ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR 3,3'-DICHLOROBENZIDINE

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CONTENTS

ADDENDUM for 3,3'-DICHLOROBENZIDINE
Supplement to the 1998 Toxicological Profile for 3,3'-Dichlorobenzidine

Background Statement

This addendum to the Toxicological Profile for 3,3'-Dichlorobenzidine supplements the Toxicological Profile that was released in 1998.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the CERCLA Priority List of Hazardous Substances and that the profiles be revised "no less often than once every three years." CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide to the public and other federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1998.

Chapter numbers in this addendum coincide with the Toxicological Profile for 3,3'-Dichlorobenzidine (1998). This document should be used in conjunction with the profile. It does not replace it.

2. HEALTH EFFECTS

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

2.2.1 Inhalation Exposure

2.2.1.8 Cancer Effects

Rosenman and Reilly (2004) identified 22 bladder cancer cases among a cohort of 538 workers exposed to benzidine and/or 3,3'-dichlorobenzidine from a chemical manufacturing plant. The plant produced benzidine from 1960 to 1972 and 3,3'-dichlorobenzidine from 1961 to 2001. The employees who had worked at the facility between 1960 and 1977 were identified. Standardized mortality ratios (SMRs) were significantly increased for all cancers: 1.54 (95% confidence interval [CI] 1.04–2.19); bladder cancer: 8.34 (95% CI 1.72–24.78); and lymphohematopoietic cancer: 2.84 (95% CI 1.04–6.18). Of the 538 workers, 202 had exposure to 3,3'-dichlorobenzidine alone. Only one case of bladder cancer was identified among those workers, while three employees died from lymphohematopoietic cancer (SMR 6.62, 95% CI 1.37–19.36).

2.3 TOXICOKINETICS

2.3.3 Metabolism

Formation of N-hydroxy-dichlorobenzidine and N-hydroxy-acetyl-dichlorobenzidine could arise from either direct N-oxidation of the amino group or deacetylation of the hydroxamic acid. In either case, the N-oxidation of 3,3'-dichlorobenzidine may lead to DNA adducts and subsequently to DNA lesions and mutations. This accumulation of non-acetylated adducts has been associated with tumor-initiating properties; thus, the balance between acetylation and deacetylation may greatly influence the biological effect. For this reason, Zwirner-Baier and Neumann (1998) analyzed hydrolysable hemoglobin adducts representing the bioavailability of N-hydroxylamines and the corresponding nitroso-derivatives, following oral administration to female Wistar rats of 3,3'-dichlorobenzidine. The results showed that deamination did not take place (low adduct levels were found); the monoacetamide (N-acetyl-3,3'-dichlorobenzidine) was readily deacetylated *in vivo*, whereas the diacetamide (N,N-diacetyl-3,3'-dichlorobenzidine) was not. In addition, acetylation polymorphism was studied with 3,3'-dichlorobenzidine in slow-acetylating A/J mice and rapid-acetylating C57BL/6J mice (Zwirner-Baier and Neumann 1998). The slow acetylator

genotype was associated with significantly higher hemoglobin-adduct levels. The results provide additional support for the use of hemoglobin adducts in biomonitoring as a dosimeter for the biologically active dose of arylamines/arylacetamides, and the results support the epidemiological finding of susceptibility of slow acetylators to developing occupational bladder cancer. Zwirner-Baier and Neumann (1998) reported that in Wistar rats the equilibrium between 3,3'-dichlorobenzidine and its metabolite, N-acetyl-3,3'-dichlorbenzidine, was 5:1.

2.3.4 Elimination and Excretion

2.3.4.2 Oral Exposure

In rats treated orally with 20 mg of 3,3'-dichlorobenzidine/kg body weight over 2 weeks, the urinary excretion rate of the parent compound was nearly constant (Lee et al. 2003). However, the excretion rate of its metabolites, N-acetyl 3, 3'-dichlorobenzidine and N,N'-diacetyl 3,3'-dichlorobenzidine, increased during the 14 days they were monitored.

2.5 RELEVANCE TO PUBLIC HEALTH

Cancer. See section 2.2.1.8.

Genotoxic Effects. Recent studies examined the genotoxicity of 3,3'-dichlorobenzidine *in vitro*. DNA damage in human lymphocytes from 3,3'-dichlorobenzidine was tested by use of a modified comet assay (Chen et al. 2003). The comet assay is a single-cell test of DNA damage by electrophoresis in which broken DNA fragments migrate more quickly than undamaged DNA, thus forming a blur resembling a comet. After 2 hours of exposure at 100 µmolar, 3,3''-dichlorodenzidine showed significant DNA-damaging potential or effect in a dose-dependent manner (Chen et al. 2003).

3,3'-Dichlorobenzidine was weakly mutagenic in tester strain *Salmonella typhimurium* TA7004 (0.01 revertants/µg), indicating that the compound induced GC->AT transitions (base-pair mutation) (Claxton et al. 2001). In another study, 3,3'-dichlorobenzidine was mutagenic to *S. typhimurium* test strain TA102, both directly (i.e., in the absence) and in the presence of rat S9 mix, and it was more potent in the presence of the S9 mix. The greatest mutagenic response was observed at 100 µg/plate, the highest dose tested (Makena and Chung 2007).

Simultaneous exposure to 3,3'-dichlorobenzidine and light (2.3 Joules/cm^2 of Ultraviolet A light and 4.2 joules/cm^2 of visible light) causes photomutagenicity and phototoxicity in *S. typhimurium* TA102. Using light as an activation system caused a greater increase in the number of revertant colonies compared to using liver S9 homogenate (Wang et al. 2005). Similar effects were observed with Jurkat cells, human leukemic T-lymphocytes used to study cell susceptibility to cancers, radiation, and drugs. Photogenotoxicity and phototoxicity in Jurkat cells increased with the dose increase of 3, 3'-dichlorobenzidine under light irradiation.

3,3'-Dichlorobenzidine given in single intraperitoneal doses of 125, 250, and 500 mg/kg or double intraperitoneal doses of 0, 75, 150, and 300 mg/kg did not result in micronucleated reticulocyte induction in the peripheral blood of CD-1 male mice (Morita et al. 1997). Gavage of 3,3'-dichlorobenzidine at doses of 67.5, 125, and 250 mg/kg did not result in micronucleated reticulocyte induction in the peripheral blood of CD-1 male mice (Morita et al. 1997). However, MS/Ae mice (male and female) did show micronucleated reticulocyte induction after double intraperitoneal injection (doses of 120, 180, and 270 mg/kg), but these responses were weak. Bone marrow examination was not reported in this study.

Sasaki et al. (1999a) evaluated the genotoxicity of 3,3'-dichlorobenzidine. The researchers administered a single gavage to four male mice at the maximum tolerated dose, which was set at about half the LD_{50} (300 mg/kg), and animals were sacrificed at 0 (zero-time control group), 3, 8, and 24 hours after treatment. Differences in length of DNA migration between control (zero-time) groups vs. other groups were measured with the comet assay and tested for statistical significance. Among cells of eight organs examined (stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow), 3,3'-dichlorobenzidine induced a statistically significant increase in DNA damage in the stomach, liver, urinary bladder, lung, brain, and bone marrow compared with controls.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

2.7.1 Biomarkers Used to Identify or Quantify Exposure to 3, 3'-Dichlorobenzidine

Two 3,3'-dichlorobenzidine metabolites, N-acetyl-dichlorobenzidine and N,N'-diacetyl-dichlorobenzidine, can form hemoglobin adducts in rats; these metabolites can be quantified by use of gas chromatography/mass spectrometry-selected ion monitoring (GS/MS-SIM) developed by Lee and Shin (2002).

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

There is some evidence that genetically slow acetylators can be susceptible to bladder cancer from 3,3'-dichlorobenzidine (see Section 2.3.3, Metabolism).

5. POTENTIAL FOR HUMAN EXPOSURE

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

3,3'-Dichlorobenzidine can become bound to sediment and be transported over long distances. An 11-year field study (Harden et al. 2005) measured the highest level of 3,3'-dichlorobenzidine as far as 6 km from its single known source in Lake Macatawa, Michigan.

5.3.2.2 Water

Fenton's reagent (Fe(II) + H_2O_2) for treatment of wastes contaminated with various carcinogenic aromatic amines was investigated by Casero et al. (1997). Under bench top experimental conditions, Fenton's reagent showed 99.99% efficiency in converting 3,3'-dichlorobenzidine to ring-cleavage products by oxidation after 1 hour of treatment at room temperature, demonstrating that this technology is potentially useful as a waste water treatment technique (Casero et al. 1997).

5.3.2.3 Sediment and Soil

Laboratory experiments designed to probe biodegradation and photodegradation pathways showed that 3,3'-dichlorobenzidine undergoes sequential dehalogenation to yield 3-chlorobenzidine and then benzidine under exposure to microorganisms and under simulated tropospheric solar radiation (Nyman et al. 1999). Dechlorination is expected to yield higher total concentrations of aromatic amines in the solution (Nyman et al. 1999).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

5.4.2 Water

Onuska et al. (2000) extracted 3,3'-dichlorobenzidine from industrial effluent samples close to an industrial pigment site in Toronto, Canada. Water concentrations near the Toronto pigment site were up to 654 µg/L). Harden et al. (2005) detected up to 26.83 µg/L of 3,3'-dichlorobenzidine in the water phase and up to 8.71 µg/L in sediment pore water. 3,3'-Dichlorobenzidine concentrations tended to be higher in the pore water of silty clay sediments.

Several studies (Mizuno et al. 2007; Ohe et al. 2008) reported 3,3'-dichlorobezidine contamination of the Waka River in Japan. Mizuno et al. (2007) detected high levels of 3,3'-dichlorobenzidine and 4-amino-3,3-dichloro-5,4-dinitrobiphenyl (ADDB), a mutagenic compound and endocrine disruptor, from samples collected at chemical plants' waste water discharge in the river. ADDB is formed by oxidation and nitration from 3,3'-dichlorobenzidine during the process of wastewater treatment of drainage . Mutagenicity of water samples was evaluated in the *Salmonella* assay by use of the *O*-acetyltransferase-overexpressing strain YG1024. Water samples from the discharge site showed stronger mutagenicity than water samples collected from upstream and downstream sites. Similarly, Ohe et al. (2008) identified two mutagenic fractions (YG1024 strain) in the water adsorbate, 3,3'-dichlorobenzidine and a novel chemical, a 5-nitro derivative of 3,3'-dichlorobenzidine (4,4'-diamino-3,3'-dichloro-5-nitrobiphenyl), supposedly formed from 3,3'-dichlorobenzidine during the waste water treatment process.

5.4.3 Sediment and Soil

Harden et al. (2005) measured 3,3'-dichlorobenzidine from nondetectable to 69.663 mg/kg, the highest level found 6 km from the single known source of 3,3'-dichlorobenzidine in Lake Macatawa, indicating that sediments contaminated with 3,3'-dichlorobenzidine are highly mobile. This finding was supported by the oscillatory pattern of 3,3'-dichlorobenzidine distribution within Lake Macatawa where sampling sites of nondetectable 3,3'-dichlorobenzidine concentrations were adjacent to sites of high concentrations. The pattern was explained by sediment resuspension and transport mostly due to wind-driven resonant motions (Nyman et al. 2003).

6. ANALYTICAL METHODS

6.1 BIOLOGICAL SAMPLES

Two 3, 3'-dichlorobenzidine metabolites, N-acetyl-dichlorobenzidine and N,N'-diacetyl-dichlorobenzidine, can form hemoglobin adducts in rats; these metabolites can be quantified by use of GC/MS-SIM developed by Lee and Shin (2002). The relative standard deviation reported was <12%, and the detection limit was reported as 0.01 ng/mL (Lee et al. 2003). After oral exposure to 20, 30, and 40 mg 3,3'-dichlorobenzidine/kg/day for 3 weeks in young female Sprague-Dawley rats, the GC/MS-SIM detected two hemoglobin adducts, 3,3'-dichlorobenzidine and N-acetyl 3,3'-dichlorobenzidine. While the adduct levels increased in dose-proportional pattern, the ratio of 3,3'-dichlorobenzidine and N-acetyl 3,3'-dichlorobenzidine stayed similar in all treatment groups at the third week (Lee et al. 2003).

Guerbert et al. (2007) studied 47 workers from a major chemical plant that produces 3,3'-dichloro-benzidine and azo dyes. Urinary mutagenicity was defined with the Ames fluctuation test and GS/MS. Urine samples were collected after a 1-month holiday (non-exposure) and again after 4 months of work (exposure). There were no significant differences between exposure and non-exposure samples: three of the non-exposure and six of the exposure samples were positive in at least one mutagenicity assay, and 3,3'-dichlorobenzidine traces were detected in several exposed and non-exposed samples.

6.2 ENVIRONMENTAL SAMPLES

A Fourier transform ion cyclotron resonance mass spectrometer was utilized to measure 3,3'-dichlorobenzidine in sediment and water samples (Nyman et al. 1999). Onuska et al. (2000) extracted 3,3'-dichlorobenzidine and other aromatic amines from aqueous media by using either methylene chloride or a solid phase extraction cartridge containing divinylbenzene-vinylpyrrolidone polymer. Aromatic amines were converted to their N-fluoroacetyl derivatives and analyzed by use of GC/MS detection. In tap water, the detection limit for this method was reported to be 0.2 ppb, with a relative standard deviation of 2.9% and a percent recovery of 90.8%.

Vera-Avila et al (2001) proposed a new, simpler, and faster (about 2 hours) method for extraction and analysis of 3,3'-dichlorobenzidine in natural waters by use of reversed-phase gradient-elution chromatography with coulometric detection. Recovery of 95% and a relative standard deviation of

approximately 5% were achieved at low µg/L concentration levels. The detection limit of the method was 50 ng/L for 3,3'-dichlorobenzidine.

Because of recent concerns over the presence of dyes in leather, both microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) followed by detection with high-performance liquid chromatography coupled with ultraviolet diode array detection have been investigated for analyzing aromatic amines (Sparr Eskilsson et al. 2002). 3,3'-Dichlorobenzidine recovery in spiked leather samples was 63% for MAE and 52% for SFE. For genuine leather samples, recoveries decreased to 55% for MAE and 52% for SFE. The quantification limits in leather samples by use of MAE or SFE were <1 mg/kg for all amines investigated. The within-laboratory precision was >10%.

Typically, analytical samples used for looking for 3,3'-dichlorobenzidine must be pre-concentrated. Wu and Huang (1998) developed a simpler method to determine aromatic amines (including 3,3'-dichlorobenzidine) at 10 µg/g levels in commercial dyestuff samples utilizing cloud point preconcentration. Cloud point preconcentration heats a liquid with nonionic surfactants until they become turbid (called the cloud point), the two phases of the solution are separated, and the surfactant rich portion is analyzed by use of liquid chromatography. The dyestuff was dissolved in water and pre-cleaned with a SAX cartridge packed with an anion-exchange resin. The effluent was then analyzed by use of the cloud point preconcentration, with subsequent determination by liquid chromatography with ultraviolet absorption detection. Among the aromatic amines, the recovery from spiked dyestuffs was lowest for 3,3'-dichlorobenzidine (range 55–66%), with relative standard deviations ranging from 2 to 4%, depending on conditions and the dye analyzed. The method detection limit was 0.9 µg/g for 3,3'-dichlorobenzidine.

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