Canadian Environmental Protection Act

Priority Substances List
Assessment Report

3,3'-Dichlorobenzidine

Government of Canada / Gouvernement du Canada
Environment Canada / Environnement Canada
Health Canada / Santé Canada

Canada

CANADA'S GREEN PLAN

PRIORITY SUBSTANCES LIST ASSESSMENT REPORT

3,3'-DICHLOROBENZIDINE

Government of Canada
Health and Welfare Canada
Environment Canada

Also available in French
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Synopsis

The substance 3,3'-dichlorobenzidine is not produced in Canada, but amounts of up to approximately 100 tonnes are imported each year. It is used in Canada primarily for the production of pigments for printing inks, textiles, paints, plastics and crayons. Entry of this substance into the Canadian environment is expected to occur in association with this industrial activity, although this cannot be confirmed. 3,3'-Dichlorobenzidine is not expected to be persistent in the environment, with overall half-lives in water, soil and air being less than a few weeks.

No information was identified on the concentrations of 3,3'-dichlorobenzidine in Canadian air, surface waters, ground water, biota, soil or sediment. Therefore, concentrations of 3,3'-dichlorobenzidine have been predicted for the various media to which humans and other organisms may be exposed. The predicted concentration for surface water is approximately 11 orders of magnitude lower than the concentration that induces adverse effects in bacteria, the most sensitive aquatic species identified.

Due to its relatively low volatility, very short persistence and low concentrations in the atmosphere, 3,3'-dichlorobenzidine is not expected to contribute to the greenhouse effect, depletion of the ozone layer or the formation of ground-level ozone.

PA though available information is considered inadequate to assess the carcinogenicity of 3,3'-dichlorobenzidine in humans, it has been shown to cause cancer in a number of animal species. 3,3'-Dichlorobenzidine is, therefore, considered to be a "non-threshold toxicant" (i.e., a substance for which there is believed to be some chance of adverse effect at any level of exposure). For such substances, estimated exposure is compared to quantitative estimates of cancer potency to characterize risk and provide guidance in establishing priorities for control options analysis and assessment of the adequacy of specific control measures to protect human health.

Based on these considerations, the Ministers of Environment and National Health and Welfare have concluded that the predicted concentrations of 3,3'-dichlorobenzidine in the environment in Canada do not constitute a danger to the environment or to the environment upon which human life depends, but may constitute a danger to human life and health. Therefore, 3,3'-dichlorobenzidine is considered to be "toxic" as defined under section 11 of the *Canadian Environmental Protection Act* (CEPA).

1.0 Introduction

CEPA requires the Ministers of the Environment and of National Health and Welfare to prepare and publish a Priority Substances List that identifies substances, including chemicals, groups of chemicals, effluents and wastes, that may be harmful to the environment or constitute a danger to human health. The Act also requires both Ministers to assess these substances and determine whether they are "toxic" as interpreted in section 11 of the Act, which states:

> "...a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions
>
> (*a*) having or that may have an immediate or long-term harmful effect on the environment;
>
> (*b*) constituting or that may constitute a danger to the environment on which human life depends; or
>
> (*c*) constituting or that may constitute a danger in Canada to human life or health."

Substances assessed as "toxic" according to section 11 may be placed on the List of Toxic Substances (Schedule I of the Act). Consideration can then be given to developing guidelines, codes of practice or regulations to control any aspect of their life cycle, from the research and development stage through manufacture, use, storage, transport and ultimate disposal.

The assessment of whether 3,3'-dichlorobenzidine is "toxic", as interpreted under CEPA, was based on the determination of whether it enters or is likely to enter the Canadian environment in a concentration or quantities or under conditions that could lead to exposure of humans or other biota to levels that could cause harmful effects.

Data relevant to the assessment of whether 3,3'-dichlorobenzidine is "toxic" under CEPA were identified through evaluation of existing review documents (ATSDR, 1989; U.S. EPA, 1980; 1988; IARC, 1982), as well as an unpublished review of the environmental behaviour and health effects of this substance prepared under contract by Cambridge Environ-mental Inc. (Croy and DeVoto, 1990), supplemented with information from published reference texts and literature identified through on-line searches of various databases (HSDB, RTECS, IRIS, CCRIS, TOXLINE, TOXLIT, ENVIROLINE, CHEMICAL ABSTRACTS, BIOLOGICAL ABSTRACTS, ELIAS, AQUAREF, MICROLOG, CODOC). Information was also obtained from the CEPA Domestic Substances List, a CEPA subsection 16(1) Notice and from Statistics Canada. In addition, a number of provincial authorities were requested to provide any available information on the levels of 3,3' -dichlorobenzidine in the drinking water in their provinces. Data relevant to the assessment of the effects of 3,3'-dichlorobenzidine on the environment and human health obtained after April, 1992 and October, 1992, respectively, were not considered for inclusion.

Review articles were consulted where appropriate; however, all original studies that form the basis for determining whether 3,3'-dichlorobenzidine is "toxic" under CEPA have been critically evaluated by the following staff of Health and Welfare Canada (human exposure

and effects on human health) and Environment Canada (entry and environmental exposure and effects):

R.G. Liteplo (Health and Welfare Canada)
R.J. Maguire (Environment Canada)
M.E. Meek (Health and Welfare Canada)

Quantitative estimates of carcinogenic potency were provided by M. Walker of Health and Welfare Canada.

A summary of technical information critical to the assessment, and which is presented in greater detail in an unpublished Supporting Document, is presented in Section 2. The assessment of whether 3,3’-dichlorobenzidine is "toxic" is presented in Section 3.

The environmental sections of this report were reviewed by Drs. C.M. Auer and W.H. Farland of the U.S. Environmental Protection Agency. Sections related to the assessment of effects on human health were approved by the Standards and Guidelines Rulings Committee of the Bureau of Chemical Hazards of Health and Welfare Canada. The entire Assessment Report was reviewed and approved by the Environment Canada/Health and Welfare Canada CEPA Management Committee.

Copies of this Assessment Report and the Supporting Document are available upon request from:

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2.0 Summary of Information Critical to Assessment of "Toxic"

2.1 Identity, Properties, Production and Uses

3,3'-Dichlorobenzidine is a chlorinated primary aromatic amine with the Chemical Abstracts Service registry number 91-94-1 and the molecular formula $C_{12}H_{10}N_2C1_2$. Synonyms for 3,3' -dichlorobenzidine include 4,4'-diamino-3,3'-dichlorobiphenyl, *o,o'*-dichlorobenzidine, 3,3'-dichlorobiphenyl-4,4'-diamine and 3,3'-dichloro-4,4'-diamino(1,1-biphenyl). 3,3' -Dichlorobenzidine is usually available as the dihydrochloride salt, 3,3'-dichlorobenzidine dihydrochloride ($C_{12}H_{10}N_2C1_2 \cdot 2HCl$). 3,3' -Dichlorobenzidine is a grey to purple crystalline solid (Banerjee *et al*., 1978) with a melting point of 133^0C (Ferber, 1978). It has a relatively low vapour pressure ($1.33x10^{-3}$ Pa at 22^0C) (Mabey *et al*., 1982), a low water solubility (4 mg/L at 20^0C as the dihydrochloride salt) (Banerjee *et al*., 1978) and a log n-octanol/water partition coefficient of 3.02 (Callahan *et al*., 1979).

3,3'-Dichlorobenzidine can be produced by the alkaline reduction of o-chloronitro-benzene and rearrangement of the resulting hydrazo compound (Ferber, 1978).

3,3'-Dichlorobenzidine is not produced in Canada (Environment Canada, 1980; 1991a; 1991b) but has been imported on a regular basis (Statistics Canada, 1990). The reported volumes of importation figures since 1986 are as follows: 1986 (21 tonnes); 1987 (60 tonnes); 1988 (not available); and 1989 (109 tonnes). The destinations in Canada are generally not known, except for the year 1989, in which 80 of the 109 tonnes imported went to Ontario.

In Canada, 3,3'-dichlorobenzidine (and some of its derivatives) are used primarily as intermediates in the manufacture of pigments for printing inks, textiles, paints, plastics and crayons (Environment Canada, 1991a; 1991b). It can also be used as a curing agent in the synthesis of polyurethane elastomers, and in the analytical determination of gold (Ferber, 1978; Budavari, 1989).

2.2 Entry into the Environment

3,3'-Dichlorobenzidine can enter the Canadian environment from any stage in the production, storage, transport, use and disposal of 3,3'-dichlorobenzidine-containing materials in Canada, or possibly by atmospheric and water-borne transport from other countries. While there are no supporting data, the largest releases would likely be through direct emissions from plants that manufacture 3,3'-dichlorobenzidine-containing materials, and from the degradation of 3,3'-dichlorobenzidine-containing pigments. In polymers, the residual 3,3'-dichlorobenzidine content would be generally so low that loss from this source would not constitute a significant route of entry. For those 3,3'-dichlorobenzidine-containing materials disposed by incineration, it is likely that this process would destroy unreacted 3,3'-dichlorobenzidine.

Data on the release of 3,3'-dichlorobenzidine into the Canadian environment were not identified. Total industrial emissions of 3,3'-dichlorobenzidine into the environment in the United States in 1988 were estimated to be 6 tonnes (U.S. EPA, 1990). If it is assumed that 3,3'-dichlorobenzidine use in the United States amounted to $5x10^3$ tonnes in 1988, losses would be estimated as 0.1% of total use. Based on data of Canadian uses in 1989, losses would be estimated as 0.1 tonne.

2.3 Exposure-related Information

2.3.1 *Fate*

Owing to the relatively short half-life (i.e., less than a few weeks) of 3,3'-dichlorobenzidine in water, soil and air, this substance is not expected to persist in the environment. Photolysis, photooxidation, partitioning to soil, biota and sediment, and slow microbiological degradation are expected to be the main pathways of distribution and transformation of 3,3'-dichlorobenzidine in the environment (Sikka *et al*., 1978; Callahan *et al*., 1979; Howard, 1989).

The half-life for volatilization of 3,3' -dichlorobenzidine from surface water (1 m deep, flowing at 1 m/s, with wind velocity of 3 m/s, at 20^0C) to the atmosphere was estimated to be 72 days (Thomas, 1982). In most natural waters, 3,3'-dichlorobenzidine will be present almost entirely as the free base, based on the pK_a's of 4.5 and 3.3 for this substance (Korenman and Nikolaev, 1974). In water, 3,3'-dichlorobenzidine may be degraded by photooxidation (Mill and Mabey, 1985), photolysis (Banerjee *et al*., 1978) and biodegradation (Sikka *et al*., 1978). In an aqueous medium, 3,3' -dichlorobenzidine is very rapidly photodegraded by sunlight, producing monochlorobenzidine, benzidine and a number of brightly coloured, water-insoluble compounds (Banerjee *et al*., 1978); the half-life of 3,3'-dichlorobenzidine in water is less than 10 minutes. The photodegradation of 3,3'-dichlorobenzidine may involve its sequential dechlorination, yielding benzidine.

3,3'-Dichlorobenzidine is fairly resistant to degradation by naturally occurring aquatic microbial communities (Sikka *et al*., 1978) and by a sewage sludge inoculum (Brown and Laboureur, 1983) over a 4-week period. Half-lives of 4-26 weeks and 16-101 weeks have been estimated for the biodegradation of 3,3'-dichlorobenzidine in surface water and anaerobic ground water, respectively (Syracuse Research Corp., 1989).

3,3'-Dichlorobenzidine is strongly bound to soil and is, therefore, highly immobile. The formation of covalent linkages between 3,3' -dichlorobenzidine and soil humic components may be the predominant fate of this substance in the soil. 3,3'-Dichlorobenzidine quickly became unextractable in soil after application in municipal sewage sludge, with none recovered by organic solvent extraction after 4 months (Demirjian *et al*., 1987). In soil, 3,3'-dichlorobenzidine is mineralized very slowly under aerobic and anaerobic conditions (Boyd *et al*., 1984; Chung and Boyd, 1987). The half-life for the aerobic degradation of 3,3'-dichlorobenzidine in soil has been estimated to range from 4 to 26 weeks (Syracuse Research Corp., 1989). 3,3'-Dichlorobenzidine may also be oxidized by metal ions such as iron (III) present in clay. The products formed by the degradation of 3,3'-dichlorobenzidine in soil have not been identified.

Half-lives of 1.5-5 minutes and 0.9-9 hours have been estimated for the photolysis and photooxidation of 3,3'-dichlorobenzidine in air, respectively (Syracuse Research Corp., 1989).

2.3.2 *Concentrations*

No data on the levels of 3,3'-dichlorobenzidine in drinking water, surface water, ground water, biota, soil or sediment, foodstuffs or products containing pigments or dyes derived from this substance within Canada were identified.

No information on the levels (or occurrence) of 3,3'-dichlorobenzidine in drinking water or foodstuffs in the United States was identified. In the United States, 3,3'-dichlorobenzidine was not found in the ambient air surrounding two dye-stuff production facilities, at the detection limits of 0.1 and 5 mg/m^3 (Narang *et al*., 1982; Riggin *et al*., 1983, both cited in ATSDR, 1989). 3,3'-Dichlorobenzidine was not detected in samples of biota or sediment within the United States, although it was detected (but not quantitated) in 1% of 1 239 samples of industrial effluent and 0.1% of 863 samples of natural water (Staples *et al*., 1985).

3,3'-Dichlorobenzidine can accumulate in aquatic biota. Appleton and Sikka (1980) reported a bioconcentration factor of approximately 500 for bluegill sunfish (Lepomis macrochirus), based on a study in which the fish were exposed to 5 or 100 µg/L [^{14}C]3,3'-dichlorobenzidine; equilibria were achieved within 96-168 hours. Freitag *et al*. (1985) reported a 3-day bioaccumulation factor in fish (golden orfe, *Leuciscus idus melanotus*) of 610, a 5-day bioaccumulation factor in activated sludge of 3 100, and a 1-day bioaccumulation factor in algae (*Chlorella fusca*) of 940.

Owing to the absence of data on fate and concentrations in the Canadian environment, the likely distribution of 3,3'-dichlorobenzidine in the environment was predicated based on the level III fugacity computer model of Mackay and Paterson (1991) applied to southern Ontario using worst-case assumptions. It was assumed that all the 3,3'-dichlorobenzidine imported into Canada in 1989 was for use only in southern Ontario, and that it would be released into the water at a rate of 0.05 mol/h (based on a loss of 0.1 tonne (see Section 2.2)). The results indicated that, at steady-state, the proportion of released 3,3' -dichlorobenzidine found in the environment would be as follows: air (< 0.001%); surface water (99.75%); sediment (0.254%); and soil (< 0.001%). This would result in predicted steady-state concentrations of $7.6x10^{-16}$ $µg/m^3$ in air, $3.44x10^{-7}$ µg/L in water, $1.1xl0^{-16}$ µg dry weight in soil and $3.1x10^{-12}$ µg/g dry weight in sediment. Since this model does not address the possibility of formation of bound residues in sediment, the concentrations in sediment may be underestimated, while the concentrations in water may be overestimated.

2.4 Effects-related Information

2.4.1 *Experimental Animals and* In Vitro

It is generally believed that the hepatic metabolism of 3,3'-dichlorobenzidine in rodents (i.e., rats) involves its oxidation to highly reactive N-oxygenated intermediates, by the microsomal cytochrome P_{450d} and flavin monooxygenase enzyme complexes (reviewed in Iba, 1990). Although the identity of the 3,3'-dichlorobenzidine derived N-oxygenated intermediates has not been unequivocally identified, they are believed to be responsible for the mutagenic and genotoxic effects (i.e., related to DNA binding) of 3,3'-dichlorobenzidine in bacterial and mammalian systems (Iba, 1990; Iba and Thomas, 1988).

No quantitative information on the metabolism of 3,3'-dichlorobenzidine in humans was identified; however, small amounts (approximately 1-2%) of free and (glucuronide)-conjugated N-hydroxyacetyl-derivatives of 3,3' -dichlorobenzidine were excreted in the urine of volunteers administered (orally) dichlorobenzidine (Belman *et al*., 1968). 3,3'-Dichloro-N-acetylbenzidine and 3,3'-dichloro-N,N'-diacetylbenzidine, as well as conjugated metabolites (the identities of which were not confirmed), have been detected in the urine of rats adminis-

tered 3,3'-dichlorobenzidine orally (Tanaka, 1981; Hsu and Sikka, 1982). The covalent binding of 3,3'-dichlorobenzidine (metabolites) to haemoglobin (Birner *et al*., 1990), hepatic lipids (Iba and Lang, 1988; Iba *et al*., 1990) and DNA in the intestinal and bladder epithelium or liver (Ghosal and Iba, 1990), has been reported in experimental animals (i.e., rodents) exposed in vivo.

The LD_{50} for the oral administration of 3,3'-dichlorobenzidine to rats, either as the free base or dihydrochloride salt, Was reported to be 7 070 and 3 820 mg/kg b.w., respectively (Gerarde and Gerarde, 1974). Reported LD_{50} values for the oral administration of 3,3'-dichlorobenzidine to male and female mice were 676 and 488 mg/kg b.w., respectively (Gaines and Nelson, 1977, cited in U.S. EPA, 1980).

No compound-related histopathological effects were observed in Sprague-Dawley rats (n = 20) administered 3,3'-dichlorobenzidine dihydrochloride (30 mg, by gastric intubation) once every 3 days over a 30-day period, and subsequently maintained for a further 9 months; 5/132 animals administered vehicle alone (negative control) had mammary tumours (carcinomas and fibroadenomas), while 100% of surviving animals (n = 29) administered a single dose of dimethylbenz[a]anthracene (positive control) had mammary tumours (carcinomas and fibroadenomas) (Griswold *et al*., 1968).

The incidence of mammary carcinomas in ChR-CD rats administered diets containing 0 or 1 000 ppm (0.1% w/w) 3,3'-dichlorobenzidine for up to 488 days was 3/44 and 26/44, and 0/44 and 7/44, in the females and males, respectively (Stula *et al*., 1975); in male rats, the incidence of Zymbal gland tumours was 0/44 and 8/44, and the incidence of granulocytic leukemias was 2/44 and 9/44, in the control and 3,3'-dichlorobenzidine-exposed animals, respectively.

Stula *et al*. (1978) administered 3,3'-dichlorobenzidine (100 mg in gelatin capsules) 3 times/week for 6 weeks, and then 5 times/week for as long as 7.1 years (the mean dose for all exposed animals was approximately 10.4 mg/kg b.w./exposure) to a group of six female beagle dogs; six unexposed animals (controls) were sacrificed after 8.3 to 9.0 years on study. The incidence of bladder carcinomas and hepatocellular tumours in 3,3'-dichlorobenzidine-exposed animals (that survived longer than 6.6 years) was 5/5 and 4/5, respectively. No liver or bladder tumours were found in the unexposed (control) animals, although 4/6 were reported to have developed mammary carcinomas (a result attributed to the greater age of the control animals rather than to an effect related to 3,3'-dichlorobenzidine) (Stula et al., 1978).

In the first reported study on the carcinogenicity of 3,3'-dichlorobenzidine in rats, tumours (in the Zymbal and mammary glands, bladder and the hematopoetic system) were observed in 79% (23/29 male and female Rappolovo rats) of animals surviving at the time (not specified) the first tumours were detected following administration of a diet containing 3,3'-dichlorobenzidine (0.5 to 1.0 mL of a 4.4% solution) for 6 days/week for 12 months; no tumours were detected in a group of 130 controls (Pliss, 1959). Subsequently, Pliss (1963) reported that 86% of Rappolovo rats (number not stated) administered a diet containing an amount (not clearly specified) of 3,3'-dichlorobenzidine for 10-13 months had tumours (in bone, mammary glands, bladder, "etc."), while only one of 50 rats in a control group had developed a tumour. The studies by Pliss (1959; 1963) were limited by inadequate protocols (e.g., small group sizes, lack of appropriate controls) and incomplete reporting of data.

The incidence of "hepatomas" in male ICR/JC1 mice administered a diet containing 0 or 0.1% (w/w) (1 000 ppm) 3,3'-dichlorobenzidine for 12 months was 2/21 and 18/18 ($p < 0.001$), in the control and 3,3'-dichlorobenzidine-exposed groups, respectively (Osanai, 1976). In the first reported study on the carcinogenicity of 3,3'-dichlorobenzidine in mice, in which male and female strain D mice were administered 3,3'-dichlorobenzidine (0.1 mL of 1.1% solution) in the diet for 10 months, amongst 18 animals surviving at the time at which the first tumour appeared (i.e., after 18.5 months), 2 animals had hepatomas and 2 had hemangiomas; however, information on the nature of, and results in, an appropriate group of controls was not presented (Pliss, 1959).

Saffiotti *et al*. (1967) reported no carcinogenic or histopathological effects in the bladder of male and female Syrian Golden hamsters administered a diet containing 0.1% (w/w) (1 000 ppm) 3,3'-dichlorobenzidine (technical grade; 40% dihydrochloride and 60% free base) for their entire lives, compared to unexposed controls. In a subsequent publication (Sellakumar *et al*., 1969), however, these authors reported the administration of a diet containing 0.3% (w/w) (3 000 ppm) 3,3'-dichlorobenzidine to male and female hamsters "induced 4 transitional cell bladder carcinomas, some liver-cell and cholangiomatous tumors and diffuse chronic intrahepatic obstructing cholangitis (63%)," although little additional information was provided. No evidence of histopathological effects in either the liver or bladder of male Wistar rats (n = 18) was observed following administration of diets containing 0% or 0.03% (w/w) (300 ppm) 3,3'-dichlorobenzidine for a period of 40 weeks (Tsuda *et al*., 1977), although the small group sizes and limited period of exposure limit the significance of these results.

Based on the results of a number of studies in bacterial and mammalian cells exposed in vitro and experimental animals exposed in vivo, there is convincing evidence to indicate that 3,3'-dichlorobenzidine is genotoxic. 3,3'-Dichlorobenzidine was mutagenic (i.e., the frequency of his^+-revertants was increased) in a variety of strains of *Salmonella typhimurium*, in the absence (Savard and Josephy, 1986; Lazear *et al*., 1979; Messerly *et al*., 1987; Garner *et al*., 1975) or presence (Savard and Josephy, 1986; Lazear *et al*., 1979; Messerly *et al*., 1987; Reid *et al*, 1984; Anderson and Styles, 1978; Garner *et al*., 1975) of metabolic activation. Iba *et al*. (Iba, 1987; Iba and Thomas, 1988) concluded that more than 50% of the mutagenic activity of 3,3'-dichlorobenzidine in Salmonella typhimurium strain TA98 (incubated in the presence of metabolic activation) was dependent upon cytoclirome P_{450} activity. The frequency of sister chromatid-exchange in human B-lymphoblastoid cells was increased following incubation in vitro with 3,3'-dichlorobenzidine (Shiraishi, 1986); unscheduled DNA synthesis in HeLa S3 cells was increased following exposure in vitro to 3,3'-dichlorobenzidine (Martin *et al*., 1978). 3,3'-Dichlorobenzidine was reported to "morphologically transform" rat embryo cells (Freeman *et al*., 1973). The oral administration of 3,3'-dichlorobenzidine (1 000 mg/kg b.w.) to male ICR-SPF mice increased the frequency of bone marrow cells with micronuclei, compared to controls administered vehicle alone (Cihak and Vontorkova, 1987). The oral administration of 3,3'-dichlorobenzidine (500 or 1 000 mg/kg b.w.) to male Alpk:AP rats increased unscheduled DNA synthesis in liver cells, compared to animals administered vehicle alone (Ashby and Mohammed, 1988).

3,3'-Dichlorobenzidine has the potential to adversely affect developing embryos, based on studies in which the incidence of "hyperplastic changes" in kidney explants obtained from embryos exposed *in utero* to 3,3'-dichlorobenzidine was increased compared to embryos exposed (*in utero*) to vehicle alone (Shabad *et al*., 1972), and the incidence of "lymphoid leukemias" in offspring born to female mice administered five (subcutaneous) injections of

3,3'-dichlorobenzidine during the last week of pregnancy was increased (significance unspecified), compared to animals administered vehicle alone (Golub *et al*., 1975, cited in U.S. EPA, 1988). Data on neurotoxic effects are limited to the observation in the study by Stula *et al*. (1978) of convulsions in one female beagle dog; at sacrifice, slight neuronal degeneration was noted after histopathological examination. No other information concerning the reproductive, developmental, neurological or immunological effects of 3,3'-dichlorobenzidine in experimental animals was identified.

2.4.2 *Humans*

Quantitative data on the toxicological effects of 3,3'-dichlorobenzidine in humans were limited to the incidence of tumours or death due to cancer associated with exposure to 3,3'-dichlorobenzidine in three limited studies in which the health of production workers was examined. No increase in the incidence or death due to bladder cancer was reported in studies involving groups of 109 (Maclntyre, 1975), 35 (Gadian, 1975) or 207 (Gerarde and Gerarde, 1974) workers occupationally exposed to 3,3'-dichlorobenzidine.

2.4.3 *Ecotoxicology*

There are very few data on the acute toxicity of 3,3'-dichlorobenzidine to aquatic organisms. An IC_{50} value of 0.06 mg/L was reported for bacteria in the Microtox assay (Dutka and Kwan, 1981). Sikka *et al*. (1978) reported a 48-h LC_{100} value for bluegill sunfish (*Lepomis macrochirus* Raf.) of 2 mg/L; following exposure to 0.5 mg/L 3,3'-dichlorobenzidine for 96 and 120 h. one half of the test group died. Kaiser (1992) estimated the following 96-h LC_{50} values: > 3 mg/L for fathead minnow (*Pimephales promelas*); 3 mg/L for rainbow trout (*Oncorhynchus mykiss*); and 1.5 mg/L for golden orfe (*Leuciscus idus melanotus*), based on quantitative structure-activity relationships.

No data were identified for the toxicity of 3,3'-dichlorobenzidine to wild mammals, terrestrial organisms, birds, sediment or soil biota.

3.0 Assessment of "Toxic" under CEPA

3.1 CEPA 11(*a*): Environment

The most sensitive aquatic species of those examined or those for which estimates have been made was bacteria in the Microtox assay, with an IC_{50} value of 0.06 mg/L. There are no data on the occurrence of 3,3'-dichlorobenzidine in Canada with which to compare this value; however, this value is about 1.7×10^{11} times greater than the concentration predicted in water using worst-case assumptions regarding release in southern Ontario (3.44×10^{-7} ng/L).

Therefore, on the basis of the limited available data, 3,3'-dichlorobenzidine is not considered to be "toxic" as defined under paragraph 11(*a*) of CEPA.

3.2 CEPA 11(*b*): Environment on Which Human Life Depends

Due to its relatively low volatility, very short residence time and low concentrations in the atmosphere, 3,3'-dichlorobenzidine is not expected to contribute to the greenhouse effect, the depletion of the ozone layer or the formation of ground-level ozone.

Therefore, on the basis of available data, 3,3'-dichlorobenzidine is not considered to be "toxic" as defined under paragraph 11(*b*) of CEPA.

3.3 CEPA 11(*c*): Human Life or Health

Population Exposure

Based on the predicted levels of 3,3'-dichlorobenzidine in air, water and soil, as well as the estimated intakes by the population of Canada (EHD, 1992), the estimated daily intake of 3,3'-dichlorobenzidine by various age groups of the population can be calculated. For example, the total intake of 3,3'-dichlorobenzidine by adults[1] ($\geq$ 20 years of age) was predicted to be approximately 7.4×10^{-9} ng/kg b.w./day. For infants up to 6 months of age[2], the group with the greatest predicted exposure on a body-weight basis, the total intake of 3,3'-dichlorobenzidine estimated on the basis of predicted concentrations in environmental media was approximately 3.6×10^{-8} ng/kg b.w./day.

Effects

Epidemiological investigations concerning the carcinogenicity of 3,3'-dichlorobenzidine are restricted to three limited studies, in which the health of occupationally exposed individuals was examined (MacIntyre, 1975; Gadian, 1975; Gerarde and Gerarde, 1974). Although no evidence of a relationship between occupational exposure to 3,3'-dichlorobenzidine and an increased incidence of tumours or death due to cancer was reported in these investigations, owing to the limited power of these studies to detect an effect due to the small sizes of the

[1] Assumed to weigh 70 kg, breathe 23 m^3 air, drink 1.5 L of water and consume 20 mg soil per day.

[2] Assumed to weigh 7 kg, breathe 2 m^3 air, drink 750 mL of infant formula (water) and consume 35 mg soil per day.

populations examined following short periods of exposure (i.e., less than 16 years) and methodological limitations such as lack of appropriate control groups, the available information is considered inadequate to assess the carcinogenicity of 3,3'-dichlorobenzidine in humans.

3,3-Dichlorobenzidine has been shown to be carcinogenic in rats, mice, dogs and possibly hamsters (Stula *et al*., 1975; Osanai, 1976; Stula *et al*., 1978; Pliss, 1959; 1963; Sellakumar *et al*., 1969). It should be noted, however, that all of these studies were limited, due to small group sizes, single dose levels, relatively short periods of exposure and/or, in some cases, inadequate histopathological analysis and reporting of data.

In view of the sufficient evidence for the carcinogenicity of 3,3'-dichlorobenzidine in a number of animal species, this substance has been classified in Group II ("Probably Carcinogenic to Humans") of the classification scheme developed for the determination of "toxic" under paragraph 11(*c*) of CEPA (EHD, 1992). Confidence in the classification of 3,3'-dichlorobenzidine as a probable human carcinogen is strengthened by substantial evidence for the genotoxicity of 3,3'-dichlorobenzidine in bacterial and mammalian cells, both *in vitro* and *in vivo*. The genotoxic effects of 3,3'-dichlorobenzidine are believed to be dependent upon the formation of highly reactive N-oxygenated intermediates that arise following the (hepatic) cytochrome P_{450}-mediated oxidation of 3,3'-dichlorobenzidine (Iba, 1990; lba and Thomas, 1988).

For such substances, where possible, estimated total daily intake by the general population in Canada is compared to quantitative estimates of carcinogenic potency to characterize risk and provide guidance for further action (i.e. analysis of options to reduce exposure) under the Act. The carcinogenic potency ($TD_{0.05}$) of 3,3'-dichlorobenzidine was derived based on the increased incidence of mammary tumours ("fibroadenomas" and adenocarcinomas (combined) in males and females), granulocytic leukemias (males) and zymbal gland carcinomas (males) in ChR-CD rats administered 3,3'-dichlorobenzidine in the diet in the study conducted by Stula *et al*. (1975). This study was considered most appropriate for quantitative assessment, owing to the size (n = 50) of the study groups, which were relatively large compared to that in other available investigations, and to the relative adequacy of documentation of the protocol and results. It should be noted, however, that only one dose level was administered in this study, and that the period of administration was less than 2 years (up to 488 days).

Based on the study conducted in rats by Stula *et al*. (1975), $TD_{0.05}$s calculated based on linear interpolation, incorporating a body weight:surface area correction (owing to the lack of identification of the active metabolite(s), and correcting for less than 2 years exposure, range from 0.74 to 1.4 mg/kg b.w./day. Calculated exposure/carcinogenic potency indices for the age group with greatest exposure on a body-weight basis estimated based on predicted concentrations in environmental media range from 2.6×10^{-14} to 4.9×10^{-14} (3.6×10^{-8} ng/kg b.w./day divided by 0.74 to 1.4 mg/kg b.w./day). The priority for further action (i.e. analysis of options to reduce exposure) is, therefore, considered to be very low.

3,3'-Dichlorobenzidine has been classified as being "Probably Carcinogenic to Humans" and is therefore considered to be "toxic" under paragraph 11(*c*) of CEPA.

This approach is consistent with the objective that exposure to non-threshold toxicants should be reduced wherever possible, and obviates the need to establish an arbitrary *de minimis* level of risk for determination of "toxic" under CEPA.

3.4 Conclusion

Based on the available data, 3,3'-dichlorobenzidine is not considered to be "toxic" as defined under paragraphs 11(*a*) or 11(*b*) of CEPA. 3,3'-Dichlorobenzidine is considered to be "toxic" as defined under paragraph 11(*c*) of CEPA.

4.0 Recommendations for Research

Although a number of data gaps on the environmental and human health effects of 3,3'-dichlorobenzidine were identified, because of the negligible exposure of biota and the general population of Canada to this substance the priority for additional research on 3,3'-dichlorobenzidine is considered to be low.

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