white, crystalline powder with low vapor pressure and moderate water solubility.

1.2 Physical-Chemical Properties

The physical-chemical properties of MBOCA are summarized in Table 1.

Table 1. Physical-Chemical Properties of MBOCA[1]

Property	Value
CASRN	101-14-4
Molecular Weight	267.16
Physical State	White, needle-like, crystalline powder
Melting Point	110.5–111.0°C (measured);
Boiling Point	195–200°C at 20–26.7 Pa (measured) >300°C (estimated)[2]
Vapor Pressure	1.0×10^{-5} mm Hg at 25°C (measured);
Dissociation Constant (pK_a)	pK_{a1} = 3.17 (estimated)[3]; pK_{a2} = 2.36 (estimated)[3]
Henry's Law Constant	$<1\times10^{-10}$ atm-m^3/mole (estimated)[4]
Water Solubility	13.9 mg/L at 24°C (measured)
Log K_{ow}	3.91 (measured)[5]

[1]MBOCA Consortium. 2005. Test Plan and Robust Summary for 4,4'-Methylenebis(2-chlorobenzenamine). Available online at http://www.epa.gov/oppt/chemrtk/pubs/summaries/44methyl/c16124tc.htm as of March 6, 2012.

[2]NOMO5. 1987. Programs to Enhance PC-Gems Estimates of Physical Properties for Organic Compounds. The Mitre Corp.

[3]SPARC. October 2011. Online pK_a/Property Calculator, w4.2.1405-s4.2.1408. University of Georgia, Athens, GA, USA.

[4]U.S. EPA. 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.10. U.S. Environmental Protection Agency, Washington, DC, USA. Available online at http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm as of March 6, 2012.

[5]SRC. 2012. The Physical Properties Database (PHYSPROP). Syracuse, NY: Syracuse Research Corporation. Available online at http://esc.syrres.com/fatepointer/search.asp, as of September 8, 2015.

2. General Information on Exposure

2.1 Production Volume and Use Pattern

MBOCA had an aggregated production and/or import volume in the United States between 500,000 pounds to 1 million pounds during calendar year 2005 (U.S. EPA, 2010).

Non-confidential information in the IUR indicated that the industrial processing and uses of the chemical include urethane and other foam product (except polystyrene) manufacturing listed as “other.” Non-confidential commercial and consumer uses of this chemical were reported to be Not Readily Obtainable (NRO.)

2.2 Environmental Exposure and Fate

MBOCA is expected to have low mobility in soil. MBOCA is not readily biodegradable since it achieved 0% of its theoretical biochemical oxygen demand (BOD) in 28 days using an activated sludge inoculum and the MITI (OECD TG 301C) test. It was not degraded over 6 weeks in a semi-continuous activated sludge (OECD TG 302A) test. However, 96% degradation was observed within 24 hours following acclimation for 7 days after adding water contaminated with MBOCA to the continuous feed reactor. Volatilization is expected to be low. The rate of hydrolysis is negligible. The rate of atmospheric photooxidation is rapid. MBOCA is expected to have moderate persistence (P2) and low bioaccumulation potential (B1).

The environmental fate characteristics of MBOCA are summarized in Table 2.

Table 2. Environmental Fate Characteristics of MBOCA[1]	
Property	**Value**
CASRN	101-14-4
Photodegradation Half-life	1.7 hours (estimated)[2]
Hydrolysis Half-life	Stable
Biodegradation	0% after 42 days (not inherently biodegradable); 0% after 28 days (not readily biodegradable)[3]
Bioaccumulation Factor	BCF = 130–398 (measured in carp at 50 ppb)[3]; BCF = 114–232 (measured in carp at 5 ppb)[3]; BAF = 220.2 (estimated)[2]
Log K_{oc}	3.8 (estimated)[2]
Fugacity (Level III Model)[2]	
Air (%)	<0.1
Water (%)	8.3
Soil (%)	88.2
Sediment (%)	3.5
Persistence[4]	P2 (moderate)
Bioaccumulation[4]	B1 (low)

[1]MBOCA Consortium. 2005. Test Plan and Robust Summary for 4,4'-Methylenebis(2-chlorobenzenamine). Available online at http://www.epa.gov/oppt/chemrtk/pubs/summaries/44methyl/c16124tc.htm as of March 6, 2012.
[2]U.S. EPA. 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v4.10. U.S. Environmental Protection Agency, Washington, DC, USA. Available online at http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm as of March 6, 2012.
[3]National Institute of Technology and Evaluation. 2002. Biodegradation and Bioaccumulation of the Existing Chemical Substances under the Chemical Substances Control Law. Available online at http://www.nite.go.jp/en/chem/index.html as of September 8, 2015.
[4]Federal Register. 1999. Category for Persistent, Bioaccumulative, and Toxic New Chemical Substances. *Federal Register* 64, Number 213 (November 4, 1999) pp. 60194–60204.

Conclusion: MBOCA is a white, crystalline powder with low vapor pressure and moderate water solubility. It is expected to possess low mobility in soil. Volatilization is low based on the Henry's Law constant. The rate of hydrolysis is negligible. The rate of atmospheric photooxidation is rapid. MBOCA is expected to have moderate persistence (P2) and low bioaccumulation potential (B1).

3. Human Health Hazard

A summary of health effects data is provided in Table 3.

Acute Oral Toxicity

(1) Rats (strain, sex and number per dose unspecified) were administered MBOCA via an unspecified oral route at unspecified doses. The length of the observation period following dosing was not specified. **LD_{50} = 1140 mg/kg**

(2) Mice (strain, sex and number per dose unspecified) were administered MBOCA via an unspecified oral route at unspecified doses. The length of the observation following dosing was not specified.
LD_{50} = 640 mg/kg

(3) See SIAP at: http://webnet.oecd.org/HPV/UI/handler.axd?id=3c245da6-4669-4341-bf18-9182b9972ec8
LD_{50} (female rat) > 2000 mg/kg

Acute Dermal Toxicity

Rabbits (strain, sex and number per dose unspecified) were administered MBOCA via the dermal route at unspecified doses under unspecified conditions. Duration and observation following dose administration were unspecified.
LD_{50} > 5000 mg/kg-bw

Repeated-Dose Toxicity

In a combined repeated-dose/reproductive/developmental toxicity screening test, Sprague-Dawley rats (12/sex/dose) were administered MBOCA by gavage at 0, 0.4, 2, 10 or 50 mg/kg-bw/day for 42 days (males) or up to 52 days (females). In the 50 mg/kg-day group, salivation was observed in males and females and low body weight in late gestation was observed in females. In addition, at 50 mg/kg-day, hematological analysis revealed increased methemoglobin and decreased red blood cells in both sexes. Moreover, decreased hemoglobin and hematocrit and increased reticulocyte and platelet counts were observed in the males and an increase in red blood cells containing Heinz bodies was observed in females. Blood biochemical examination revealed decreases in total protein and albumin in both sexes, increases in total cholesterol, triglycerides and inorganic phosphorus in males and increases in lactate dehydrogenase and γ-glutamyl transpeptidase, as well as decreases in the A/G ratio, in females. Organ weight measurements revealed increases in the absolute and relative liver weights and relative spleen weight in males and increases in the absolute and relative spleen weights and relative weight of the liver, kidneys and thyroid gland in females. Additionally, grossly, the liver was bleached in both sexes. Histopathological examination of the liver revealed swelling and fatty degeneration of hepatocytes in the central zone and intermediate zone, respectively, in both sexes. Single cell necrosis of hepatocytes in the central zone and an increased trend for basophilic changes of renal tubules were observed in males. Increased trends of hemosiderin deposition and extramedullary hemopoiesis in female spleens were also observed. In the 10 mg/kg-day group, increased trends of both basophilic changes of the renal tubules and splenic hemosiderin deposition were observed in males, and females exhibited decreases in total serum protein and albumin and an increase in the relative weight of kidneys. These changes were reversible, and in recovery groups, all of these changes recovered or showed a tendency to recover.
LOAEL = 10 mg/kg-day (based on effects on the spleen, liver and kidneys)
NOAEL = 2 mg/kg-day

Reproductive/Developmental Toxicity

In the 42-52-day combined repeated-dose/reproductive/developmental toxicity screening test described previously, no effects on the estrous cycle, pairing days until copulation, copulation index, fertility index, gestation length, number of corpora lutea, implantation index, live birth index, delivery index, parturition or parenting state were observed in dams. In addition, no effects on the total number of pups,

number of live pups, sex ratio, live birth index, body weight, body formation or viability index of live pups on day 4 were observed.
LOAEL (parental systemic toxicity) = 10 mg/kg-bw/day (based on toxicity to the spleen, liver and kidneys)
NOAEL (parental systemic toxicity) = 2 mg/kg-bw/day
NOAEL (reproductive/developmental toxicity) = 50 mg/kg-bw/day (based on no adverse treatment-related effects on reproductive and developmental parameters at the highest dose tested)

Genetic Toxicity – Gene Mutations

In vitro
(1) The National Toxicology Program (NTP) conducted 10 bacterial mutagenicity assays, exposing *Salmonella typhimurium* strains TA97 (2/10 studies), TA98, TA100, TA1535 and TA1537 to MBOCA at concentrations up to 3333 μg/plate with and without S9 activation. No mutagenic response was seen without S9 activation in any strain, but with S9 activation, mutagenicity was induced in strains TA98, TA100 and TA97.
MBOCA was mutagenic in these assays.

(2) NTP conducted a mammalian cell mutagenicity assay exposing mouse L5178Y TK+/-cells to MBOCA at concentrations ranging from 41.8 to 108 μg/mL without S9 activation or 3.3 to 70 μg/mL with S9 metabolic activation. A mutagenic response was seen only with S9 metabolism.
MBOCA was mutagenic in these assays.

(3) *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to MBOCA at concentrations ranging from 10 to 500 μg/mL. Precipitation of the test substance was observed at 500 μg/mL. TA100 showed an increase in the number of revertants with metabolic activation, while TA98, TA1535, TA1537 and TA1538 had no increases in the number of revertants relative to controls with or without metabolic activation. Data are from TSCATS (OTS0535307; 8EHQ-0292-2150).
MBOCA was mutagenic in this assay.

Genetic Toxicity – Chromosomal Aberrations

In vitro
NTP conducted assays for chromosomal aberrations in Chinese hamster ovary (CHO) cells exposed to MBOCA at 0.005, 0.05, 0.5 or 5.0 μg/mL with and without S9 activation. Chromosomal aberrations were not detected in the concentrations tested in the study.
MBOCA did not induce chromosome aberrations in these assays.

Genetic Toxicity - Other

In vitro
(1) NTP conducted a sister chromatid exchange (SCE) assay in CHO cells exposed to MBOCA at 0.005, 0.05, 0.5 or 5.0 μg/mL without S9 activation or 0.08, 0.4, 2, 10 or 50 μg/mL with S9 activation. SCEs were induced without metabolic activation and the results were questionable with metabolic activation.

MBOCA induced SCEs in this assay.

(2) In an additional SCE assay conducted by NTP, CHO cells did not show signs of SCE after cells were cells exposed to MBOCA at 0.003, 0.03, 0.3, 3.0 or 30 μg/mL with and without S9 activation.
MBOCA did not induce SCEs in this assay.

(3) In a mitotic recombination assay, *Saccharomyces cerivisiea* D_7 were exposed to MBOCA at 30, 80, 150, 200 or 250 μg/mL. Incidences of mitotic recombinations were increased over controls at 0.25 mg/mL with a 22% survival rate. Data are from TSCATS (OTS0535307; 8EHQ-0292-2150).
MBOCA induced mitotic recombination in yeast in this assay.

Conclusion: Acute oral (rats and mice) and dermal (rabbits) toxicity of MBOCA is low. In an oral gavage combined repeated-dose/reproductive/developmental toxicity study in rats, effects on the spleen, liver and kidneys were observed at 10 mg/kg-day; the NOAEL for systemic toxicity is 2 mg/kg-day. No adverse treatment-related effects were observed on reproductive and developmental parameters; the NOAEL for reproductive and developmental toxicity is 50 mg/kg-day (highest dose tested) and for parental systemic toxicity is 2 mg/kg-day. MBOCA was mutagenic in bacteria and mammalian cells *in vitro*, but did not induce chromosomal aberrations *in vitro*. It induced sister chromatid exchanges in Chinese hamster ovary cells *in vitro*.

Table 3. Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data

Endpoint	4,4'-Methylenebis(2-chlorobenzenamine) (MBOCA; CASRN 101-14-4)
Acute Oral Toxicity **LD_{50} (mg/kg)**	**1140 (rat)** **640 (mouse)**
Acute Dermal Toxicity **LD_{50} (mg/kg)**	**>5000**
Repeated-Dose Toxicity **NOAEL/LOAEL** **Oral (mg/kg-day)**	**(rat; 28-d)** **NOAEL= 2** **LOAEL = 10**
Reproductive Toxicity **NOAEL/LOAEL** **Oral (mg/kg-day)**	**NOAEL= 50** **(highest dose tested)**
Developmental Toxicity **NOAEL/LOAEL** **Oral (mg/kg-day)** **Maternal Toxicity** **Developmental Toxicity**	 **NOAEL= 2** **LOAEL = 10** **NOAEL = 50** **(highest dose tested)**

Table 3. Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Human Health Data	
Endpoint	**4,4'-Methylenebis(2-chlorobenzenamine) (MBOCA; CASRN 101-14-4)**
Genetic Toxicity – Gene Mutation *In vitro*	**Positive**
Genetic Toxicity – Chromosomal Aberrations *In vitro*	**Negative**
Genetic Toxicity – Other *In vitro*	
SCE	**Positive**
Mitotic exchange	**Positive**

Measured data in BOLD

4. Hazard to the Environment

A summary of aquatic toxicity data submitted for SIDS endpoints is provided in Table 4.

Acute Toxicity to Fish

Japanese medaka fish (*Oryzias latipes)* were exposed to nominal concentrations of MBOCA under semi-static conditions. See SIAP at: http://webnet.oecd.org/HPV/UI/handler.axd?id=3c245da6-4669-4341-bf18-9182b9972ec8
96-h LC_{50} = 0.61-0.66 mg/L

Acute Toxicity to Aquatic Invertebrates

Daphnia magna were exposed to measured concentrations of MBOCA under static conditions. See SIAP at: http://webnet.oecd.org/HPV/UI/handler.axd?id=3c245da6-4669-4341-bf18-9182b9972ec8
48-h EC_{50} = 0.25-0.92 mg/L

Toxicity to Aquatic Plants

Algae (*Pseudokirchneriella subcapitata)* were exposed to measured concentrations of MBOCA under static conditions. See SIAP at: http://webnet.oecd.org/HPV/UI/handler.axd?id=3c245da6-4669-4341-bf18-9182b9972ec8
72-h EC_{50} (growth rate) = 0.85 mg/L

Chronic Toxicity to Aquatic Invertebrates

Daphnia magna were exposed to nominal concentrations of MBOCA under semi-static conditions. See SIAP at: http://webnet.oecd.org/HPV/UI/handler.axd?id=3c245da6-4669-4341-bf18-9182b9972ec8
21-d EC_{50} = 0.052 mg/L (reproduction)
21-d NOEC = 0.01 mg/L (reproduction/mortality)
21-d LOEC = 0.03 mg/L (reproduction/mortality)

Conclusion: For MBOCA, the 96-h LC_{50} for fish ranges from 0.61 to 0.66 mg/L, the 48-h EC_{50} for aquatic invertebrates ranges from 0.25 to 0.92 mg/L, and the 72-h EC_5 to aquatic plants is 0.85 mg/L, which is based on growth rate. For chronic toxicity to aquatic invertebrates, the 21-d NOEC is 0.01 mg/L, and the 21-d LOEC is 0.03 mg/L, and both values are based on reproduction and mortality.

Table 4. Summary Table of the Screening Information Data Set as Submitted under the U.S. HPV Challenge Program – Aquatic Toxicity Data

Endpoint	4,4'-Methylenebis(2-chlorobenzenamine) (MBOCA; CASRN 101-14-4)
Fish **96-h LC_{50} (mg/L)**	**0.61-0.66**
Aquatic Invertebrates **48-h EC_{50} (mg/L)**	**0.25-0.92**
Aquatic Plants **72-h EC_{50} (mg/L)** **Growth rate**	 **0.85**
Chronic Aquatic Invertebrates **21-d NOEC (mg/L) reproduction/mortality** **21-d LOEC (mg/L) reproduction/mortality**	 **0.01** **0.03**

Bold = measured data (i.e. derived from testing)

5. References

U.S. Environmental Protection Agency (2010) Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information for CASRN 101-14-4. Inventory Update Reporting (IUR); Version 6: Updated May 12, 2010. Available online at http://www.epa.gov/cdr/tools/previouslycollected.html