CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of 3,3'-dichlorobenzidine is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of 3,3'-dichlorobenzidine.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

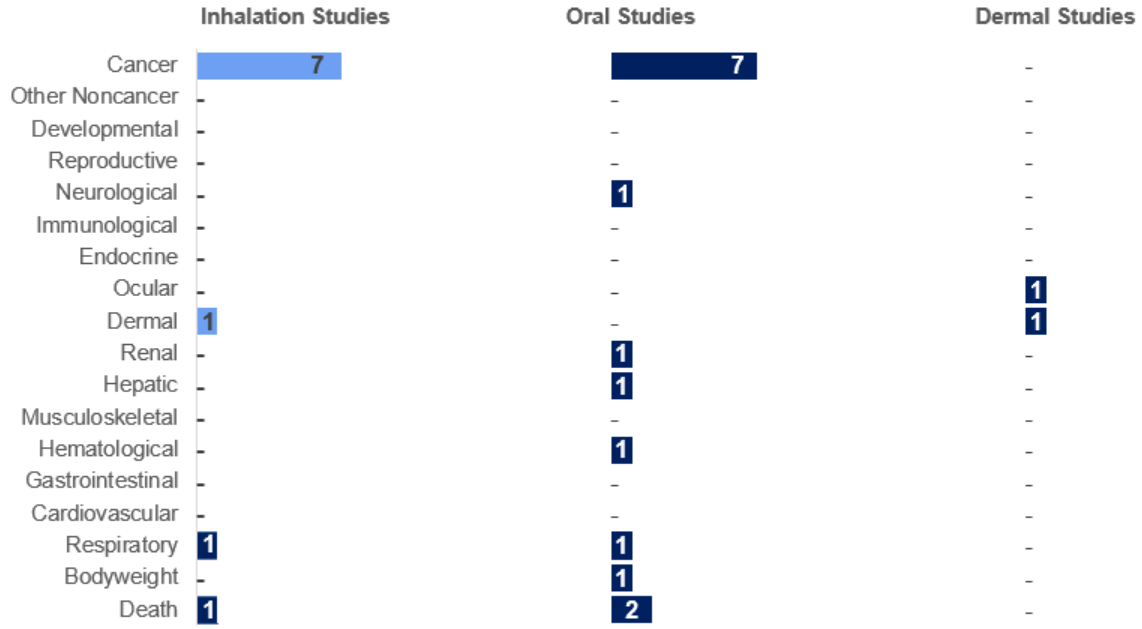
6.1 INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to 3,3'-dichlorobenzidine that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of 3,3'-dichlorobenzidine. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

Figure 6-1 illustrates that a majority of the toxicity data available for 3,3'-dichlorobenzidine comes from oral studies on laboratory animals. Cancer is the most commonly studied endpoint. Very few studies were found on humans, and the majority of these studies examined cancer outcomes among occupationally exposed groups. Dermal and inhalation studies focused on a very small number of endpoints, as oral studies examined seven different endpoints, followed by inhalation and dermal, examining five and three endpoints, respectively.

Figure 6-1. Summary of Existing Health Effects Studies on 3,3'-Dichlorobenzidine By Route and Endpoint*

Potential cancer and hematological effects were the most studied endpoints
The majority of the studies examined oral exposure in **animals** (versus **humans**)



*Includes studies discussed in Chapter 2; the number of studies include those finding no effect; some studies examined multiple endpoints

6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Acute-Duration MRLs. One study in humans showed that application of 3,3'-dichlorobenzidine base causes skin irritation (Gerarde and Gerarde 1974). The limited information in humans is insufficient to conclusively identify target organs, other than the skin, following exposure by any route. Acute-duration exposure can cause eye damage (erythema, pus, cornea1 opacity) in rabbits following conjunctival application. However, the relevance of these findings for the general population is unknown, since conjunctival application is not a likely route of exposure, and inhalation exposure is unlikely. 3,3'-Dichlorobenzidine can be lethal following oral and dermal exposure at very high doses. In most animal studies, comprehensive gross and histopathological evaluations have not been conducted, and clinical signs have not been monitored. Such studies may provide insight into systemic toxicity and potential health threats associated with acute-duration exposure. With the exception of effects caused by direct contact of 3,3'-dichlorobenzidine with the skin or the eyes, the limited pharmacokinetic data do not suggest route-specific target organs. The available data were inadequate for derivation of either inhalation or oral acute-duration MRLs.

Intermediate-Duration MRLs. No intermediate-duration studies in humans were located. Intermediate-duration oral studies have been performed in rats but no adverse systemic effects were reported. However, only one dose level was examined in all studies reviewed (Griswold et al. 1968; Ito et al. 1983; Osanai 1976; Pliss 1959, 1963). Organs and/or tissues from the reproductive, neurological, and immunological systems have not been examined in the available intermediate-duration studies; such information would be useful. No intermediate-duration inhalation or dermal studies were found. Animal studies evaluating toxicological parameters at several dose levels would provide dose-response data that could help better assess potential adverse effects in humans following intermediate-duration exposure. No oral intermediate-duration MRL was derived, because the available studies did not identify relevant noncancer effects.

Chronic-Duration MRLs. Studies examining noncancer endpoints in humans following chronic exposure to 3,3'-dichlorobenzidine are limited and insufficient for derivation of a chronic MRL. Available chronic-duration oral studies provide information regarding systemic and carcinogenic effects in rats and dogs (Stula et al. 1975, 1978). These studies employed one dose level and the toxicological parameters measured were limited. Serious effects were seen at the lowest dose tested. The inadequacies of these studies precluded derivation of a chronic oral MRL. No chronic-duration animal inhalation or dermal exposure studies were located. Well conducted chronic-duration inhalation, dermal, and oral studies involving low-dose exposure in animals might provide dose-response data on potential systemic effects that could be extrapolated to humans. The available data are insufficient to establish a relationship between the concentration of 3,3'-dichlorobenzidine and/or its metabolites in the body and the levels that are associated with adverse effects. Studies that provide data on the body burden of 3,3'-dichlorobenzidine associated with toxicity may prove useful.

Various studies have assessed the potential carcinogenicity of 3,3'-dichlorobenzidine in exposed workers (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Millerick-May et al. 2021; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004). However, many confounders have rendered the results inconclusive. A major difficulty in such studies is the simultaneous exposure to several potential or known carcinogens. The carcinogenicity of 3,3'-dichlorobenzidine has been well established in animals after oral administration of the compound (Osanai 1976; Pliss 1959, 1963; Stula et al. 1975, 1978), but no information is available regarding inhalation and dermal exposure. There is suggestive evidence that 3,3'-dichlorobenzidine may cause cancer in animals when applied dermally since tumors were found in rats injected with the compound subcutaneously (Pliss 1963). Of particular interest would be additional studies, using relevant routes of exposure, to confirm the findings that 3,3'-dichlorobenzidine causes cancer in offspring of rats injected with the chemical subcutaneously during pregnancy (Golub et al. 1975).

Health Effects. Few studies on human exposure to 3,3'-dichlorobenzidine were located, especially regarding noncancerous endpoints for all exposure routes. There is a need for studies examining noncancerous endpoints for 3,3'-dichlorobenzidine, particularly inhalation and dermal routes, which are more likely for workers. There is also need of human studies that identify the biological fate of 3,3'-dichlorobenzidine in the human body (see Absorption, Distribution, Metabolism, and Excretion below) to better distinguish if observed effects are due to the chemical. Additionally, no animal or human studies identified a specific target organ for 3,3'-dichlorobenzidine; studies examining this are needed.

Hepatic. Liver injury was seen in one of six dogs chronically exposed to 3,3'-dichlorobenzidine, noted by marked fatty change in the liver (Stula et al. 1978). All dogs exhibited modestly elevated ALT levels. Further intermediate- or chronic-duration oral animal studies testing a range of doses would help to determine the dose and time dependency of hepatotoxicity in animals.

Neurological. The nervous system has not been evaluated after exposure to 3,3'-dichlorobenzidine. A chronic-duration oral study in dogs reported convulsions in one of six dogs treated orally with 3,3'-dichlorobenzidine (Stula et al. 1978). Upon necropsy, the authors noticed slight neuronal degeneration in tissues (unspecified) of the nervous system from the convulsing dog. However, the effect was seen in only one of the six dogs and only one dose level was tested. The limited information available does not suggest that 3,3'-dichlorobenzidine is a neurotoxicant. However, any future long-term toxicity study on 3,3'-dichlorobenzidine in animals should include histological evaluation of representative elements of the nervous system. Furthermore, evaluation of neurological endpoints in offspring from animals exposed during gestation would provide information that may be relevant to children of pregnant women exposed to 3,3'-dichlorobenzidine in the workplace.

Developmental. Animal studies have shown that 3,3'-dichlorobenzidine and/or its metabolites may be transferred across the placenta and/or through maternal milk to the offspring and may affect the growth of the kidneys after parenteral exposure during pregnancy (Golub 1969; Shabad et al. 1972) or induce tumors in the offspring (Golub et al. 1975). Future animal studies examining various dose levels and relevant exposure routes during critical developmental periods may provide information on potential fetotoxicity, embryotoxicity, and teratogenic effects in humans. Also, cross-fostering studies may help determine the relative impacts of in utero transfer of the chemical and transfer through breast milk. Further animal data may provide dose-response information if studies are conducted to determine what dose of 3,3'-dichlorobenzidine, or its metabolites, reaches the fetus.

Cancer. Cancer studies of manufacturing workers suggest 3,3'-dichlorobenzidine may lead to the formation of bladder tumors and bladder cancer (Millerick-May et al. 2021; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004). Although these studies did not have information on cigarette smoking status, they were able to indirectly control for smoking by examining other smoking related cancers such as lung cancer, which were not statistically increased in the cohort (Rosenman and Reilly 2004). Moreover, they were not able to

distinguish between the effect of 3,3'-dichlorobenzidine or other chemicals that workers were exposed to (Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996). Studies on animals have also observed the development of tumors and carcinomas in various locations following oral exposure to 3,3'-dichlorobenzidine (Pliss 1959, 1963; Stula et al. 1975, 1978). At the same time, several studies did not report formations of tumors or carcinomas in animals, especially in the bladder (Griswold et al. 1968; Saffiotti et al. 1967). Additional studies of animals are needed to better establish whether exposure to 3,3'-dichlorobenzidine and the formation of cancerous tumors and carcinomas is an endpoint of concern among exposed workers.

Genotoxicity. Available studies in animals and in bacterial systems show that 3,3'-dichlorobenzidine did alter genetic material (Ashby and Mohammed 1988; Bratcher and Sikka 1982; Chen et al. 2003, 2014; Chung et al. 2000; Cihak and Vontorkova 1987; Garner et al. 1975; Hering et al. 2018; Iba 1987; Imaoka et al. 1997; Lazear et al. 1979; Makena and Chung 2007; Sasaki et al. 1999; Savard and Josephy 1986; Shiraishi 1986; Wang et al. 2005). Studies involving additional test systems may allow a better assessment of mutagenic potential.

Epidemiology and Human Dosimetry Studies. The potential for occupational exposure exists in the use of 3,3'-dichlorobenzidine in the synthesis of 3,3'-dichlorobenzidine-based pigments for printing ink applications and to a lesser extent in paints. Dermatitis is the only noncancerous adverse health effect that appears to be associated with 3,3'-dichlorobenzidine exposure attributed to a manufacturing process change that resulted in exposure to 3,3'-dichlorobenzidine-free base (Gerarde and Gerarde 1974). Studies of occupationally exposed individuals have been complicated by the fact that there is usually simultaneous exposure to other chemicals. Based on available data, the potential for nonindustrial exposure to the general population by air, soil, or water is expected to be negligible. Epidemiological studies of people who live in areas where 3,3'-dichlorobenzidine has been detected in groundwater, near industries releasing 3,3'-dichlorobenzidine, or near hazardous waste sites are not likely to provide information on whether 3,3'-dichlorobenzidine exposure produces effects in humans given the likelihood that the levels of exposure to these populations has been low. In the unlikely event that exposure of the general population (in the past or present) to 3,3'-dichlorobenzidine occurs, individuals should be monitored for dermal effects (as reported earlier by Gerarde and Gerarde 1974).

No studies were located that monitored human tissues for content of 3,3'-dichlorobenzidine or its metabolites. 3,3'-Dichlorobenzidine is excreted in urine. If 3,3'-dichlorobenzidine and metabolites can be

detected and correlated with exposure, it may be possible to correlate urinary levels of 3,3'-dichlorobenzidine or its metabolites, with systemic effects.

Biomarkers of Exposure and Effect. Methods for the determination of 3,3'-dichlorobenzidine in urine and serum have been reported (Birner et al. 1990; Bowman and Nony 1981; Knoell et al. 2012; Lee et al. 2003; Nony and Bowman 1980; Nony et al. 1980; Zwirner-Baier and Neumann 1994). Some of the methods have been shown to be suitable for the determination of the acetylated metabolites (Bowman and Nony 1981; Nony and Bowman 1980; Nony et al. 1980). The methods of Birner et al. (1990), Joppich-Kuhn et al. (1997), and Zwirner-Baier and Neumann (1994) permit the analysis of hemoglobin adducts of 3,3'-dichlorobenzidine and its monoacetyl metabolite. Monitoring of hemoglobin adducts combined with measuring urinary 3,3'-dichlorobenzidine concentrations showed that both tests together were effective tools of biological monitoring in humans (Knoell et al. 2012). Limits of detection for 3,3'-dichlorobenzidine in urine and serum were reported to be as low as 1–5 ppb (Bowman and Rushing 1981; Hofman and Schmidt 1993; Roberts and Rossano 1982), with detectable concentrations of the acetylated metabolites somewhat higher. Defining the levels of these biomarkers associated with exposures to 3,3'-dichlorobenzidine of toxicological concern can increase their utility.

There are no specific disease states in humans or animals that have been associated with exposure to 3,3'-dichlorobenzidine. Hemoglobin adducts have been isolated from the blood of 3,3'-dichlorobenzidine-treated animals (Birner et al. 1990; Joppich-Kuhn et al. 1997; Lee and Shin 2002) and exposed humans (Knoell et al. 2012). It is not known what relationship exists between adduct levels in the blood and 3,3'-dichlorobenzidine toxicity. Further research in animal models is needed to determine if these adducts could be correlated with effects of 3,3'-dichlorobenzidine exposure. Further studies to identify more sensitive toxic effects (noncancer) that are specific for 3,3'-dichlorobenzidine would be useful in monitoring effects in people living near hazardous waste sites containing 3,3'-dichlorobenzidine.

Absorption, Distribution, Metabolism, and Excretion. Available data are insufficient to allow accurate evaluation of absorption, metabolism, or persistence of 3,3'-dichlorobenzidine in human tissues. Additional studies to identify and quantify metabolites of 3,3'-dichlorobenzidine in humans and animals would be useful to establish the relevance of animal studies in predicting human health effects. Metabolic handling of 3,3'-dichlorobenzidine in humans needs to be better characterized to be able to utilize urinary levels of the compound or its metabolites to quantitate human exposure.

Comparative Toxicokinetics. Pharmacokinetics studies have not been performed under conditions analogous to those of the carcinogenicity studies. Therefore, it is not possible to determine systemic levels of the compound associated with the reported effects. Pharmacokinetic data developed under exposure conditions associated with biological effects would markedly increase the possibility of improved species extrapolation for evaluating the true potency of 3,3'-dichlorobenzidine in humans.

Children's Susceptibility. The information on health effects of 3,3'-dichlorobenzidine in humans is derived exclusively from studies of occupational exposure (Gadian 1975; Gerarde and Gerarde 1974; MacIntyre 1975; Millerick-May et al. 2021; Myslak et al. 1991; Ouellet-Hellstrom and Rench 1996; Rosenman and Reilly 2004). In one occupational study, it was reported that contact with the free base form of 3,3'-dichlorobenzidine caused dermatitis (Gerarde and Gerarde 1974); it is reasonable to assume that children will respond in a similar manner under similar exposure conditions, although such exposure scenarios for children seem unlikely. There is no information available to determine whether children and adults are equally susceptible to the toxic effects of 3,3'-dichlorobenzidine. No studies in animals have addressed this issue.

There is no information on whether the developmental process is altered in humans exposed to 3,3'-dichlorobenzidine. Studies in animals have been inadequate (Golub 1969; Golub et al. 1975; Shabad et al. 1972), and further well-conducted research would be helpful to clarify whether the developmental process can be affected in animals exposed to 3,3'-dichlorobenzidine by a relevant exposure route. This includes information on whether 3,3'-dichlorobenzidine (or its metabolites) can cross the placenta and/or be transferred to offspring via breast milk. There are no data to evaluate whether the pharmacokinetics of 3,3'-dichlorobenzidine in children is different from adults. There are no PBPK models for 3,3'-dichlorobenzidine. There is no information to evaluate whether metabolism of 3,3'-dichlorobenzidine in children is different than in adults.

Continued research into the development of sensitive and specific biomarkers of exposure and effect for 3,3'-dichlorobenzidine would be valuable. There are no biomarkers of exposure or effect for 3,3'-dichlorobenzidine that have been validated for children or adults exposed as children. There are no biomarkers in adults that identify previous childhood exposure. There are no data on interactions of 3,3'-dichlorobenzidine with other chemicals in children or adults. There are neither adult nor pediatric-specific methods to reduce peak absorption of 3,3'-dichlorobenzidine following exposure, to reduce body burdens, or to interfere with 3,3'-dichlorobenzidine's mechanism of action. However, it is reasonable to assume that if children have the potential to be exposed, avoidance measures should be used.

Physical and Chemical Properties. It has been demonstrated that 3,3'-dichlorobenzidine is strongly adsorbed by soils and sediments, and that it may not readily desorb. Adsorption cannot be accurately predicted a priori; such data are soil-system specific and must be determined experimentally for each system under study. Because there is some discrepancy regarding the volatility of the free base form of 3,3'-dichlorobenzidine (CPMA 1998; Gerarde and Gerarde 1974), research in this area is indicated.

Production, Import/Export, Use, Release, and Disposal. 3,3'-Dichlorobenzidine is no longer used to produce dyes in the United States (alternative dyes based on other chemicals are available) (NLM 2019). Its use is not expected to increase or return to past levels of use in the future due to its classification as a carcinogen. There is evidence that it can be brought into the home on the shoes and clothing of adults who work with 3,3'-dichlorobenzidine (ATSDR 1996) but the quantity that might be present is unknown. In the workplace, OSHA regulations require that 3,3'-dichlorobenzidine be handled in closed systems and that shipping containers be cleaned thoroughly (again, within a closed system) before disposal (DCMA 1989).

3,3'-Dichlorobenzidine is most likely to be found in sediments and soils near current or former industrial sites where the chemical was used. Since the chemical's use in U.S. manufacturing has decreased, releases to the environment are expected to be very low. Citations regarding disposal techniques for 3,3'-dichlorobenzidine are found in the Hazardous Substances Data Bank (NLM 2019). Small quantities can be destroyed by chemical reaction, for example, with sodium hypochlorite solution, which converts 3,3'-dichlorobenzidine to a quinone-type compound. Incineration at high temperatures can be used to destroy work garments and miscellaneous solid wastes exposed to the compound. Presumably, only small amounts would need to be disposed of since the compound is mainly consumed during the manufacture of pigments.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA.

Environmental Fate. 3,3'-Dichlorobenzidine does not appear to biodegrade easily, but the few studies in this area did not state the type(s) or concentrations of microorganisms used in each study. More systematic studies with other organisms may prove useful. 3,3'-Dichlorobenzidine has been observed up to 6 km from its primary source, suggesting that the chemical travels when bound to sediments. Further

research on the chemical's ability to transport would provide needed data on exposure levels to the general population, especially from contact with contaminated soils.

A study by Nyman et al. 1997 provides evidence that in the span of a year, up to 80% of 3,3'-dichlorobenzidine can degrade to benzidine in anaerobic mixtures of sediment/water. Further research to identify the pathways and products of decomposition of 3,3'-dichlorobenzidine in various soils is needed.

Bioavailability from Environmental Media. The Canadian Government's Priority Substances List Assessment Report for 3,3'-dichlorobenzidine (Government of Canada 1993) reported that no data on the levels of 3,3'-dichlorobenzidine in drinking water or foodstuffs were identified within either Canada or the United States. 3,3'-Dichlorobenzidine has been found to bind strongly to soil constituents (Berry and Boyd 1985; Chung and Boyd 1987; Harden et al. 2005). Law (1995) concluded that it would also bind strongly to sedimentary material in the marine aquatic environment and may therefore have limited bioavailability. A study by Paraiba et al. (2006) suggested that 3,3'-dichlorobenzidine has the potential to bioconcentrate in orange orchards and its fruit, when the soil used is cultivated in sludge from industrial wastewater effluents. Further research into the chemical's bioavailability in fruits and other vegetation would provide evidence of possible exposure levels for the general public.

Food Chain Bioaccumulation. 3,3'-Dichlorobenzidine is bioconcentrated by aquatic organisms under experimental conditions. Whole-fish BCFs of around 500, with equilibration occurring in 96–168 hours, have been published (Appleton and Sikka 1980). In view of the n-octanol water partition coefficient for 3,3'-dichlorobenzidine, limited bioaccumulation could be expected (Law 1995) because the retention time of the chemical in exposed fish is short (Appleton and Sikka 1980). The ability of aquatic organisms to concentrate the compound could present a human health hazard if contaminated fish were eaten. However, 3,3'-dichlorobenzidine was not found in fish taken from waters in the vicinity of dye or textile manufacturing plants on the Buffalo and Delaware rivers in the United States (Diachenko 1979). It was concluded that monitoring for 3,3'-dichlorobenzidine in marine waters of the United Kingdom is unwarranted at present (Law 1995).

Exposure Levels in Environmental Media. There were no quantitative data on current atmospheric levels of 3,3'-dichlorobenzidine emissions or on the chemical's potential to act as a surface contaminant of soil environments. It is difficult to determine 3,3'-dichlorobenzidine levels in the aquatic environment because the concentrations tend to be at or below analytical detection limits. In general, it may only be possible to ascertain fully the environmental fate of 3,3'-dichlorobenzidine as analytical advances permit

the routine determination of very low concentrations. Moreover, determination of the nature and environmental fate of breakdown products of 3,3'-dichlorobenzidine would be useful. Reliable monitoring data for the levels of 3,3'-dichlorobenzidine in contaminated media at hazardous waste sites are needed so that the information obtained on levels of 3,3'-dichlorobenzidine in the environment can be used in combination with the known body burdens of 3,3'-dichlorobenzidine to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. It has been speculated that the 1974 OSHA regulations have reduced workplace air levels of 3,3'-dichlorobenzidine (CPMA 1998). However, it would be important to conduct exposure studies to monitor air levels in the workplace or conduct biomonitoring in workers to confirm this premise. There is continued need for more information on the extent of air, water, and soil contamination by industrial plant emissions or waste sites containing 3,3'-dichlorobenzidine. There is little information on exposure of children to 3,3'-dichlorobenzidine (or products derived from the compound). The compound has a very limited distribution and has only been detected in consumer goods sold abroad (other than in insoluble pigmented forms). Exposure may also occur from dietary sources, including aquatic organisms and fruit, but extent of the exposure is unknown. This information can be useful for assessing the need to conduct health studies on these populations.

Exposures of Children. There is no available information on exposure of children to 3,3'-dichlorobenzidine (or products derived from the compound). The compound has been found in trace amounts in some cosmetics, skin care, and personal hygiene products available for consumer use abroad (Hailong et al. 2014). Quantifying child exposure to the chemical by dermal use of these products, or accidental ingestion, would be useful in determining if exposure is of concern. Inadvertent take-home exposure by occupationally exposed parents could also be explored. A public health assessment (ATSDR 1996) found measurable levels of 3,3'-dichlorobenzidine (10.5 ppm in vacuum cleaner bags and 0.74 ppm in clothes dryer lint) in the homes of workers who were employed in manufacturing or processing the compound.

6.3 ONGOING STUDIES

No ongoing studies were identified for 3,3'-dichlorobenzidine during the literature search or in the NIH Research Portfolio Online Reporting Tools (RePORTER 2021).