Canadian Environmental Protection Act

Priority Substances List

Supporting Documentation - Environmental Sections

BENZIDINE

(Unedited Version)

Environment Canada   March 1993

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1.0 Identity of substance

1.1 Name of substance

Benzidine (Chemical Abstracts Service Registry Number 92-87-5) is a primary aromatic amine with a molecular formula of $C_{12}H_{12}N_2$. Synonyms for benzidine include 4,4'-bianiline, 4,4'-biphenyldiamine, 4,4'-diaminodiphenyl and 4,4'-diphenylenediamine. Its chemical structure is shown in Fig. 1.

1.2 Characteristics of substance

Benzidine is white or slightly red in the form of either crystals, powder or leaflets at room temperature (Ferber, 1978).

1.3 Analytical methodology

Benzidine in water, sediments and tissues can be determined by standard techniques of extraction and gas chromatography. Detection limits depend upon the detector used. For industrial and municipal wastewater discharges, the method detection limit by gas chromatography-mass spectrometry is 44 μg/L for a 1 L water sample (Clesceri et al., 1989).

2.0 Physical and chemical properties

Table 1 gives the physical and chemical properties of benzidine.

3.0 Sources and releases to the environment

3.1 Natural sources

No natural sources of benzidine have been identified.

3.2 Anthropogenic sources

3.2.1 Production and market trends

In recognition of the danger of benzidine to human health, many countries have prohibited or severely limited its production, use and importation. For example, as of 1982 benzidine was no longer manufactured for sale in the United States or Japan (International Agency for Research on Cancer, 1982). Although there are 254 dyes or pigments that can be produced from benzidine, in 1983 only 1 benzidine-based pigment and 16 benzidine-based dyes were in production in the United States (U.S. Agency for Toxic Substances and Disease Registry, 1988), presumably using imported benzidine. Estimated U.S. production of benzidine-based dyes was 200-300 tonnes in 1983, but there have been no imports of benzidine into the U.S.A. for such syntheses in recent years (U.S. Agency for

Toxic Substances and Disease Registry, 1988). No benzidine was produced in the U.S.A. in 1986 or 1990 (U.S. Environmental Protection Agency, 1992).

In 1982 it was believed that one or more companies in South Korea and one company in France still produced benzidine dihydrochloride as a captive dye intermediate. Other countries that were also believed to produce this intermediate at that time were Argentina, Brazil, Mexico, China, Poland, Romania and the USSR (International Agency for Research on Cancer, 1982).

3.2.2 Canadian consumption, imports and exports

Benzidine is not produced in Canada. Available data indicate sporadic importation, and for 1985 appear to be contradictory. Statistics Canada (1990) records show the following importation figures: 1980 (4 tonnes); 1981 (12 tonnes); 1982 (0.1 tonne); 1983 (0 tonne); 1984 (0 tonne); 1985 (0.3 tonne); 1986 (0 tonne); 1987 (1.9 tonnes). Imports were mainly from the U.S.A., but 2 tonnes were also imported in 1981 from Japan. The destinations in Canada are not known, nor the ultimate use. In 1988 Statistics Canada changed the system it used for statistical reporting purposes. The new "Harmonized System" does not have a specific category for benzidine. Consequently, Statistics Canada importation data for benzidine are not available from 1988 onwards (Brien, 1992). Benzidine was not reported on the CEPA Domestic Substances List (Canada Department of the Environment, 1990), which designates chemicals imported or produced in quantities greater than 100 kg and for commercial manufacturing purposes in Canada in the period 1984-1986. Benzidine and its salts were the subjects of a notice in *The Canada Gazette* under CEPA Subsection 16.1 requiring any person who, in 1990, was engaged in any commercial activity involving more than 10 kg, whether alone or in a mixture, to provide information on quantities, Canadian suppliers, use patterns, Canadian customers, losses, presence in products and human exposure (Canada Department of the Environment, 1991a). No company reported the use of benzidine in 1990 (Canada Department of the Environment, 1991b). It is concluded that benzidine is not currently produced in, or imported into, Canada.

3.2.3 Manufacturing processes

Benzidine can be produced through the alkaline reduction of nitrobenzene in either one stage to hydrazobenzene, or stepwise, changing conditions at the azoxybenzene or azobenzene stages. Zinc or sodium amalgams, iron and the electrolytic cathode have been used as reducing agents; however, powdered zinc or methanol was preferred. The hydrazobenzene was separated from the reaction mass and rearranged to benzidine and several by-products with hydrochloric acid (Ferber, 1978).

3.2.4 Uses

Benzidine and several of its derivatives have been of commercial value primarily as intermediates in the manufacture of dyes and pigments. They were also used in minor amounts in inorganic qualitative and quantitative analysis for the determination of various cations and anions, in various organic analyses, in the determination of blood in forensic and clinical medicine, and as stains in microscopy (Ferber, 1978; Budavari, 1989). It is assumed that benzidine was used for similar purposes in Canada.

3.2.5 Releases

No data were identified on the environmental release of benzidine in Canada. Benzidine can enter the environment from any stage in the production, storage, transport, use and disposal of benzidine itself or benzidine-containing materials (such as dyes and pigments), or possibly by atmospheric and water-borne transport from other countries. In water, benzidine can be produced by the photodegradation of 3,3'-dichlorobenzidine (Banerjee et al., 1978). No information was identified on the extent to which benzidine may be formed and released into the environment by this mechanism. Approximately 100 tonnes of 3,3'-dichlorobenzidine were imported into Canada in 1989 (Statistics Canada, 1990). 3,3'-Dichlorobenzidine is on the CEPA Priority Substances List.

4.0 Environmental transport, transformation and concentrations

4.1 Transport and distribution between and within media

In general, partitioning to sediment or soil is expected to be the main pathway of distribution of benzidine in the environment (Sanders, 1979; Callahan et al., 1979; Howard, 1989).

4.1.1 Water

The pK_a values for benzidine are 4.7 and 3.6 (Weast et al., 1984). Therefore, dissolved benzidine will be present almost entirely as the free base in most natural waters. Volatilization from water is not expected to be significant for such an involatile chemical with a Henry's Law constant value of 0.2 torr L mole^{-1} (Smith et al., 1980).

4.1.2 Air

Because of the low volatility of benzidine, significant concentrations are not expected to be found in air. Consequently, it is not expected to be a greenhouse gas, to contribute to stratospheric ozone depletion, or to contribute to the formation of ground level ozone.

4.1.3 Soil

The relatively low log K_{ow} value of 1.34 for benzidine (Lu et al., 1977) suggests only a modest potential for binding to the organic part of sediment and soil. However, benzidine can be strongly bound to other soil constituents (e.g., Graveel et al., 1985). Zierath et al. (1980) noted that benzidine adsorption to soil or sediment was largely controlled by the pH of the aqueous phase, and highly correlated with the surface area of the soil or sediment. It has also been shown that the protonated forms of benzidine are more strongly bound to estuarine colloidal organic matter than is the neutral form (Means and Wijayaratne, 1987). Graveel et al. (1986) found no volatilization of benzidine from various soils over a 1 year period. Sorption experiments indicated that benzidine was strongly retained by all soils studied, and that minimal amounts of sorbed benzidine were desorbed by a neutral salt.

4.1.4 Biota

The relatively low log K_{ow} value of 1.34 for benzidine (Lu et al., 1977) suggests only a modest potential for bioaccumulation. Lu et al. (1977) observed 3-d bioconcentration factors of 55 for mosquito fish (Gambusia affinis), 293 for Daphnia magna, 456 for mosquito larva (Culex pipiens quinquefasciatus), 645 for snail (Physa sp.) and 2617 for a filamentous green alga (Oedogonium cardiacum). Freitag et al. (1985) observed a 5-d bioaccumulation factor for benzidine in activated sludge of 1200, a 1-d bioaccumulation factor in algae (Chlorella fusca) of 850, and a 3-d bioaccumulation factor in fish (golden ide, Leuciscus idus melanotus) of 83.

4.2 Transformation

In general, oxidation, photochemical transformation and microbial degradation (with slow mineralization) are expected to be the main pathways of transformation of benzidine in the environment (Sanders, 1979; Callahan et al., 1979; Howard, 1989). Benzidine itself is not expected to be persistent (with overall half-lives in water, soil and air less than a few weeks) since strongly bound residues in soils and sediments are formed quickly, but the biological availability of such residues has not been investigated. In addition, products of degradation have not been well characterized.

4.2.1 Biodegradation

Benzidine is relatively easily biodegraded in sewage treatment plants. Benzidine at 20 mg/L was readily degraded by activated sewage sludge (Baird et al., 1977). It had some inhibitory effect on the respiration of organisms in activated sludge even while

being degraded, which suggested that a metabolite or metabolites may be responsible for the observed toxicity. Tabak and Barth (1978) showed that acclimated extended aeration sludges could completely oxidize continuous doses of 1 mg/L benzidine in less than two weeks (controls indicated that the removal was not simply adsorption). Less complete oxidation occurred at higher dosage levels and intermediate oxidation products began to accumulate in the system. One of the products of chlorination of benzidine in a sewage treatment plant appeared to be a polymer (Ontario Ministry of the Environment, 1982). No information was found on the biodegradation of benzidine in natural waters. However, the Syracuse Research Corporation (1989) estimated half-lives for the biodegradation of benzidine in surface water and groundwater of 31-192 h and 96-384 h, respectively.

In soil benzidine is strongly bound and subject to microbial degradation (e.g., Graveel et al., 1985). Lu et al. (1977) found that 21% of benzidine remained in a Drummer soil after 4 weeks. Methylated and acetylated metabolites were among those found. Graveel et al. (1986) studied the decomposition of ^{14}C-labelled benzidine in soil by monitoring $^{14}CO_2$ evolution over a 10-12 month period. More benzidine decomposition was observed in non-sterile soils than in sterile soils. After 1 year of incubation, 8-12% of the original benzidine was evolved as CO_2. Almost all the rest was residual activity in the soil. Soil environmental conditions had only a limited effect on the decomposition of benzidine. Modifying soil environmental conditions such as water content, temperature or addition of metabolizable organic substrates did not markedly affect the short-term rate of benzidine degradation to CO_2 in soils.

4.2.2 Abiotic degradation

In water, although both oxidation (by hydroperoxyl radical or molecular oxygen) and photolysis may be significant processes, the most important process controlling the fate of benzidine appears to be oxidation by naturally-occurring metal cations, with a half-life of the order of a few hours (Callahan et al., 1979). Benzidine is very rapidly oxidized by iron (III) and several other naturally-occurring cations (Lahav and Raziel, 1971) which are found in natural waters as solvated cations, complexes of fulvic acids, and as parts of the structure of microcrystalline clays (Gould, 1968). Little is known of the products of oxidation. Adsorption to clays with subsequent oxidation is very fast. Benzidine is oxidized by the iron (III) in montmorillonite clay to an intensely blue-coloured benzidine radical-cation (Tennakoon et al., 1974a,b). Although the environmental fate of such complexes is not known with certainty, by analogy with other relatively easily oxidized semiquinone-like structures, it is assumed that further oxidation would be facile (Callahan et al., 1979). However, this has not been demonstrated.

In air benzidine is expected to photooxidize fairly rapidly, with a half-life < 1 d (Howard, 1989; Syracuse Research Corp., 1989).

The absorption maximum for benzidine in aqueous ethanol is at 287 nm with significant absorption in the ultraviolet region extending to 340 nm (Bilbo and Wyman, 1953), which indicates the possibility of sunlight photolysis (*i.e.*, absorption at λ > 290 nm). However, there are no studies on the sunlight photolysis of benzidine. Larson and Zepp (1988) have shown that light of wavelength 313 nm directly photolyzed benzidine, with a half-life of < 60 min in the pH range 4.2 - 11.9. In addition, carbonate radicals generated by the photolysis of hydrogen peroxide at 313 nm in aqueous sodium bicarbonate (pH 8.3) or carbonate (pH 11.6) solution can react with benzidine. No products were identified in this study. Lu *et al.* (1977) found that the photolysis half-life of benzidine in methanol under 254 nm light was about 2 h. Within 0.5 h the colour of the solution changed from clear to red to brown. After 4 h 23% of the benzidine remained, with a number of unidentified degradation products. The formation of unidentified and polar degradation products was proportional to the length of the time of exposure to light. Freitag *et al.* (1985) found that irradiation of benzidine on silica gel for 17 h with 290 nm UV light resulted in the mineralization of 41% of the radiolabel to $^{14}CO_2$.

4.3 Environmental concentrations

4.3.1 Surface water

Benzidine was not detected (detection limit 2 μg/L) in 34 samples of raw water and 1015 samples of treated drinking water obtained in Alberta between 1987 and 1991 (Alberta Environment, 1992). No other information was identified on the occurrence of benzidine in surface water in Canada. Benzidine was reported in the Sumida River in Tokyo in 1964 at concentrations around 0.2 mg/L (Takemura *et al.*, 1965). Its presence was ascribed to dye and pigment factories along the river. However, the identification of benzidine should be regarded as tentative because it was made by thin layer chromatography, which does not have the resolving power of gas or liquid chromatography. The authors reported that another compound, dianisidine, had the same Rf value as benzidine under the TLC conditions used. Benzidine has been detected (but not quantitated) in 1.1% of 1235 industrial effluent samples, and 0.1% of 879 natural water samples in the United States (Staples *et al.*, 1985).

4.3.2 Groundwater

No information was identified on the occurrence of benzidine in groundwater in Canada. In the United States, benzidine has been

found at 1 μg/L in groundwater near a pit where benzidine and 3,3'-dichlorobenzidine wastes were dumped (Rice and Kissinger, 1982).

4.3.3 Air

No information was identified on the occurrence of benzidine in air in Canada or elsewhere.

4.3.4 Soil and sediment

No information was identified on the occurrence of benzidine in soil in Canada or elsewhere. No information was identified on the occurrence of benzidine in sediment in Canada. Benzidine was not detected in any of 3240 sediment samples in a survey in the U.S.A. (Staples *et al.*, 1985).

4.3.5 Biota

No information was identified on the occurrence of benzidine in biota in Canada. In the United States it was not detected in any of 110 biota samples (Staples *et al.*, 1985).

5.0 Population exposures

Refer also to supporting documentation from Health and Welfare Canada.

5.1 Exposures of wildlife populations

No data were identified for the exposure of benzidine to wild mammals, terrestrial organisms, birds, sediment or soil biota in Canada or elsewhere.

5.2 Exposures of the general human population

Refer to supporting documentation from Health and Welfare Canada.

6.0 Toxicokinetics and metabolism

Refer to supporting documentation from Health and Welfare Canada.

7.0 Mammalian toxicology

Refer to supporting documentation from Health and Welfare Canada.

8.0 Effects on humans

Refer to supporting documentation from Health and Welfare Canada.

9.0 Effects on the ecosystem

9.1 Aquatic toxicity

Table 2 shows those acute toxicity data for benzidine to aquatic organisms which were identified. They are primarily for fish. Acutely lethal concentrations ranged from a 96-h LC_{50} value, as well as a 72-h LC_{50} value, of 2.5 mg/L for the red shiner, *Notropis lutrensis* (Jones, 1980) to a 96-h LC_{50} value of 64 mg/L for sheepshead minnow (*Cyprinodon variegatus*) (Martin, 1982).

Baird *et al.* (1977) noted that benzidine at 20 mg/L had some inhibitory effect on the respiration of organisms in activated sludge even while being degraded, which suggested that a metabolite or metabolites may have been responsible for the observed toxicity.

Some data were identified on the chronic toxicity of benzidine to aquatic organisms. Pliss and Khudoley (1975) found that benzidine at 30 mg/100 g dry weight in food produced no tumours in guppies (*Poecilia reticulata*) or zebra fish (*Danio rerio*) over 56 weeks. However, toxic effects on the liver were noted, such as foci of necrosis, fatty dystrophy and diffuse hyperplasia of the hepatocytes. Khudoley (1977) observed tumours of the liver and haemopoetic system of grass frogs (*Rana temporaria*) administered (subcutaneously or *per os*) 1-2.5 mg benzidine per week for 38 weeks. Martin (1982) found large fibrotic regions in livers of sheepshead minnows (*Cyprinodon variegatus*) exposed to 1 mg/L benzidine after 25-29 weeks. Above 50 mg/L the following abnormalities were observed: tubed heart syndrome with distended pericardia, poor circulation, sparse distribution of melanophores around yolks, inability to hatch, abnormal head morphology, scoliosis and faint red blood cell pigmentation.

9.2 Terrestrial toxicity

No data were identified for the acute or chronic toxicity of benzidine to wild mammals, terrestrial organisms, birds, sediment or soil biota.

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Table 1. Chemical and physical properties of benzidine

Property		Reference
molecular formula	$C_{12}H_{12}N_2$	
molecular weight	184.24	
appearance	white or slightly red crystals, powder or leaflets	Ferber (1978)
melting point	128 °C	Weast et al. (1984)
boiling point	400 °C	Weast et al. (1984)
density	1.250 g/cm^3	Sax (1968)
vapour pressure	5×10^{-4} mm Hg at 25 °C	Mabey et al. (1982)
aqueous solubility	500 mg/L at 25 °C	Bowman et al. (1976)
pK$_a$	4.66, 3.57	Weast et al. (1984)
log (octanol-water partition coefficient)	1.34	Lu et al. (1977)

Table 2. Acute toxicity of benzidine to aquatic organisms[*]

Compound	Species	Size / age	Static / flow-through	Temp. °C	Hardness mg/L	pH	Response	Conc. mg/L	Nominal / measured	Reference
	bacteria Photobacterium phosphoreum		stat	15			5-30 min IC_{50} (light output)	44-65	n	Kaiser (1992)
	flagfish Jordanella floridae		stat				24-h LC_{50}	> 50	n	Jones (1980)
							48-h LC_{50}	32.5	n	Jones (1980)
							72-h LC_{50}	25	n	Jones (1980)
							96-h LC_{50}	16.2	n	Jones (1980)
	fathead minnow Pimephelas promelas		stat				24-h LC_{50}	> 20	n	Jones (1980)
							48-h LC_{50}	> 20	n	Jones (1980)
							72-h LC_{50}	> 20	n	Jones (1980)
							96-h LC_{50}	> 20	n	Jones (1980)
	emerald shiner Notropis atherinoides		stat				96-h LC_{50}	5	n	Jones (1980)
	bluegill sunfish Lepomis macrochirus		stat				96-h LC_{50}	15	n	Jones (1980)
	red shiner Notropis lutrensis		stat				24-h LC_{50}	> 20	n	Jones (1980)
							48-h LC_{50}	10	n	Jones (1980)
							72-h LC_{50}	2.5	n	Jones (1980)

Table 2 cont'd

Compound	Species	Size / age	Static / flow-through	Temp. °C	Hardness mg/L	pH	Response	Conc. mg/L	Nominal / measured	Reference
							96-h LC_{50}	2.5	n	Jones (1980)
	lake trout Salvelinus namaycush		stat				24-h LC_{50}	8.7	n	Jones (1980)
							48-h LC_{50}	5	n	Jones (1980)
							72-h LC_{50}	4.4	n	Jones (1980)
							96-h LC_{50}	4.4	n	Jones (1980)
	rainbow trout Oncorhynchus mykiss		stat				24-h LC_{50}	> 20	n	Jones (1980)
							48-h LC_{50}	14.1	n	Jones (1980)
							72-h LC_{50}	10	n	Jones (1980)
							96-h LC_{50}	7.4	n	Jones (1980)
	scud Gammarus pseudolimnaeus		stat				24-h LC_{50}	> 20	n	Jones (1980)
							48-h LC_{50}	> 20	n	Jones (1980)
							72-h LC_{50}	> 20	n	Jones (1980)
							96-h LC_{50}	> 20	n	Jones (1980)
	sheepshead minnow Cyprinodon variegatus		stat				96-h LC_{50}	64	n	Martin (1982)

*Abbreviations used: IC_{50} - concentration which effectively inhibits 50% of function/growth; LC_{50} - concentration which kills 50% of test organisms.

Figure 1.   Chemical structure of benzidine.

BENZIDINE