ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR BENZIDINE

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CONTENTS

ADDENDUM FOR Benzidine
Supplement to the 2001 Toxicological Profile for Benzidine

Background Statement

This addendum for Benzidine supplements the Toxicological Profile for Benzidine that was released in 2001.

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 Priority List 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 2001.

Chapter numbers in this addendum coincide with the Toxicological Profile for Benzidine (2001). This document should be used in conjunction with the profile. It does not replace it.

2. RELEVANCE TO PUBLIC HEALTH

3. HEALTH EFFECTS

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

3.2.1 Inhalation Exposure

3.2.1.7 Cancer

Kim et al. (2007) examined the bladder cancer incidence of factory workers exposed to benzidine or benzidine dyes. Their study investigated 650 benzidine-exposed subjects employed at a company manufacturing benzidine-based dye or at facilities where subjects handled benzidine. Two confirmed bladder cancer cases were diagnosed among the 650 subjects. One study subject was 53 years old and had been exposed to benzidine for 24 years. The latency period for this man's cancer was 30 years from the time of initial exposure. The other study subject was 60 years old and had been exposed to benzidine for 30 years. The latency period for this man's cancer was 35 years from the time of initial exposure. The usual latency period for bladder cancer due to benzidine exposure is over 20 years. The investigators attributed the longer latency period of their study subjects to the lower exposure level from the company's probable use of benzidine chloride. Benzidine chloride does not tend to break up into small particles, so that high inhalation exposure levels of this substance would not be as great from breathing the ambient air.

Rosenman and Reilly (2004) investigated cancer mortality and incidence among a cohort of benzidine and dichlorobenzidine dye manufacturing workers. A cohort of 538 workers employed from 1960 to 1977 was identified from a single chemical manufacturing facility producing benzidine. The company produced benzidine from 1960 to 1972 and dichlorobenzidine from 1961 through the 1990s. The cohort was identified from Social Security records for the years 1960 to 1977. The Social Security records had the names and Social Security numbers of workers employed by this facility. Due to ownership changes, no personnel records were available to link the Social Security information to personnel records.

Demographic information was, therefore, obtained from death certificates, from the Michigan Tumor Registry, and from an Agency for Toxic Substances and Disease Registry (ATSDR) surveillance program of bladder cancer screening. Because of the limited numbers of white females and African-Americans, the cohort consisted of white males only. The purpose of the study was to determine the risk of bladder cancer, the risk for other types of cancer, and the risk of cancer from dichlorobenzidine in this population. Twenty-two bladder cancer cases were detected. The standardized mortality ratios (SMR) were elevated for all cancer 1.54 (95% confidence interval or CI 1.04–2.19), for bladder cancer 8.34 (95% CI 1.72–24.78), and for lymphohematopoietic cancer 2.84 (95%CI 1.04–6.18). The standardized incidence ratio (SIR) for bladder cancer was 6.85 (95% CI 4.30–10.4). Only a single worker exposed to dichlorobenzidine was identified as having bladder cancer. An elevated risk for lymphohematopoietic cancer was detected for workers exposed to dichorobenzidine (SMR 6.62 with 95% CI 1.37–19.36). No information was available with regard to job title and exposures. This made it impossible to determine the percentage of workers exposed to benzidine or to dichlorbenzidine and also to identify the high-risk group.

Miyakawa et al. (2001) re-evaluated the latency period of bladder cancer in Japanese dyestuff-plant workers. Benzidine and beta-naphthylamine are known bladder carcinogens. Although it was considered unlikely for bladder cancer to develop more than 20 years after exposure to these chemicals, new clinical cases of bladder cancer have been observed 30 years after exposure. The purpose of this study was to once again evaluate the latency period for carcinogenicity to determine a safety period after which development of bladder cancer would be unlikely. The safety period would be used to develop a health surveillance system. The study subjects consisted of 236 Tokyo dyestuff-exposed workers. The group's incidence of bladder cancer and its histopathology was evaluated for the period 1962 to 1996. Nineteen (8.1%) of the subjects had bladder cancer, with an exposure period of 82.0±50.2 months. The latency period (mean and standard deviation [SD]) for initial and final exposure and tumor development was 29.5±8.2 years and 20.1±10.6 years, respectively. A significant negative correlation was detected between the exposure period and the latency period from the end of exposure to cancer onset. All but one tumor was transitional carcinoma. These findings demonstrate the need to survey the onset of bladder cancer in exposed workers for more than 30 years after the initial exposure.

3.3 GENOTOXICITY

Chen et al. (2003) assessed the genotoxicity of benzidine and structural analogues to human lymphocytes by using the Comet Assay. Benzidine and its structural analogues (e.g., 2-aminobiphenyl, 4-aminobipenyl, 3,3'-diaminobenzidine, 3,3'-dichorobenzidine, 3,3'-dimethoxybenzidine, and 3,3'-dimethylbenzidine) were tested for DNA (deoxyribonucleic acid) damage in human lymphocytes by use of the Alkaline Comet Assay. DNA damage from the structural analogues had no relationship with regard to some physicochemical parameters (e.g., oxidation and ionization potentials). However, free radical scavengers (e.g., catalase and histidine) influenced in varying degrees inhibitory effects on DNA damage caused by benzidine.

Claxton et al. (2001) used base-specific Salmonella (S) tester strains to characterize mutation induced by benzidine and benzidine congeners following reductive metabolism. Using the base-specific tester strains of S. typhimurium (TA7001-TA7006) with a reductive metabolic activation system (i.e., hamster liver S9 preparation with glucose-6-phosphate dehydrogenase, flavin mononucleotide [FMN], nicotamide adenine dinucleotide phosphate monosodium salt [NADH], and four times glucose 6 phosphate), the researchers investigated whether these compounds had the ability to produce specific base-pair substitutions after reductive metabolism. The findings demonstrated that benzidine was a weak mutagen in TA7005. The 4-aminobiphenyl, the monodeaminated benzidine, was found to be mutagenic in TA7002, TA7004, TA7005, and TA7006. 3,3'-Dichlorobenzidine HCl was weakly mutagenic in TA7004.

Chung et al. (2000) used the Ames Salmonella/microsome assay to determine the mutagenicity of benzidine and its analogs by using strains TA98 and TA100 in the presence and absence of Arolor 1254-induced rat S9 mix. A direct mutagenicity to TA 98 was observed with 3,3'-dichlorobenzidine-2HCl and 4,4'-dinitro-2-biphenylamine. A direct mutagenicity to TA98 and TA100 was observed with 4,4'-dinitro-2-biphenylamine in the absence of S9 mix. Mutagenicity to TA98 was observed for 4-aminobiphenyl, benzidine, 3,3'-dichlorobenzidine-2HCl, 3,3'-dimethoxybenzidine, 3,3'-4,4'-tetraaminobiphenyl, o-tolidine, N,N-N',N'-tetramethylbenzidine,

and 4,4'-dinitro-2-biphenylamine in the presence of S9 mix. Mutagenicity to TA100 was observed for 4-aminobiphenyl, 3,3'-dichlorobenzidine-2HCl, 3,3'-dimethoxybenzidine, and 4,4'-dinitro-2-biphenylamine. Physiocochemical parameters (e.g., oxidation and ionization potentials) had no association with bacterial mutagenic activities.

3.4 TOXICOKINETICS

3.4.3 Metabolism

Carreon et al. (2006) conducted a study of N-acetyltransferase 2 (NAT2) polymorphisms and bladder cancer in male subjects occupationally exposed only to airborne benzidine. The study subjects from a Chinese cohort of production workers exposed to benzidine consisted of 68 cases and 107 controls. The subjects included 30 new cases and 67 controls not previously studied. NAT2 enzymatic activity phenotype was measured by use of the urinary caffeine metabolite ratios. Polymerase Chain Reaction (PCR)-based methods allowed the identification of genotypes for NAT2, N-acetyltransferase 1 (NAT1), and glutathione S-transferase μ1 (GSTM1). After adjustment for cumulative benzidine exposure and lifetime smoking, a protective association was observed between the presence of the slow NAT2 genotype and the occurrence of bladder cancer (odds ratio or OR = 0.3, 95% CI of 0.1–1.0). A higher risk of bladder cancer was observed in study subjects with NAT1wt/*10 and NAT1*10/*10 genotypes (OR = 2.8, 95% CI = 0.8–10.1 and OR = 2.2, 95% CI = 0.6–8.3, respectively). The findings demonstrate that slow acetylation (NAT2 genotype) is protective against the development of bladder cancer, as demonstrated in an earlier study.

Lin et al. (2005) conducted a study examining UDP-Glucuronyltransferase 2B7 (UGT2B7) involved in benzidine metabolism. A group of benzidine-exposed workers (n=36) employed in the Chinese dyestuff industry and diagnosed with bladder cancer was evaluated for the possible association of UGT2B7 gene polymorphism at locus $C_{802}T$ ($His_{268}Tyr$) and bladder cancer risk. The PCR-RFLP (Restriction Fragment Length Polymorphism)-based procedure was used to detect UGT2B7 polymorphism. Work-related, non-diseased cohort members acted as controls for the study and consisted of 156 males and 95 females. Unexposed, healthy community individuals acted as a second control group comprised of 113 males and 105 females.

Polymorphism at locus UGT2B7 $C_{802}T$ in the general Chinese population differed significantly, with a lower frequency of T/T genotypes than a Caucasian population (p = 0.00018). A higher prevalence of T/T genotype carriers was detected in the cancer cases than in the unexposed, healthy controls (25% vs 9%, OR = 3.30, 95% CI = 1.37–7.98, p = 0.006). A higher presentation of T allele carriers was observed in the patient group (46% vs 33%, OR = 1.73, 95% CI = 1.05–2.87, p = 0.03). A higher portion of the T/T genotype was detected in bladder cancer patients than in non-diseased individuals of the same benzidine-exposed cohort. This study revealed the newly discovered finding of an association between a homozygous mutant genotype of human UDP-glucuronosyltranserase 2B7, catalyzing the biotransformation of benzidine, and the subsequent elevated bladder cancer risk for workers formerly exposed to benzidine from employment in the dyestuff industry.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Benzidine

Xiang et al. (2007) investigated the mutant p53 protein in workers occupationally exposed to benzidine. The study population consisted of 331 benzidine-exposed healthy workers who had been a part of a medical surveillance program in 1999. The group was the Shanghai cohort of benzidine–exposed dyestuff industry workers. The immuno-PCR assay was employed to examine the expression of the mutant p53 protein in the serum of workers occupationally exposed to benzidine. Exfoliated urothelial cells in urine samples from study subjects were classified by use of the Papanicoloau grading (PG). Through this method, urothelial cells were graded from grade I (normal) to grade III (suspicious malignant cells). The study subjects were classified into high, medium, and low exposure groups by use of an exposure intensity index. The findings demonstrated that mutant p53 protein was significantly higher in the medium and high exposure groups than in the low exposure group ($p<0.05$). In addition, the incidence and/or quantity of mutant p53 protein in the PG II and/or III grades for the medium and high exposed groups were significantly higher than for the PG I group ($p<0.05$).

Beyerbach et al. (2006) studied a group of benzidine-exposed Indian workers (n=15), workers exposed to azo dyes (n=18), and controls (n=15) with regard to the presence of hemoglobin adducts. Benzidine has been used to synthesize azo dyes and may be released by metabolism from azo dyes. Hemoglobin adducts were measured by using gas chromatography-mass spectrometry (GC-MS); the measured adducts included benzidine, N-acetyl-benzidine, 4-aminobiphenyl, and aniline. Quantitatively, the major adducts consisted of 4-aminobiphenyl and aniline. Adduct levels were 10–17-fold higher in workers exposed to benzidine than in workers exposed to dyes. Metabolic release of 4-aminobiphenyl may be observed with benzidine and azo dyes. 4-Aminobiphenyl is a human carcinogen, so that tumor formation in workers may be caused by benzidine and benzidine dyes. Genetic variants in NAT1 and NAT2 need to be understood in terms of their ability to modify the impact of benzidine on bladder cancer risk. Gaining such an understanding may be difficult, because N-acetylation detoxifies 4-aminobiphenyl but activates benzidine.

Hemstreet et al. (2001) investigated a cohort of Chinese workers occupationally exposed to benzidine. The investigators wanted to determine whether biomarker profiles could be used for risk assessment and cancer detection. The cohort of 1,788 exposed and 373 non-exposed workers was followed from 1991 through 1997. Urothelial cells from voided urine samples were assayed for DNA ploidy (expressed as 5C-exceeding rate – [DNA 5CER]), the bladder tumor-associated antigen p300, and a cytoskeletal protein (G-actin). Workers were categorized as high, moderate, and low risk at each examination on the basis of a predefined biomarker profile. Tumor risk assessment for workers developing bladder cancer was analyzed from samples collected 6–12 months prior to cancer diagnosis. Statistical models, including Cox proportional hazard regression analysis and logistic analysis, after adjustment, were used to analyze the association between risk group and subsequent development of bladder cancer. Two-sided statistical tests were used. The findings demonstrated that exposed workers had a higher number of diagnosed bladder cancers (28) than did the unexposed (2). For risk assessment, the sensitivity of DNA 5CER was 87.5%, with a specificity of 86.5%. The OR for DNA 5CER was 46.2 (95% CI=8.1 to 867.0) and a risk ratio (RR) of 16.2 (95% CI= 7.1 to 37.0). The p300 had a sensitivity of 50% and a specificity of 97.9%. The OR for p300 was 40 (95% CI= 9.0–177.8) and the RR was 37.9 (95% CI=16.8–85.3). The risk of developing bladder cancer was 19.6

times (95% CI=8.0–47.9) greater for those workers positive for either the DNA 5CER or p300 biomarkers than for workers negative for both biomarkers. The risk of developing bladder cancer in workers having both biomarkers was 81.4 (95% CI=33.3 to 199.3). G-actin proved to be a poor biomarker of individual risk. For workers at risk for bladder cancer, individuals can be stratified, screened, monitored, and diagnosed on the basis of predefined molecular biomarker profiles.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

Guo et al. (2004) investigated the possible association between NAT1*10 and NAT1*14A genotypes and the risk of bladder cancer in an occupational study of Chinese workers exposed to benzidine. Stratified subgroups for study participants were developed by use of the Papanicolaou cytological gradings of exfoliated urothelial cells. Polymorphism in the polyadenylation signal at the locus NAT1 $T_{1088}A$ was detected by use of the allele-specific PCR-based procedure. In addition, NAT1*14A ($T_{1088}A$, $C_{1095}A$, and $G_{560}A$) was differentiated from NAT1*10 ($T_{1088}A$, $C_{1095}A$) by use of the nested PCR-RFLP procedure. No significant differences in the frequency of homozygous and heterozygous NAT1*10 alleles were detected with (i) Papanicolaou gradings ≤II (18.3 and 40.2%, respectively, (ii) higher Papanicolaou gradings (>II; 28.0 and 34.1%, respectively), and (iii) bladder cancer (26.3 and 34.2%, respectively). The findings demonstrate that NAT1*10 did not show an association with an elevated grading of urothelial cells, nor did they show a clear impact on bladder cancer risk in benzidine-exposed Chinese workers. This finding, which is discrepant with findings in European populations, may be due to ethnic differences in the disposition of aromatic amines.

Arylamine N-aceyltransferases (NATs) are engaged in the detoxification of aromatic amines and hyrazines. Ma et al. (2004) evaluated occupational and non-occupational bladder cancer subjects for polymorphism of NAT2. They investigated the possible association of NAT2 polymorphism with bladder cancer risk from benzidine exposure by selecting three sets of subjects. They included a group of healthy subjects, subjects with bladder cancer who had been exposed to benzidine after being employed in the Shanghai dyestuff industry, and a group of bladder cancer

patients without known occupational exposure to aromatic amines. All the subjects were genotyped for NAT2 gene polymorphism. NAT2 genotyping was done by use of a set of RFLP procedures at seven major polymorphic loci of gene coding area: G191A, C282T, T341C, C481T, G590A, A803G, and G857A. The most prevalent allele in healthy individuals was the wild allele NAT2*4. The most common alleles seen were the NAT2*6A and NAT2*7B. The percentage of slow acetylators was significantly higher in Caucasian than in the Chinese subjects. Healthy subjects and both groups of bladder cancer subjects had no relevant differences for homogenous rapid, heterogeneous rapid/slow and homogeneous slow acetylation genotypes. Chinese subjects had a lower percentage of slow acetylators than did Caucasian subjects. The research did not demonstrate an association of slow acetylating genotypes of NAT2 gene with an elevated risk of bladder cancer in Chinese. It was, however, an important genetically determined risk factor in Caucasians. The researchers surmised that different mechanisms in individual susceptibility to bladder cancer associated with aromatic amine exposure may be a factor in racial or ethnic differences.

Ma et al. (2003) investigated the role of GSTP1 polymorphism in the development of benzidine-related bladder cancer. Polymorphism at codon 105 (IIe/Val) in the GSTP1 gene was related to an increased risk for different types of cancer. Benzidine-exposed Chinese workers from the Shanghai region without known disease and benzidine-exposed bladder cancer patients from the same cohort were assessed for GSTP1 AA, AG, and GG genotypes in order to determine the role of GSTP1 polymorphisms in the development of benzidine-related bladder cancer. An increase was observed in the frequency of GSTP1 AG or GG carriers in the occupationally exposed bladder cancer group, but this increase was not statistically significant—OR = 1.95, 95% CI = 0.70–5.46). Of the non-genetic risk factors for bladder cancer in males (i.e., age, smoking status, duration of exposure, and high exposure), duration of exposure was the only statistically significant risk factor for bladder cancer (OR = 1.19, 95% CI = 1.10–1.29). A significant difference was observed between benzidine-exposed workers without known disease but with modified exfoliated urothelial cells (grade ≥II) and all workers without known disease with minor changes to the urothelial cells (<II) as demonstrated by Papanicolaou (OR = 1.90, 95% CI = 1.13–3.20). The findings demonstrate the association between the GSTP1 AG or GG genotype

and higher cytological gradings of exfoliated urothelial cells from workers previously exposed to benzidine.

Zhang et al. (2002) undertook a study to assess two aryl hydrocarbon receptor (AHR) genes, $G_{1721}A$ ($R_{554}K$) and $G_{1768}A$ ($V_{570}I$), in a Chinese population. The researchers were investigating the possible association of human AHR gene polymorphism and elevated incidence of bladder cancer among Chinese Han subjects residing in East China. An allele-specific PCR-based procedure for AHR gene polymorphism genotyping was created by this work. The Shanghai area subjects were divided into three groups by genotype: occupationally-exposed benzidine bladder cancer group, a non-occupational bladder cancer patient group with no obvious aromatic amine exposure record, and a normal population from Shanghai serving as controls. A significant difference was observed in the frequency distribution at locus $G_{1721}A$ between the normal Chinese population in Shanghai and a Caucasian population studied by other authors. There was no finding of a mutant allele (A_{1768}) at locus G_{1768}. No significant differences were observed by gender in the normal population or by case group and control group. A significant difference in distribution at locus $G_{1721}A$ of human AHR gene was observed between Chinese Han and Caucasian subjects. The study did not demonstrate an association of the AHR $G_{1721}A$ polymorphism with a higher risk of bladder cancer in an occupationally benzidine-exposed group or among individuals who never had an obvious exposure to aromatic amines.

Lin et al. (2001) examined the polymorphic alleles distribution of the genes coding for glutathione S-transferases (GSTs) M1 and T1 with the cytological grading of exfoliated urothelial cells (Pap test) in a non-diseases high-risk group of workers. These workers (n=317) had been exposed to benzidine in the Shanghai dyestuff industry. The allele-specific PCR was used to genotype the subjects for GSTT1 and M1 gene polymorphism. Subjects were categorized according to their job and duration of exposure. A subgroup consisting of 78 subjects with cytological gradings of grade III or higher in the Pap test had a significant under-representation of the combination of GSTT1 0/0 and M1 0/0 genotypes when compared to 238 subjects with a cytological classification lower than grade III (OR = 0.55, 95%CI 0.31-0.98, p=0.04). These results indicate that neither GSTM1 0/0 or GSTT1 0/0 genotype separately nor

in combination were correlated with cytopathological changes in exfoliated urothelial cells from subjects previously exposed to benzidine in Shanghai. This result is in contrast to research indicating that GSTM1 0/0 genotype is correlated with an increased risk for bladder cancer in the general population, for the most part, outside of China.

4. CHEMICAL AND PHYSICAL INFORMATION

No updated data.

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

No updated data.

6. POTENTIAL FOR HUMAN EXPOSURE

6.3 ENVIRONMENTAL FATE

6.3.2 Transformation and Degradation

6.3.2.2 Water

Harden et al. (2005) investigated the release to the environment of benzidine. The investigators conducted an 11-year field study to clarify the fate and behavior of benzidine in the Lake Macatawa sediment-water system, located in Michigan. The results of the field study established that benzidine is conveyed over long distances. In the water phase, the concentration of benzidine was detected at a level approximately 12,300 times higher than the EPA [recommended water quality] guideline (0.000086 ug/l). In the sediment phase, benzidine was detected at levels as high as ~63 mg/kg (1994) to a low of 0.148 mg /kg (2003). The concentrations of benzidine for both phases have decreased in recent years.

Muneer et al (2002) investigated the photocatalysed degradation of benzidine under a number of conditions, employing a pH-stat technique. The substances were monitored for degradation by

examining the change in substrate concentration of the model compound using high pressure liquid chromatography (HPLC) analysis and ascertaining the decrease in total carbon content, respectively, as a function of irradiation time. The degradation kinetics was investigated under different conditions (e.g., reaction pH and substrate and photocatalyst concentration). The degradation rates and the photonic efficiencies were strongly affected by the studied conditions (e.g., reaction pH). Toxicity tests were done for the irradiated samples of benzidine by measuring the luminescence of the Vibrio fischeri bacteria after 30 minutes of incubation. 4-Amino-biphenyl and hydroquinone were found to be intermediate products by GC/MS technique. The investigators are considering probable pathways for the formation of these products.

6.3.2.3 Sediment and Soil

Donaldson and Nyman (2006) investigated the short-term interactions of aniline and benzidine for three soils in natural and artificial matrices. The investigators noted the importance of understanding the fate of aromatic amines in natural systems due to their persistence and toxicity. Laboratory experiments were done on silty-clay, sandy loam, and sandy soils as well as six background matrices (i.e., rainwater, 12.5 mM $CaCl_2$, 25 mM $CaCl_2$, passing each through soil columns). The study's intent was to establish the validity of using $CaCl_2$ as a laboratory simulation for rainwater and to determine how short-term sorption (24 hours) of aniline and benzidine transformed when passed through the soil columns. Neither of the $CaCl_2$ solutions was predictive of the sorption of aniline and benzidine in a rainwater solution. This finding may be attributed to the varying soil properties that may influence sorption. Statistical analysis revealed that rainwater or $CaCl_2$ solution through soil columns did not influence significantly the sorption of aniline and benzidine. Cation exchange was shown to play a role in the sorption of both aniline and benzidine under all conditions. Solubility plots demonstrated a higher association for benzidine due to its lower aqueous solubility.

Harden et al. (2006) investigated benzidine transformation processes in natural environmental sediments. The investigators collected water and sediment samples from Lake Macatawa in Michigan and spiked these samples with benzidine. The sediment samples consisted of sandy to

silty-clay types. The spiked samples of sediment and water were incubated under anaerobic conditions at 4, 15, and 23^{o} C for 211 d. Degradation of benzidine was investigated over the time-course analysis in the sediment and water mixtures. Gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry revealed three possible metabolites (aniline, 2-ethyl-1-hexanol, and 1-amino-2-hexene). Because no metabolites were seen in autoclaved bottles, it was suggested that the benzidine transformation in the sediment-water mixtures was due to microbial activity. Benzidine was shown to have a higher sorption affinity for the different sediment phases than aniline, its degradation product. Aniline, microbial transformed from benzidine, would be expected to result in a greater concentration in the water phase and a greater chance of transport in the water phase.

Donaldson and Nyman (2005) investigated sorption of benzidine and 3,3'-dichlorobenzidine (DCB) in lake sediments. Benzidine and DCB batch isotherms were produced and evaluated. The sediment samples that were used ranged from sandy to silty-clay soil. The batch isotherms were examined by use of high-performance liquid chromatography. A multiparameter model (MPM) that examined partitioning, covalent bonding, and cation exchange was developed and tested. The MPM was effective in predicting sorption of aromatic amines (benzidine and DCB) to lake sediments. The results indicated that MPM can give a better understanding of the sorption process of aromatic amines than more conventional models.

7. ANALYTICAL METHODS

7.1 BIOLOGICAL SAMPLES

Patel and Agrawal (2003) used the supercritical fluid chromatography to separate and make trace estimates of benzidine and its macromolecular adducts in blood plasma. Benzidine and its acetylated metabolite, N-OH-N,N'-diacetylbenzidine, were derived from plasma by use of ether. The components were divided by use of the Nucleosil 10 um, Nucleosil-RP-C_{18} column with 7.4% methanol-modified supercritical fluid carbon dioxide as mobile phase. The UV-Vis detector was used for detection. The procedure resulted in a 98.6% mean extraction recovery of benzidine. When compared with the HPLC-UV (ultraviolet) method, the supercritical fluid

chromatography was found to be superior with regard to characteristics of sensitivity, specificity, and accuracy.

8. REGULATIONS AND ADVISORIES

No updated data.

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