United States Environmental Protection

Office of Prevention, Pesticides and Toxic Substances Agency (7510C)

Pesticide Fact Sheet

Name of Chemical: Bis-(N-cyclohexyldiazeniumdioxy) -copper
Reason for Issuance: Registration
Date Issued:
Fact Sheet Number:

Description of Chemical

Chemical Name:	Bis-(N-cyclohexyldiazeniumdioxy) -copper
Common Name:	None
Trade Name:	Cu-HDO
Chemical Abstract Number (CAS):	312600-89-8
Empirical Formula:	$C_{12}H_{22}N_4O_4Cu$
Molecular Weight:	349.9g/mol
EPA Chemical Code:	235500
Year of Initial Registration:	2005
Pesticide Type:	Wood Preservative, Outdoor
Mode of Action:	Affect sulfhydryl groups of essential amino acids of fungi and cause protein denaturation.
Chemical Family:	Copper-Diazenium-Compound
U. S. Producer:	BASF Corporation

Use Patterns and Formulation

Bis-(N-Cyclohexyldiazeniumdioxy)-copper (Cu-HDO) (EPA File Symbol 241-UEN), an MUP

containing 97% of active ingredient (a.i.) for use in “formulating wood protectant end use products”; and Protectol CX Type A (EPA File Symbol 71406-E), containing a mixture of 3.5% Cu-HDO, 16.3% Copper Carbonate, and 5.0% Boric Acid as a.i.

Types and Methods of Application

Protectol CX Type A (EPA File Symbol 71406-E) is used for pressure-treatment applications and used in conjunction with a vacuum-pressure-treating vessel for impregnating forest products such as lumber, millwork, poles, and piles. It is restricted from use in aquatic areas, construction of beehives, and any application associated with the packaging of food or feed.

Science Findings:

All data required to support the proposed wood preservative use pattern are acceptable. Bis-(N-cyclohexyl-diazeniumdioxy)-copper is toxic to aquatic organisms. The MOEs calculated for dermal and incidental oral ingestion of surface residues are above the target MOE (dermal MOE of 1,699 and an oral MOE of 5,058), and thus are not of concern. The MOE from the child soil ingestion scenario is also above the Agency target (oral MOE of 41,269) and thus is not of concern. Bis-(N-cyclohexyl-diazeniumdioxy)- Copper is not considered carcinogenic. Bis-(N-cyclohexyl-diazeniumdioxy)-Copper is not considered to be mutagenic in this micronucleus assay.

Physical and Chemical Characteristics

Color:	Blue Violet
Physical State:	solid
Odor:	Odorless
Melting Point:	149 ^{0}C
Boiling Point:	Not applicable (solid)
Density:	D_4^{20} = 1.514
Water Solubility:	<10 ppm @pH< 7
Vapor Pressure:	$<10^{-6}$ hPa
Dissociation Constant:	Cannot be determine (due to low solubility)
Partition Coefficient:	log P_{ow} =2.6
pH:	7.3
Stability:	Stable (>45 months)
Oxidizing or Reducing Action:	Not reactive to strong oxidizing/reducing agents
Flammability:	Non-flammable

Explodability:	Not explosive by DOT criteria
Viscosity:	Not Applicable (solid)
Miscibility:	Not Applicable (solid)
Corrosion Characteristics:	Non-corrosive
Dielectric Breakdown Voltage:	Not Applicable (Not intended to be used around electrical equipment)

Toxicology Profile:

Technical Acute Toxicity:	Status	Toxicity Category
Acute Oral/Rat	LD_{50} =380 mg/kg	II
Acute Dermal:	$LD_{50} \geq$2500 mg/kg	III
Acute Inhalation:	Waived	II
Primary Eye Irritation:	Corrosive	I
Dermal Irritation:	Not a skin irritant	IV
Dermal Sensitization:	Not a dermal sensitizer	N/A

Subchronic Oral Toxicity:

In a 90 day subchronic oral toxicity study, Cu-HDO (99% a.i.) was administered to 40 Beagle dogs (5/sex/dose) in the diet at dose levels of 0, 300, 900, and 2,700 parts per million (ppm) (equivalent to approximately 0, 9, 26, and 69 mg/kg bw/day in all animals, respectively). No compound related changes in mortality, hematology (except prothrombin time) or ophthalmology occurred.

The NOAEL for this study is 900 ppm (26 mg/kg bw/day) for male and female animals. The LOAEL is 2,700 ppm (69 mg/kg bw/day) based on clinical observations, decrease in body weight food consumption, decrease in body weight and body weight gain, increased liver weight, and hematologic and clinical chemistry parameter changes observed at this dose level.

Chronic Toxicity:

In a 12 month chronic dietary toxicity study, Bis-(N-cyclohexyl-diazeniumdioxy)-copper (Cu-HDO) (95% a.i.) was administered to 20 male and 20 female Wistar rats each in the diet at dose levels of 0, 100, 300, 1,000, and 3,000 ppm (equivalent in both sexes to 0,6.1, 18.3, 61, and 183 mg/kg bw/day). The 3000 ppm Cu-HDO group was compared to an equimolar concentration of copper as contained in CuSO4 (1350 ppm) . At the end of the administration period (1 year) all animals were subjected to a gross pathology followed by

histopathology examination of selected organs.

The LOAEL of 1,000 ppm (67 mg/kg body weight for females and 54 mg/kg body weight males) and the NOAEL of 300 ppm (20 mg/kg/body weight for females and 16 mg/kg body weight males) was assigned based on treatment related pathology in the liver, kidneys, and digestive tract and increases in clinical chemistry parameters (ALT and AST).

Developmental Toxicity

In a developmental toxicity study, Bis-(N-cyclohexyl-diazeniumdioxy)-Copper (99% a.i.) in 0.5% aqueous carboxymethyl cellulose was administered to mated female Himalayan rabbits (15/dose) orally by gavage at dose levels of 0 (Control), 10, 30, or 60 mg/kg bw/day from days 7 through 19 of gestation.

Maternal effects were observed at 30 and 60 mg/kg bw/day, including significantly decreased food consumption and body weight gain throughout the treatment period. Also, high-dose dams exhibited clinical signs of toxicity (constipation and blood in bedding) and reduced mean gravid uterine weight. The maternal LOAEL is 30 mg/kg bw/day, based on decreased food consumption and body weight gain. The maternal NOAEL is 10 mg/kg bw/day.

Carcinogenicity:

In a carcinogenicity study (MRID 45687211) Bis-(N-cyclohexyl-diazeniumdioxy)-copper (88-93% a.i., batch # Reu E 7360) was administered to 50 Wistar rats/sex/dose in the diet at dose levels of 0 ©, 100 (LDT), 600 (MDT), or 3000 ppm (HDT) (equivalent to 0, 6, 33, and 169 mg/kg bw-day) for 24 months in both sexes. Copper sulfate was used as a reference standard at the same molar concentration of copper (1350 ppm) as in the high dose concentration. Information on the toxicity of copper sulfate can be found in the text.

No substance related mortality was seen in either males or females. Body weight and body weight gain were reduced by 10 and 12%, respectively, in high-dose (3000 ppm) males. Body weight and body weight gain was unaffected in other male treatment groups and all treated females. Food consumption was unaffected. There was also no apparent treatment-related effect on food efficiency. Hematology was not remarkable.

Black feces was common in rats treated with the HDT and was likely due to the copper in the compound as it also occurred in rats treated with $CuSO_4$. The forestomach was determined to be a target organ in male and female rats with increases observed in both gross pathology and non-neoplastic lesions (submucosal edema, ulceration, parakeratosis, hyperkeratosis, and hyperplasia). There was a statistically significant [$p<0.05$] dose-related increase in hemangioma of the mesenteric lymph nodes of both males and females, but pair-wise comparisons were not statistically significant. The tumor incidence was similar to the historical control incidences.

The LOAEL (non-carcinogenic) is 33 mg/kg/day [♂,♀](600 ppm), based on increases in the incidence and/or severity of the non-neoplastic lesions of the forestomach (i.e., sub-mucosal edema, ulceration, parakeratosis, hyperkeratosis, and hyperplasia). The

NOAEL (non-carcinogenic) is 5 mg/kg/day [♂,♀] (100 ppm). Cu-HDO is not considered carcinogenic.

Mutagenicity - Ames assay:

In a reverse mutation assay (Ames test), Cu-HDO was tested for mutagenicity. Standard plate tests were conducted for test concentrations ranging from 1.25 μg/plate to 5000 μg/plate, both in the presence and absence of S-9 mix. Preincubation tests were performed at test concentrations ranging from 0.6 μg/plate to 25 μg/plate, both in the presence and absence of S-9 mix. The number of revertants on each plate was counted. In both protocols, the positive controls gave the appropriate response. All testing was performed up to and greater than the toxic concentration. There was no evidence that Cu-HDO is mutagenic in the Ames test.

Mutagenicity - in vivio micronucleus assay

In a study (*in vivo*), Bis-(N-Cyclohexyldiazeniumdioxy)-Copper was used to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. The test substance was suspended in polyethylene glycol 400 (PEG 400). PEG 400 was also used as the negative control. The volume administered orally was 10 ml/kg body weight (b.w.). The bone marrow cells were collected for micronuclei analysis at 24 hours, 48 hours and 72 hours after a single dose of the test article. Ten animals (5 males, 5 females) per test group were evaluated for the occurrence of micronuclei in PCEs. 1000 PCEs/animal were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test article, the ratio between polychromatic and normochromatic erythrocytes (NCE) was determined in the same sample and reported as number of NCEs per 1000 PCEs. The following dose levels of the test article were tested: 24 h preparation interval: 50, 170 and 500 mg/kg b.w.; 48 h preparation interval: 500 mg/kg b.w.; and 72 h preparation interval: 500 mg/kg b.w. In a series of pre-experiments 500 mg/kg b.w. was estimated to be the maximum tolerated dose. The animals expressed toxic reactions. After treatment with the test article cytotoxic effects were observed at the 48 hours interval. Under the experimental conditions reported, the test article did not induce micronuclei in the test system. Based on the test results Bis-(N-cyclohexyl-diazeniumdioxy)-Copper is not considered to be mutagenic in this micronucleus assay.

Unscheduled DNA Synthesis in Mammalian Cells in Culture:

In an unscheduled DNA repair assay (MRID 456872-14), the genotoxic activity of the test substance Bis-(N-Cyclohexyldiazeniumdioxy)-Copper (89% a.i.) was investigated *in vitro* in hepatocytes obtained from male rats. Unscheduled DNA-synthesis caused by the test substance can be detected via the incorporation of ^{3}H-thymidine into DNA. The exposure time of the test chemical (6 concentration levels) was for 18 hours during which time ^{3}H-thymidine was added. The cells were washed and fixed and subsequently examined for unscheduled DNA synthesis by autoradiography. The control and positive controls showed the appropriate responses. The test material did not show genotoxic activity under the test conditions employed at doses up to the toxic level.

Human Exposure:

A MOE of 100 is adequate for occupational dermal exposure assessments. For occupational inhalation assessments, a MOE of 1000 was considered adequate by the ADTC based on the use of an oral endpoint and extrapolation to an inhalation exposure. For residential dermal an incidental oral exposure assessments, a MOE of 300 is considered adequate. An additional 3X uncertainty factor was added to these exposures scenarios because of the lack of neurotoxicity and reproductive data for Cu-HDO.

Worker Handler and Post-application Exposure:

Occupational handler exposures and risks were assessed for pressure treatment plant workers. The MOEs calculated for all handler scenarios were above the target and therefore the risks do not exceed the level of concern. Occupational postapplication exposure via the dermal route was assessed and found to be not of concern (dermal MOE of 13,131). Occupational handler dermal exposures to ***PROTECTOL CX Type A*** under real use conditions should be minimized further based on the automated mix/load/application methods employed during pressure treatment. Dermal contact is anticipated during handling of wet, freshly treated wood when trams are removed from treatment cylinders and unloaded. Based on the lack of dermal irritation/sensitization for Cu-HDO and the use of product-specified PPE to protect skin and eyes, handler exposure is further mitigated. In addition, Cu-HDO has a low vapor pressure $< 1 \times 10^{-6}$ hPa and is not anticipated to produce vapor during use applications.

Residential Post-application Exposure:

Post-application exposures and risks were assessed for children in contact with Cu-HDO residues from treated-wood structures (playsets/decks) and in contaminated soils surrounding these structures. The MOEs calculated for dermal and incidental oral ingestion of surface residues are above the target MOE (dermal MOE of 1,699 and an oral MOE of 5,058), and thus are not of concern. The MOE from the child soil ingestion scenario is also above the Agency target (oral MOE of 41,269) and thus is not of concern.

Ecological Effects:

A 96 hour acute toxicity study using the rainbow trout (*Oncorhynchus mykiss* of Bis-(N-Cyclohexyldiazeniumdioxy)(Cu-HDO in a static system (96 hours). The LC_{50} was 0.29 mg a.i./L nominal concentration, Cu-HDO is considered to be highly toxic to fish. The NOEC was determined to be 0.1 mg/L.

A static acute freshwater toxicity of Bis-(N-Cyclohexyldiazeniumdioxy) to the water flea *Daphnia magna* STRAUS. Based on nominal concentrations, the 48-hour EC_{50} was 1.1 mg/L, which classifies Cu-HDO as moderately toxic to freshwater invertebrates. Bis-(N-cyclohexyl-diazeniumdioxy)-copper - Avian Single Dose Oral LD_{50} on the Bobwhite Quail (*Colinus virginianus*).

Two avian acute oral toxicity studies were submitted in support of registration of the wood preservative use of Bis-(N-cyclohexyl-diazeniumdioxy)-copper (copper HDO).

One study was conducted with copper HDO, and the other with (N-cyclohexyl-diazeniumdioxy)-potassium (potassium-HDO), in order to demonstrate that the toxicity of the compound of interest is due to the copper ion.

The copper-HDO bobwhite quail acute study (MRID#457709-01) provides an LD50 of 189 mg ai/kg, indicating that copper-HDO is moderately toxic to birds on an acute oral basis. The NOEL was 63 mg ai/kg, due to mortality in all higher treatment levels.

The potassium-HDO bobwhite quail acute study (MRID#457709-02) provides an LD50 of 1389 mg ai/kg, indicating that potassium-HDO is slightly toxic to birds on an acute oral basis. The NOEL was 250 mg ai/kg, based on mortality in all higher treatment levels.

Environmental Fate

Environmental Fate and Transport

Hydrolysis

Bis-(N-cyclohexyl-diazeniumdioxy)-copper hydrolyzed at pH 3 at temperatures $\geq$ 40°C and at pH 7 at temperatures $\geq$ 50°C. The hydrolytic half-lives of Bis-(N-cyclohexyl-diazeniumdioxy)-copper were 1090, 305, and 60 hrs in the pH 3 solution at 40, 55, and 70°C, respectively. In the pH 7 solution, the hydrolytic half-life of the test material was 1450 hrs at 55°C. Bis-(N-cyclohexyl-diazeniumdioxy)-copper was found to be hydrolytically stable at pH 11 at all test temperatures.

Leaching - Adsorption/Desorption

The K_{oc} values for both Cu and HDO indicate that Cu-HDO has a low mobility in the soil, therefore less probability to move into surface and ground waters.

Contact Person at EPA

Adam Heyward
Antimcrobials Division (7510C)
Office of Pesticide Programs
(703) 308-6422
Email Address: heyward.adam@epa.gov

Mailing Address:

By US Mail:

Document Processing Desk
Office of Pesticide Programs (7510C)

US Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

By Courier:

Document Processing Desk
Office of Pesticide Programs (7510C)
US Environmental Protection Agency
Crystal Mall II, Room 266A
1801 S. Bell Street
Arlington, VA 22202-4501