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

3.1 TOXICOKINETICS

There are limited data regarding N-nitrosodi-n-propylamine toxicokinetics in humans and laboratory animals; these data are summarized below.

- N-Nitrosodi-n-propylamine is absorbed following oral and dermal exposure, and presumably following inhalation exposure; however, no data are available on the rate or extent of absorption.
- There are limited data on the distribution of N-nitrosodi-n-propylamine. Studies of related nitrosoamines suggest that it would be widely distributed.
- The primary pathway of metabolism of N-nitrosodi-n-propylamine is hydroxylation at the α-carbon. This pathway ultimately results in the formation of propionaldehyde, 1-propanol, and 2-propanol metabolites.
- N-Nitrosodi-n-propylamine is primarily excreted in the urine as metabolites.

3.1.1 Absorption

No studies were located regarding absorption in humans or animals following inhalation exposure to N-nitrosodi-n-propylamine. However, structurally similar compounds, such as N-nitrosodimethylamine and N-nitrosodiethanolamine, are readily absorbed (70–90% of the dose) following inhalation exposure in experimental animals (Klein and Schmezer 1984; Preussmann et al. 1981). Absorption was inferred by monitoring urinary excretion of the unchanged compounds.

No studies were located regarding absorption in humans following oral exposure to N-nitrosodi-n-propylamine. Specific information regarding absorption in animals following oral exposure to N-nitrosodi-n-propylamine was not located. Gastrointestinal absorption of N-nitrosodi-n-propylamine by rodents is indicated by the occurrence of metabolites in the urine following oral treatment (Section 3.4.1) and effects in oral carcinogenicity and toxicity studies (Chapter 2). Other nitrosamines are rapidly absorbed from the gastrointestinal tract after oral exposure. Diaz Gomez et al. (1977) found that <2% of radiolabelled dimethylnitrosamine could be recovered from the stomach and intestine of rats 15 minutes after administration. Also in rats, Lijinsky et al. (1981) and Preussmann et al. (1978) estimated absorption extents of 25 and 70% of the dose for N-nitrosodiethanolamine, respectively (estimates are from urinary excretion).

Absorption of N-nitrosodi-n-propylamine through human skin *in vivo* (Edwards et al. 1979) and *in vitro* (Bronaugh et al. 1979, 1981) has been demonstrated. Diffusion of N-nitrosodi-n-propylamine through rat skin *in vitro* has been demonstrated (Wishnok et al. 1982). Information regarding dermal absorption of N-nitrosodi-n-propylamine by animals *in vivo* was not located in the reviewed literature. Dermal absorption of N-nitrosodiethanolamine has been determined in pigs (Marzulli et al. 1981), monkeys (Marzulli et al. 1981), and rats (Airoldi et al. 1984; Lijinsky et al. 1981). The degree of absorption varied greatly (4–78%); differences in the test animal species, site of the application, and the vehicle used preclude direct comparisons across the studies. Based on the data for N-nitrosodimethylamine and N-nitrosodiethanolamine, it is likely that N-nitrosodi-n-propylamine will be absorbed following dermal exposure.

3.1.2 Distribution

Route-specific distribution data for N-nitrosodi-n-propylamine in humans were not located in the reviewed literature. Quantitative analyses of six volatile nitrosamines in postmortem organs (brain, liver, kidneys, pancreas) from four human subjects were conducted (Cooper et al. 1987). N-Nitrosodi-n-propylamine was detected only in the liver of one of the subjects (female, age 84 years) at a concentration of 19.30 ng/50 g of tissue. The ages of the other subjects (two males, one female) ranged from 47 to 80 years. N-Nitrosodi-n-propylamine was not detected in the other examined tissues (brain, kidney, and pancreas) from the four subjects. Unusual sources of nitrosamine exposure or causes of death were not indicated.

Transplacental transport of N-nitrosodi-n-propylamine was shown in pregnant hamsters (Althoff and Grandjean 1979; Althoff et al. 1977a). After a single 100 mg/kg subcutaneous injection, N-nitrosodi-n-propylamine was detected in the maternal blood, placenta, fetus, and amniotic fluid. The concentration of the chemical in maternal blood reached a maximum 45 and 90 minutes after the injection, whereas a single peak at 90 minutes was observed in the fetus. Analysis for metabolites was not conducted, but 1.6 and 1.3% of the unchanged compound was found in the placenta and in the fetus, respectively, at day 14 of gestation. Detection of 0^6-methylguanine in human placental DNA by immunoassay indicate that nitrosamines, as a group, can reach the placenta in humans (Foiles et al. 1988).

The limited data available regarding the distribution of related nitrosamines suggest that they are widely distributed in the body. Daugherty and Klapp (1976) reported that after oral administration of ^{14}C-N-nitrosodimethylamine to mice, radioactivity could be detected in the homogenates of heart,

forestomach, esophagus, liver, and lungs. Radioactivity was detected in all organs and tissues of rats after oral doses of ^{14}C-N-nitrosodiethanolamine (Lethco et al. 1982). After intravenous injection of ^{14}C-N-nitrosodi-n-butylamine to rats, the highest concentrations of radiolabel occurred in the nasal mucosa, liver, and preputial gland (Brittebo and Tjalve 1982).

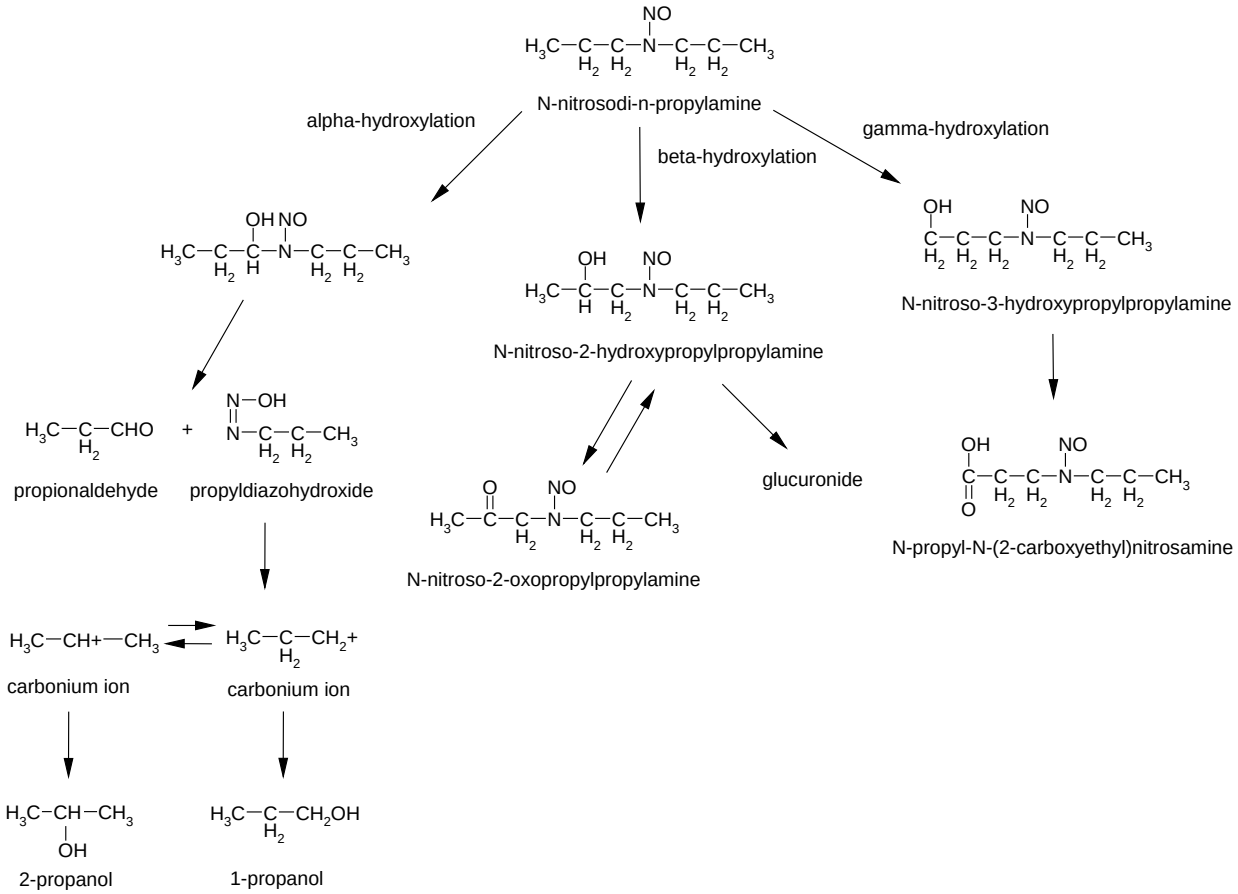
3.1.3 Metabolism

No studies were located regarding metabolism in humans following exposure to N-nitrosodi-n-propylamine. *In vitro* and *in vivo* studies with rodents have been conducted that provide evidence that N-nitrosodi-n-propylamine can be metabolized via oxidation at the alpha, beta, and gamma carbon positions (Figure 3-1). Alpha carbon oxidation (hydroxylation) is regarded as the primary pathway, resulting in formation of propionaldehyde and 1-propanol and 2-propanol as metabolites (Farrelly et al. 1984; Park and Archer 1978; Park et al. 1977). 1-Propanol and 2-propanol are formed via propyldiazohydroxide and a propyl cation (carbonium ion). It is generally believed that the carbonium ions can also react with nucleic acids to form propylated adducts, but Park et al. (1980) have suggested that propylation takes place via a bimolecular reaction. However, reaction of DNA with propylnitrosourea (a direct acting equivalent of N-nitrosodi-n-propylamine) results in formation of n-propyl and isopropyl DNA adducts, suggesting that carbonium ions are involved. Alkylation of nucleic acids and proteins by metabolites of nitrosamines has been suggested as the mechanism responsible for the toxic and carcinogenic properties of these substances.

Beta-carbon hydroxylation yields N-nitroso-2-hydroxy-propylpropylamine, which is excreted as the glucuronide or further oxidized to a small extent to N-nitroso-2-oxopropylpropylamine (Bauman et al. 1985; Leung and Archer 1981; Park and Archer 1978; Suzuki and Okada 1981). Methylated hepatic nucleic acids have been recovered from rats and hamsters treated with N-nitrosodi-n-propylamine (Althoff et al. 1977b; Kruger 1971, 1973; Kruger and Bertram 1973; Leung and Archer 1984). Putative methylating intermediates, formed from N-nitroso-2-oxo-n-propylamine, are N-nitrosomethyl-propylamine and diazomethane.

Gamma-carbon hydroxylation yields N-nitroso-3-hydroxy-propylpropylamine and its oxidation product, N-propyl-N-(2-carboxyethyl)nitrosamine (Baumann et al. 1985; Blattmann and Preussman 1973; Suzuki and Okada 1981). Urinary N-propyl-N-(2-carboxyethyl)nitrosamine amounted to approximately 5% of a 300 mg/kg oral dose of N-nitrosodi-n-propylamine in rats (Suzuki and Okada 1981).

Figure 3-1. Metabolism of N-Nitrosodi-n-Propylamine



Several studies have examined the cytochrome P450 isoforms involved in N-nitrosodi-n-propylamine metabolism. Induction of cytochrome P450 2B1 resulted in increases in dealkylation specific for α-carbons (Shu and Hollenberg 1996). Cytochrome P450 2E1 is the predominant isoform responsible for N-nitrosodi-n-propylamine α-hydroxylation (Shu and Hollenberg 1996; Teiber and Hollenberg 2000; Teiber et al. 2001), whereas cytochrome P450 isoforms 1A1/1A2 and 4A1/4A2 do not appear to be involved in N-nitrosodi-n-propylamine metabolism (Shu and Hollenberg 1996). Cytochrome P450 2E1 and 2B1 isoforms also mediate the oxidation of the metabolite, N-nitroso-2-hydroxypropylpropylamine, to N-nitroso-2-oxopropylpropylamine (Teiber et al. 2001).

Documented and postulated metabolites of N-nitrosodi-n-propylamine have been shown to be carcinogenic in hamsters and rats (IARC 1978). These include N-nitroso-bis-(2-hydroxy-n-propyl)amine, N-nitroso-2-oxo-n propylpropylamine, N-nitroso-bis(2-oxo-n-propyl)amine, and N-nitroso-bis(2acetoxy-n-propyl)amine. Main tumor sites of many of these metabolites include those associated with N-nitrosodi-n-propylamine treatment (Section 2.19).

3.1.4 Excretion

Rats excreted metabolites but not unchanged N-nitrosodi-n-propylamine in the urine following oral dosing with N-nitrosodi-n-propylamine (Blattmann and Preussmann 1973; Suzuki and Okada 1981). The principal metabolite in the Suzuki and Okada (1981) study, N-propyl-N-(2-carboxyethyl)nitrosamine, amounted to approximately 5% of the administered dose. Additional information regarding the extent or rate of excretion in either of the studies was not reported.

Excretion of unchanged N-nitrosodiethanolamine in the urine of rats has been reported in several studies after cutaneous application of N-nitrosodiethylamine (Airoldi et al. 1984; Lijinsky et al. 1981; Preussmann et al. 1981).

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 models were identified for N-nitrosodi-n-propylamine.

3.1.6 Animal-to-Human Extrapolations

No studies were identified that provide evidence to suggest differences in the toxicity or toxicokinetics of N-nitrosodi-n-propylamine. Most of the available toxicity/carcinogenicity studies have been conducted in rats, which does not allow for a comparison across species. Studies in rats and hamsters have demonstrated the N-nitrosodi-n-propylamine metabolites in both species (IARC 1978).

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-nitrosodi-n-propylamine are discussed in Section 5.7, Populations with Potentially High Exposures.

No studies have evaluated the toxicity of N-nitrosodi-n-propylamine in children; the toxicity is assumed to be similar to that in adults. Although the developmental toxicity has not been evaluated following inhalation, oral, or dermal exposure; a parenteral exposure study demonstrated the transplacental carcinogenicity of N-nitrosodi-n-propylamine (Althoff and Grandjean 1979; Althoff et al. 1977a).

No populations with unusual susceptibility to health effects of N-nitrosodi-n-propylamine have been identified. However, heavy consumers of alcoholic beverages might be considered to be a susceptible population based on a single report in which ethanol was shown to potentiate the carcinogenicity of N-nitrosodi-n-propylamine in mice (Griciute et al. 1982). Co-exposure to other compounds that induce CYP2E1 may result in increased metabolism of N-nitrosodi-n-propylamine, which could result in increased toxicity and/or carcinogenicity.

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-nitrosodi-n-propylamine 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-nitrosodi-n-propylamine 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-nitrosodi-n-propylamine 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

No biomarkers of exposure have been identified for N-nitrosodi-n-propylamine.

3.3.2 Biomarkers of Effect

No biomarkers of effect have been identified for N-nitrosodi-n-propylamine.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Ethanol was found to enhance the carcinogenicity of N-nitrosodi-n-propylamine. Mice that were treated with estimated 1 mg/kg doses of N-nitrosodi-n-propylamine dissolved in 40% ethanol by gavage, twice a week for 50 weeks, developed higher incidences of tumors than mice that were similarly treated with the same dose of compound in water (Griciute et al. 1982). The most pronounced tumor enhancement was in the forestomach (51% carcinomas versus 10% in N-nitrosodi-n-propylamine/water group), but increases in pulmonary adenomas and lymphomas also occurred.