CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

No studies were located regarding *N*-nitrosodiphenylamine in humans. Limited information available in animals is summarized below.

- *N*-nitrosodiphenylamine is absorbed through the gastrointestinal tract, but quantitative data are not available.
- No distribution data are available.
- Limited data suggest that the main metabolic pathway for *N*-nitrosodiphenylamine is cytochrome P-450-dependent denitrosation and ring hydroxylation in the liver.
- In animals, *N*-nitrosodiphenylamine was eliminated primarily in the urine and the main metabolite appeared to be nitrate.

3.1.1 Absorption

No studies were located regarding absorption of *N*-nitrosodiphenylamine in humans following any route of exposure or in animals following inhalation exposure.

The appearance of metabolites in the urine of rats and in the serum of rats and guinea pigs following oral administration provides indirect evidence of gastrointestinal absorption of *N*-nitrosodiphenylamine (Appel et al. 1984; Dodd et al. 2013; Tatsumi et al. 1983). Furthermore, the occurrence of systemic effects in rats and mice in oral studies suggests that *N*-nitrosodiphenylamine is absorbed through the gastrointestinal tract in these animals (Cardy et al. 1979; Dodd et al. 2013; NCI 1979).

3.1.2 Distribution

No studies were located regarding distribution of *N*-nitrosodiphenylamine in humans or animals following any route of exposure.

3.1.3 Metabolism

No studies were located regarding metabolism of *N*-nitrosodiphenylamine in humans.

In experiments with animals, the reaction in which *N*-nitrosodiphenylamine is denitrosated to diphenylamine and nitric oxide seems to be the first step in the metabolic activation of *N*-nitrosodiphenylamine (Appel et al. 1984). Following administration of a single dose of *N*-nitrosodiphenylamine in corn oil (1,000 mg/kg) to female Wistar rats, nitrate was identified as the major urinary metabolite, while nitrite, diphenylamine, and a monohydroxydiphenylamine were found in smaller amounts. These results suggested that *N*-nitrosodiphenylamine is denitrosated to diphenylamine and nitric oxide and then the nitric oxide is converted into nitrite and nitrate. Nitrite can be oxidized in substantial amounts to nitrate (Appel et al. 1984).

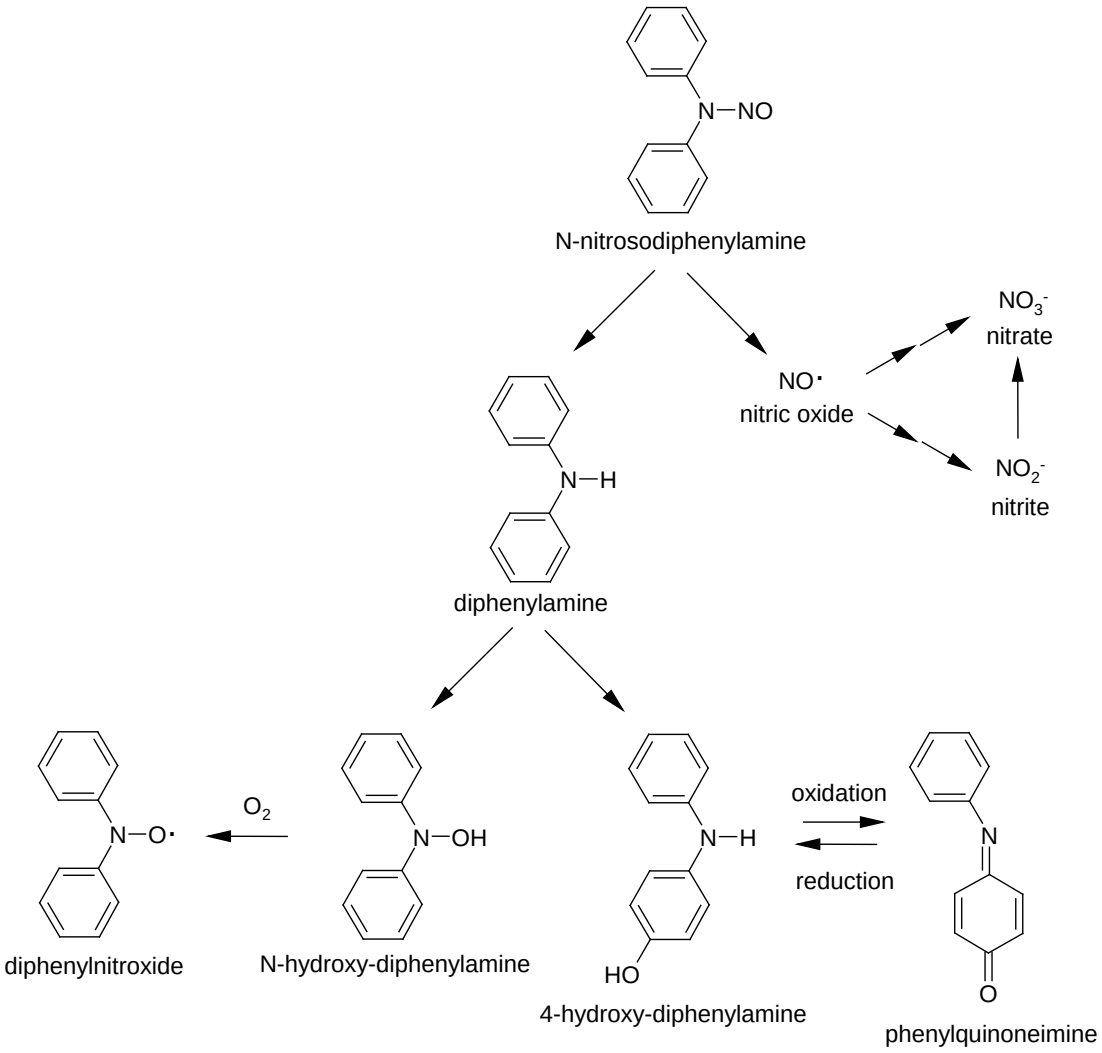
In vitro studies investigated the metabolism of *N*-nitrosodiphenylamine in phenobarbital-induced mouse liver microsomes (Appel et al. 1987a, 1987b, 1987c). The metabolites found were diphenylamine, 4-hydroxydiphenylamine, and its oxidized product, the corresponding quinoneimine. The authors concluded that diphenylamine undergoes ring hydroxylation to form 4-hydroxydiphenylamine, which is oxidized to the quinoneimine. Since *N*-hydroxylation is recognized as the initial step in the bioactivation of carcinogenic arylamines, the *N*-hydroxy derivative of diphenylamine may be a potential metabolite. This possible metabolite, however, has not been detected using microsomal incubation. A postulated metabolic scheme based on these data is presented in Figure 3-1.

In vitro studies conducted with rat and mouse liver cytochrome *P*-450 demonstrated the denitrosation of *N*-nitrosodiphenylamine (Appel et al. 1979; Schrenk et al. 1982; Wakabayashi et al. 1982).

Transnitrosation of proline by *N*-nitrosodiphenylamine occurred in male BD VI rats that were orally administered 28.28 mg/kg *N*-nitrosodiphenylamine and 50 µmol proline by gavage (Ohshima et al. 1982). The excretion of *N*-nitrosoproline was 15-fold higher than in the controls. Co-administration of thiocyanate had a catalytic effect, which resulted in a 58-fold increase in the urinary levels of *N*-nitrosoproline.

N-Nitrosodiphenylamine can undergo reductive metabolism by liver aldehyde oxidase under anaerobic conditions (Tatsumi et al. 1983). Guinea pigs received oral dosages (200 mg/kg) of *N*-nitrosodiphenylamine. Just before and 3 hours after administration of *N*-nitrosodiphenylamine, the guinea pigs were treated with oral dosages (50 mg/kg) of acetaldehyde (an electron donor). Acetaldehyde diphenylhydrazone was identified as a plasma metabolite.

Figure 3-1. Metabolic Pathways for *N*-Nitrosodiphenylamine



Source: Appel et al. 1987b

3.1.4 Excretion

No studies were located regarding excretion of *N*-nitrosodiphenylamine in humans following any route of exposure or in animals following inhalation and dermal exposure.

One study was located that investigated excretion in animals. After oral administration of a single l,000-mg/kg dose of *N*-nitrosodiphenylamine to female Wistar rats, the maximum urinary excretion of nitrate and nitrite was found 24–48 hours after administration (Appel et al. 1984). Within 36 hours of administration, 24.8 and 1.4% of the administered dose of *N*-nitrosodiphenylamine was excreted as nitrate

and nitrite, respectively. Ninety-six hours after administration, about 30% of the administered dose had been eliminated as nitrite and nitrate.

In female Wistar rats, the maximum urinary nitrate or nitrite excretion was found in the 24 hours following intraperitoneal administration of 500 mg/kg *N*-nitrosodiphenylamine (Appel et al. 1984). This is a more rapid elimination than that following oral dosing. Ninety-six hours after administration, approximately 50% of the administered dose was detected as nitrate and nitrite—almost twice as much as was found after oral administration. Diphenylamine and hydroxydiphenylamine were also present as urinary metabolites. The rate of denitrosation after intraperitoneal injection was considerably higher than after oral administration. This was probably due to an altered availability of *N*-nitrosodiphenylamine to the liver.

Results from a study of rats, rabbits, and guinea pigs receiving 50 mg/kg *N*-nitrosodiphenylamine through intraperitoneal injection suggested that the rate of excretion of *N*-nitrosodiphenylamine into the bile and elimination of the chemical from the bile varies among species (Atawodi and Maduagwu 1990). Guinea pigs showed the most rapid excretion of *N*-nitrosodiphenylamine into the bile. Rabbits had the slowest excretion of *N*-nitrosodiphenylamine into the bile, but the most rapid elimination of the chemical from the bile. Both excretion to and elimination from bile were comparatively slow in the rat. The half-lives for *N*-nitrosodiphenylamine elimination from bile for these species are as follows: 95 minutes for rabbits, 240 minutes for guinea pigs, and 510 minutes for rats.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

No PBPK model has been developed for *N*-nitrosodiphenylamine.

3.1.6 Animal-to-Human Extrapolations

There is virtually no information on the toxicity of *N*-nitrosodiphenylamine in humans and there are limited data in animals, so predicting possible adverse health outcomes in humans based on results from animal studies would be highly speculative and inappropriate.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to *N*-nitrosodiphenylamine are discussed in Section 5.7, Populations with Potentially High Exposures.

It is difficult to determine persons with increased risk because there are limited data on the toxicity of *N*-nitrosodiphenylamine. People who have bladder dysfunction or disease may be more susceptible since the primary effect of *N*-nitrosodiphenylamine in animals is bladder cancer.

The alterations in activities of phase I and phase II metabolic enzymes in the liver by *N*-nitroso-diphenylamine (Sheweita and Mostafa 1996a, 1996b) may affect the metabolism of other chemicals. Whether this will result in increased or reduced toxicity of a particular chemical will depend on the specific enzymes involved and whether metabolism leads to production of an active metabolite or is a detoxifying reaction.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to *N*-nitrosodiphenylamine are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for *N*-nitrosodiphenylamine from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by *N*-nitrosodiphenylamine are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

N-Nitrosodiphenylamine can be detected and quantitated in the blood, serum, and urine of animals, with the lowest detection limits for serum (Pylypiw and Harrington 1981). Limited animal data suggest that

suspected metabolites of *N*-nitrosodiphenylamine can also be detected in the urine. However, these methods do not appear to have been used to test humans for exposure and no monitoring data for *N*-nitrosodiphenylamine were located. Therefore, no conclusion regarding the usefulness of these potential biomarkers in humans can be made, although it is reasonable to assume that they can indicate exposure. There are no other known biomarkers of exposure to *N*-nitrosodiphenylamine.

There are no data on how long *N*-nitrosodiphenylamine persists in the body of humans or animals. In one study, 96 hours after the administration of an oral dose, 30% of the dose had been eliminated in the urine (Appel et al. 1984). However, it is not known how much was eliminated in the feces or by other routes and how much was retained in the body. No data are available regarding the exposure levels that would result in levels detectable in body fluids.

3.3.2 Biomarkers of Effect

Based on data in rats and mice, the target organ appears to be the urinary bladder. Observed effects consist of epithelial hyperplasia and squamous metaplasia of the bladder (NCI 1979). These effects were seen at the lowest dose tested (15 mg/kg/day), and the effect is only observable postmortem. In addition, these effects can occur from other circumstances such as disease, exposure to drugs, and exposure to other chemicals, and are not unique to *N*-nitrosodiphenylamine. Therefore, they are not useful as specific biomarkers of effect for *N*-nitrosodiphenylamine. There are so few data available on the chemical that it is difficult to associate specific symptoms with exposure to *N*-nitrosodiphenylamine.

3.4 INTERACTIONS WITH OTHER CHEMICALS

N-Nitrosodiphenylamine was mutagenic in *Salmonella* strains TA98 and TA1535, but not TA100, in preincubation assays with rat liver S-Y fractions only in the presence of the comutagen norharman (9H-pyrido-[3,4b]indole) (Nagao and Takahashi 1981; Wakabayashi et al. 1981, 1982).

In mice treated with *N*-nitrosodiphenylamine prior to pentobarbital administration, pentobarbital sleeping time was significantly shortened compared to control mice given only the corn oil vehicle (Nishie et al. 1972). This was believed to be due to induction of liver enzymes that could metabolize pentobarbital.