# DRAFT

## For Review Only

# Public Health Goal for
# N-Nitrosodimethylamine
# in Drinking Water

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# PREFACE

**Drinking Water Public Health Goals**
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This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to perform risk assessments and adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires that PHGs be set in accordance with the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which no known or anticipated adverse effects on health will occur, with an adequate margin of safety.
2. PHGs for carcinogens or other substances that may cause chronic disease shall be based solely on health effects and shall be set at levels that OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider potential adverse effects on members of subgroups that comprise a meaningful proportion of the population, including but not limited to infants, children, pregnant women, the elderly, and individuals with a history of serious illness.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. OEHHA shall consider additive effects of exposure to contaminants in media other than drinking water, including food and air, and the resulting body burden.
7. In risk assessments that involve infants and children, OEHHA shall specifically assess exposure patterns, special susceptibility, multiple contaminants with toxic mechanisms in common, and the interactions of such contaminants.

8. In cases of insufficient data for OEHHA to determine a level that creates no significant risk, OEHHA shall set the PHG at a level that is protective of public health with an adequate margin of safety.
9. In cases where scientific evidence demonstrates that a safe dose response threshold for a contaminant exists, then the PHG should be set at that threshold.
10. The PHG may be set at zero if necessary to satisfy the requirements listed above in items seven and eight.
11. PHGs adopted by OEHHA shall be reviewed at least once every five years and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations or technical feasibility, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. Each primary drinking water standard adopted by DHS shall be set at a level that is as close as feasible to the corresponding PHG, placing emphasis on the protection of public health. PHGs established by OEHHA are not regulatory in nature and represent only non-mandatory goals. By state and federal law, MCLs established by DHS must be at least as stringent as the federal MCL, if one exists.

PHG documents are used to provide technical assistance to DHS, and they are also informative reference materials for federal, state and local public health officials and the public. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not intended to be utilized as target levels for the contamination of other environmental media.

Additional information on PHGs can be obtained at the OEHHA Web site at www.oehha.ca.gov.

# LIST OF CONTRIBUTORS

To be added later

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# PUBLIC HEALTH GOAL FOR N-NITROSODIMETHYLAMINE IN DRINKING WATER

## SUMMARY

The Office of Environmental Health Hazard Assessment (OEHHA) has developed a proposed Public Health Goal (PHG) of $3\times10^{-6}$ mg/L (0.003 µg/L, 0.003 parts per billion, or ppb) for N-nitrosodimethylamine (NDMA) in drinking water. Studies by the California Department of Health Services (DHS) have documented the formation of NDMA during the water disinfection treatment process.

The proposed PHG is based on an extra cancer risk of 1 in 1 million for lifetime exposure to NDMA in drinking water. There is no California or federal Maximum Contaminant Level (MCL) for NDMA. The California Notification Level (previously known as Action Level) for NDMA is 0.01 µg/L. Notification levels are defined by DHS as "health-based advisory levels established by DHS for chemicals in drinking water that lack maximum contaminant levels (MCLs)."

Carcinogenic effects observed in animal studies were judged to be the most sensitive endpoint and therefore are the basis of the proposed PHG for NDMA. Significant increases in tumors have been observed in numerous species of animals administered NDMA by oral, inhalation or other routes of exposure. Evidence that specifically links exposure to NDMA to increased incidence of cancer in humans is generally lacking, but the available studies are suggestive. Studies on other nitrosamines support the presumption of potential human carcinogenicity of NDMA.

Using the findings of Peto *et al.* (1991b) a dose-response relationship was derived based on the occurrence of bile duct tumors in female rats using the linearized multistage model. The model was employed to estimate the dose associated with a 10 percent incidence of tumors. A linear relationship between dose and response was then employed to extrapolate to the one in a million cancer risk level, and corrected to human dose equivalents based on the ratio of rat and human body weights to the 3/4 power. Concentrations of NDMA associated with a lifetime theoretical extra cancer risk of $10^{-4}$ or $10^{-5}$ are 0.3 and 0.03 ppb, respectively.

Given the low volatility and skin permeability of NDMA, neither inhalation nor dermal exposure routes contribute significant amounts of exposure relative to the oral route. Therefore, the PHG was based on exposure by the oral route. A protective level has not been developed for non-cancer effects due to the lack of adequate toxicological studies that investigated other toxic endpoints. No specific sensitive populations were identified that were substantially at higher risk from the toxic effects of NDMA. The PHG level is judged to be adequately protective of lifetime exposure to NDMA in water.

## INTRODUCTION

N-nitrosodimethylamine is a chemical formed in industrial and natural processes and is also created from nitrates and nitrites in the human gut. The nitrosamines are generally considered to be classic carcinogens, and a large amount of basic research on cancer mechanisms has been carried out on them.

NDMA occurs in various foods and alcoholic beverages, and is also detected in cigarette smoke. NDMA has also been detected in California drinking water. This specific chemical has been extensively studied in experimental animals and is considered an animal carcinogen. Because of similarities in its metabolism to reactive intermediates in animals and humans, it is considered as a likely human carcinogen.

NDMA has become more important in California because of its increasing detection in drinking water. It has been associated with the chloramine drinking water disinfection process, and has also been reported to be formed in the chlorination of wastewater used for aquifer recharge. NDMA may be formed from the nitrogen species added for chloramination (DHS, 2003) or from dimethylamine functional groups on polymers used for water filtration (Mitch and Sedlak, 2004). Because of concern over the exposures and the carcinogenic properties of NDMA, California DHS requested that OEHHA develop a PHG for NDMA, to support the development of a California MCL.

California presently has only a notification level (previously known as Action Level) for NDMA, which has been established at 0.01 μg/L. If a chemical is detected above its notification level in a municipal drinking water supply, a utility must provide timely notification of the local governing bodies (e.g., city council, county board of supervisors, or both). If this water is provided to consumers, DHS recommends that the utility inform its customers and consumers about the presence of the chemical, and about health concerns associated with exposure to it. If a chemical is present at concentrations considerably higher than its notification level (the "response level" of Health and Safety Code §116455), DHS recommends that the drinking water system take the source out of service (DHS, 2005).

## CHEMICAL PROFILE

### *Chemical Identity*

N-nitrosodimethylamine, also commonly referred to as dimethylnitrosamine, is a yellow oily liquid at room temperature. The Chemical Abstracts Service Registry number for NDMA is 62-75-9; its molecular formula is $C_2H_6N_2O$. The chemical structure of NDMA is shown in Figure 1.

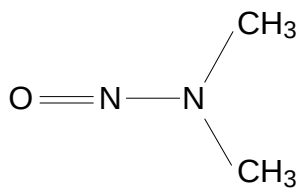



**Figure 1. Structure of N-nitrosodimethylamine**

## *Physical and Chemical Properties*

Physical and chemical properties of NDMA are summarized in the following table.

**Table 1. Physical and Chemical Properties of NDMA**

| **Property** | **Value** | **References** |
|---|---|---|
| Molecular weight | 74.08 | Weast, 1989 |
| Color | Yellow | IARC, 1978 |
| Physical state | Liquid | IARC, 1978 |
| Odor | No distinctive odor | Frank and Berry, 1981 |
| Melting point | -25 $^0$C (estimated) | Lyman, 1985 |
| Boiling point | 154 $^0$C | Weast, 1989 |
| Solubility | | |
| Water | Miscible | Mirvish *et al.*, 1976 |
| Organic solvents | Soluble in water, alcohol, ether | Weast, 1989; IARC, 1978 |
| Specific gravity | 1.0059 g/mL | Weast, 1989 |
| Partition coefficients | | |
| Log Octanol-$H_2O$ (Log $K_{ow}$) | -0.57 | Hansch *et al.*, 1995 |
| Log soil-organic carbon-$H_2O$ (Log $K_{oc}$) | 1.07 (estimate) | ATSDR, 1989 |
| Vapor pressure | 2.7 mm Hg (20$^0$C) | Klein, 1982 |
| Henry's law constant (37$^0$C) | 1.99 $10^{-6}$ atm-m$^3$/mol | Mirvish *et al.*, 1976 |
| Conversion factors**:** | | |
| mg/m$^3$ = 3.08 x ppm (air) (20$^0$C) | | ATSDR, 1989 |
| ppm (air) = mg/m$^3$ x 0.325 (20$^0$C) | | ATSDR, 1989 |

## *Production and Uses*

NDMA does not appear to be currently produced or commercially used (other than for research purposes) in the United States (HSDB, Hazardous Substance Data Base, 2004). It was formerly used in the production of rocket fuels and was used as an antioxidant,

additive for lubricants, and a softening agent for copolymers (HSDB, 2004). NDMA can form inadvertently and be released into the environment from a large array of manmade sources including tanneries, pesticide manufacturing plants, rubber and tire plants, foundries and dye manufacturers (ASTDR, 1989). It also appears to be formed in trace levels in the drinking water disinfection process (DHS, 2003; Mitch and Sedlak, 2004).

## ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

### *Air*

NDMA has a low vapor pressure and is not likely to sorb onto particulates to any extent (IPSC, 2002). In the ambient atmosphere, NDMA would be expected to rapidly degrade when exposed to sunlight, with a half-life estimate of 5 to 30 minutes (ATSDR, 1989).

### *Soil*

NDMA is highly mobile in soil and therefore has the potential to leach into groundwater (ATSDR, 1989). Little NDMA would be expected in surficial soils, as photolysis and volatilization would rapidly remove NDMA from the surface (IPSC, 2002).

### *Water*

NDMA is miscible in water. Given its low vapor pressure, very low Henry's constant and low octanol/water partition coefficient, little NDMA would be expected to sorb onto particles or volatilize from water. The California DHS conducted a survey of a number of water systems to determine the extent of NDMA occurrence in drinking water. NDMA was frequently detected in the finished drinking water (Table 2). NDMA may be occurring because it is present in sources of drinking water or may occur as a byproduct of disinfection (DHS, 2003). Chlorination of wastewater used for groundwater recharge and the chloramination process have both been associated with formation of NDMA. Some of the NDMA may be derived from interaction of chlorination chemicals with dimethylamine functional groups on polymers used for water filtration (Mitch and Sedlak, 2004).

Disinfection of drinking NDMA in liquid rocket fuels was the source of contamination of groundwater at rocket testing facilities in California at Baldwin Park in the San Gabriel Valley (see www.epa.gov/superfund/accomp/success/sangabriel.htm) and in Rancho Cordova. At the latter, concentrations of NDMA in groundwater onsite were up to 40,000 ng/L, and offsite at up to 20,000 ng/L (Mitch *et al*., 2003).

**Table 2. NDMA Results from Surface Water Treatment Plants[1]**

| Water System | Location | 1st Round NDMA Results (ppt) | 2nd Round NDMA results (ppt) |
|---|---|---|---|
| A | Plant Influent | <1 | |
| A | Distribution | <1 | |
| B | Plant Influent | <1 | <1 |
| B | Plant Effluent | <1 | <1 |
| B | Distribution | <1 | <1 |
| C | Plant Influent | <1 | |
| C | Plant Effluent | 1.8 | <1 |
| C | Distribution | 1.3 | <1 |
| D | Plant Influent | 1.3 | <1 |
| D | Plant Effluent | 3.9 | <1 |
| D | Distribution | <1 | <1 |
| E | Plant Influent | <1 | <1 |
| E | Plant Effluent | 3.3 | <1 |
| E | Distribution | <1 | <1 |
| F | Plant Influent | 1.1 | <1 |
| F | Plant Effluent | 1.5 | <1 |
| F | Distribution | 2.5 | <1 |
| G | Plant Influent | <1 | |
| G | Plant Effluent | <1 | |
| G | Distribution | <1 | |
| H | Plant Influent | 1.8 | <1 |
| H | Plant Effluent | <1 | <1 |
| H | Distribution | 1.4 | <1 |
| I | Plant Influent | <1 | |
| I | Plant Effluent | 1 | 2 |
| I | Distribution | 4.3 | 1.6 |
| J | Plant Influent | 9.4 | <1 |
| J | Plant Effluent | 2.3 | <1 |
| J | Distribution | 1.4 | <1 |
| K | Plant Influent | <1 | |
| K | Plant Effluent | <1 | 1.1 |
| K | Distribution | <1 | 1.8 |
| L | Plant Influent | 1.5 | <1 |
| L | Plant Effluent | 4.6 | <1 |
| L | Distribution | 4.4 | <1 |
| M | Plant Influent | <1 | |
| M | Plant Effluent | <1 | |
| M | Distribution | <1 | |
| N | Plant Influent | 1.2 | 1.1 |

| | | | |
|---|---|---|---|
| N | Plant Effluent | 5.3 | 4.5 |
| N | Distribution | 7.4 | 3.8 |
| O | Plant Influent | <1 | |
| O | Plant Effluent | <1 | |
| O | Distribution | 3.2 | <1 |
| P | Plant Influent | 2.4 | <1 |
| P | Plant Effluent | 3.2 | 1.8 |
| P | Distribution | 1.1 | 1.1 |
| Q | Plant Influent | <1 | |
| Q | Plant Effluent | <1 | |
| Q | Distribution | 1.8 | |
| R | Plant Influent | 1.1 | 1 |
| R | Plant Effluent | 2.4 | 1.8 |
| R | Distribution | 1.8 | 1.5 |
| S | Plant Effluent | <1 | |
| S | Distribution | 1.2 | |
| T | Plant Influent | <1 | 3.9 |
| T | Plant Effluent | 3 | 1.5 |
| T | Distribution | 3.7 | 1.3 |
| U | Plant Influent | <1 | |
| U | Plant Effluent | 2.5 | 1.9 |
| U | Distribution | 6.8 | 2.6 |
| V | Plant Influent | <1 | <1 |
| V | Plant Effluent | 1.7 | 1.8 |
| V | Distribution | 1.8 | 1.8 |
| W | Plant Influent | <1 | |
| W | Plant Effluent | <1 | |
| W | Distribution | 1.7 | |
| X | Plant Influent | 1.2 | <1 |
| X | Plant Effluent | 3.9 | 3.4 |
| X | Distribution | 4.4 | 4.6 |
| Y | Plant Influent | <1 | |
| Y | Plant Effluent | <1 | |
| Y | Distribution | <1 | |
| Z | Plant Influent | 1.2 | <1 |
| Z | Plant Effluent | 1.1 | <1 |
| Z | Distribution | <1 | <1 |
| AA | Plant Influent | <1 | 1.7 |
| AA | Plant Effluent | 63.7; 26.2; <1 | 2 |
| AA | Distribution | 2.4 | 2.2 |
| BB | Plant Influent | <1 | |
| BB | Plant Effluent | 1.2 | |
| BB | Distribution | 2.6 | 1.8 |

| | | | |
|---|---|---|---|
| CC | Plant Influent | <1 | |
| CC | Plant Effluent | 2.4 | |
| CC | Distribution | 28.3; 1.1 | |
| DD | Plant Influent | <1 | |
| DD | Plant Effluent | <1 | |
| DD | Distribution | <1 | 1.1 |
| EE | Plant Influent | <1 | |
| EE | Plant Effluent | 10.4; <1 | <1 |
| EE | Distribution | <1 | <1 |
| FF | Plant Effluent | 18.3 | 2 |
| FF | Distribution | 15.8 | 13.4 |

[1]DHS, 2003

## *Food*

NDMA has been detected in a variety of different foods, but given its low octanol/water partition coefficient, NDMA would not be expected to bioaccumulate to any great extent (IPSC, 2002). IARC (1978) provides a thorough summary of the results of studies that have detected NDMA in various foods. NDMA has been detected in foods such as cheese, meat and fish, and in alcoholic beverages such as beer, rum, whiskey and brandy (IARC, 1978, ATSDR, 1989). NDMA occurs in foods (meats) preserved by the addition of nitrate, nitrite, or cured by smoking. The cooking of cured meats is believed to result in the formation of NDMA.

Nitrates and nitrites appear to be converted to NDMA or other nitrosamines in the stomach (Mirvish, 1975; Pignatelli *et al.*, 1993; Bartsch and Montesano, 1984), and also by bacteria in the gastrointestinal tract (Mirvish, 1975). Other constituents in the diet such as dimethylamine, trimethylamine, or choline and lecithin are converted to dimethylamine or trimethylamine by bacteria, which probably results in NDMA formation (Tannenbaum, 1980). Exposure to high dietary sources of nitrosamines has been associated with higher cancer rates in some populations (ATSDR, 1989).

## *Other Sources*

NDMA has been detected in consumer products such as cosmetics and related personal care products (IPSC, 2002). The curing of tobacco is thought to facilitate the formation of nitrosamines. NDMA also has been detected in the mainstream and side stream smoke of cigarettes (IARC, 1978). The burning of tobacco is believed to foster the conversion of introduced nitrates and nitrites into NMDA.

## EXPOSURE ASSESSMENT

### Oral exposure

Important sources of exposure to NDMA include the consumption of food and beverages (ATSDR, 1989). The ingestion of drinking water that contains NDMA appears to contribute only a small fraction of the overall NDMA exposure (Environment Canada, 2001). Rough estimates of the exposure to various sources of NDMA in Canada indicate that water contributes less than 10 percent of the overall exposure (IPSC, 2002).). A report from U.S. EPA staff (Fristachi and Rice, 2005) indicates that the trace levels of NDMA in drinking water contribute from 0.001 to 0.55 percent (or less than one percent) of overall human exposure to NDMA. Higher concentrations as observed in groundwater plumes derived from rocket testing facilities could result in much higher exposures in some cases.

Because relative source contribution (RSC) is not utilized in cancer risk calculations, no attempt is made here to formally document the contribution of drinking water relative to other sources (e.g.. food, beer, formation from nitrates and nitrites in the GI tract). Estimation of non-cancer risks, for which RSC is traditionally used, is not appropriate for this chemical because of inadequate data.

### Inhalation exposure

No data are available regarding the concentration of NDMA in indoor air associated with the use of a domestic water supply. The potential for inhalation exposure to NDMA during showering can be estimated using Henry's constant, which is a measure of the relative ability of chemicals to partition into air from a water phase. Henry's constants for NDMA have been reported as $2.63 \times 10^{-7}$ atm-m$^3$/mole at 20° C, and $1.99 \times 10^{-6}$ atm-m$^3$/mole at 37° C (ATSDR, 1989). Given these very low values, negligible quantities of NDMA would be expected to partition from the shower water into the air during showering. Exposure to NDMA entrained in droplets during showering is also expected to be insignificant.

### Dermal Exposure

The contribution of dermal exposure to NDMA that occurs during showering relative to exposure due to the ingestion of drinking water can be calculated, based on the dermal permeability constant of NDMA (Kp) of 0.000265 cm/hr (Oak Ridge National Laboratory, 2004). With an average body surface area of 20,000 cm$^2$, and a showering time of 10 minutes/day, the dermal dose can be estimated for a drinking water concentration of one parts per million (ppm) as follows:

$$\text{Dose (mg/day)} = 1\ \mu\text{g/cm}^3 \times 0.000265\ \text{cm/hr} \times 20{,}000\ \text{cm} \times 1/6\ \text{hr showering/day}$$

$$= 0.88\ \mu\text{g/day or } 8.8 \times 10^{-4}\ \text{mg/day}$$

The dose from ingestion at the same NDMA concentration would be 2 mg/day (1 mg/L x 2 L/day. The relative contribution from the two exposure routes would therefore be:

$$\text{Dose}_{\text{dermal}}/\text{Dose}_{\text{ingestion}} = 8.8 \times 10^{-4}\ \text{mg/day} / 2\ \text{mg/day} = 4.4 \times 10^{-4} \text{ or } 0.04 \text{ percent}$$

The contribution to total dose by the dermal route resulting from showering is therefore judged to be negligible, compared to the oral exposure route.

## METABOLISM AND PHARMACOKINETICS

### *Absorption*

NDMA appears to be readily absorbed from the gastrointestinal tract. Less than two percent of a 2 mg/kg oral dose of NDMA was recovered in the stomach and intestine 15 minutes after its administration to fasted female rats (Diaz Gomez *et al.*, 1977). The amount of methylation of DNA in the liver was proportional to dose (up to 10 mg/kg) suggesting oral absorption was not saturated (Diaz Gomez *et al.*, 1977). Following the oral administration of NDMA to rats, approximately 50 percent of a 5 mg/kg dose was recovered as $CO_2$ after six hours (Agrelo *et al.*, 1978).

Little NDMA administered in saline to the rat was absorbed in the stomach, while the small intestine appeared to be the primary locus of NDMA absorption (Phillips *et al.*, 1975). Less than 10 percent of the dose of NDMA introduced into the small intestine of female rats remained after 20 minutes, while little NDMA introduced into the stomach disappeared after 30 minutes (Phillips *et al.*, 1975). The ligation of the stomach markedly reduced the rate of metabolism of NDMA (generation of $CO_2$ following intragastric administration) in the female rat compared to unligated animals (Phillips *et al.*, 1975). The co-administration of casein with NDMA had little effect on the adsorption of NDMA (Agrelo *et al.*, 1978).

Percutaneous absorption of NDMA was measured *in vitro* in human cadaver skin in unsealed Franz type diffusion cells using three separate vehicles (Brain *et al.*, 1995). From one to four percent of the administered dose was recovered in the receptor fluid after 48 hours. Most of the dose was not accounted for in the skin or skin wash and was presumed lost due to volatilization. Thus, the rate of absorption of NDMA was greatly influenced by the loss of the compound due to volatilization. The recovery in the receptor fluid, skin or skin wash of nitrosodiethanolamine, a less volatile nitrosamine, was effectively complete.

### *Distribution*

In early studies in rats or mice, similar concentrations of NDMA were observed in various organs one to four hours following intravenous injection (Magee (1956) or 24 hours following subcutaneous injection (Dutton and Heath, 1956), suggesting that the compound was distributed to body water. These findings were confirmed by Wishnok *et al.* (1978) and Johansson and Tjalve (1978). A uniform distribution of radioactivity was observed 30 minutes and one hour following intravenous administration of labeled NDMA to mice treated with compounds that prevented NDMA metabolism in the liver (Johansson and Tjalve, 1978).

### *Metabolism*

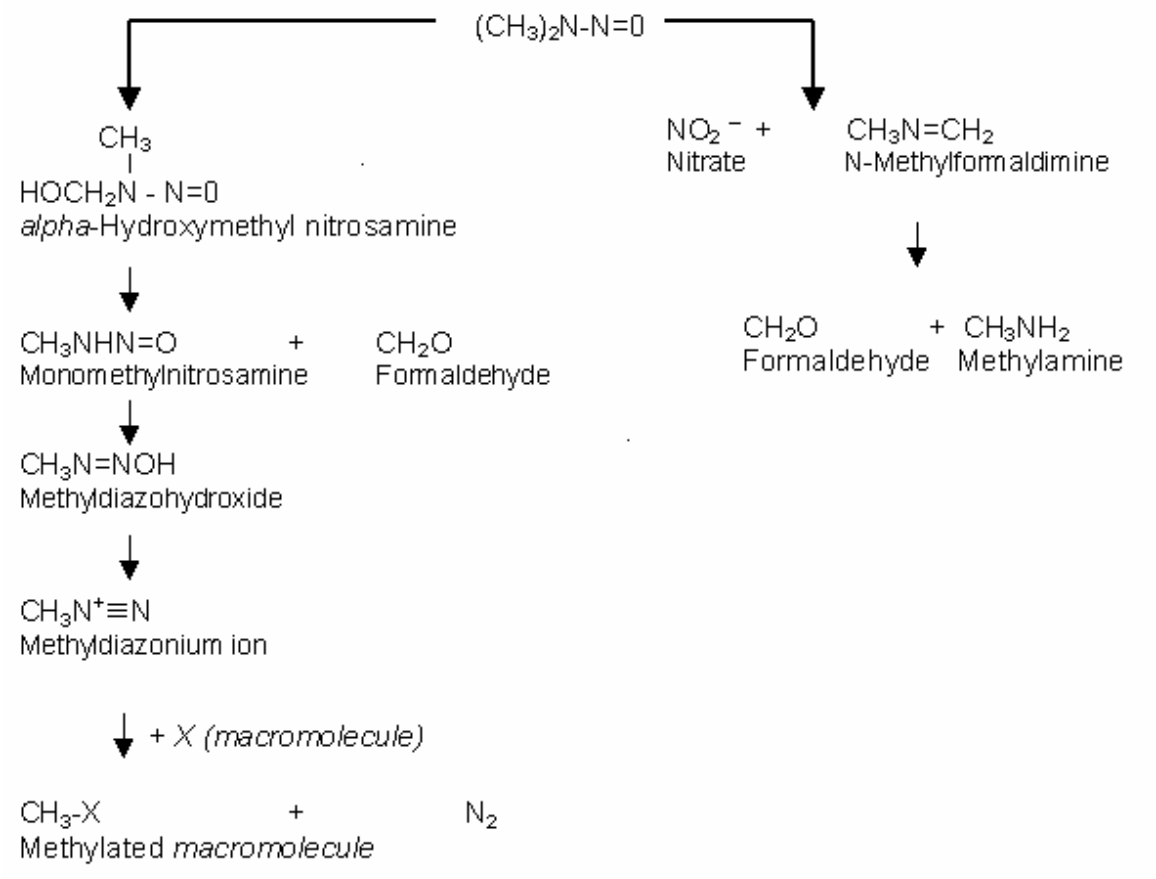
NDMA itself appears to be essentially biologically inert. However, metabolites of NDMA appear to be responsible for the host of toxic effects associated with NDMA exposure. The metabolism of NDMA and other nitrosamines have been extensively studied because this agent is considered a prototype of toxicants that produce toxicity or carcinogenicity due to metabolism to an active form(s) (Magee and Barnes, 1967; Preussmann and Stewart, 1984; Lai and Arcos, 1980). The metabolism of NDMA has been studied *in vitro* in microsomes, in tissue slices, and in cell cultures and isolated organs. I*n vivo*, metabolism has been evaluated in a number of animal species, in male and female, immature and mature animals, using inducers and inhibitors of drug metabolism, and recently by employing transfected and knockout animal models. While a comprehensive review of all these studies is beyond the scope of this document, certain pertinent findings will be discussed.

The observation by Magee (1956) of a rapid decline of NDMA levels in the whole animal (rats or mice) with little recovery of NDMA in the excreta first indicated that this compound was rapidly metabolized. Little of the administered dose was recovered in the urine and the removal of the kidney had little effect of the amount of NDMA recovered (Magee, 1956). In contrast, most of the administered dose was recovered in animals that were hepatectomized, indicating that most of the metabolism occurred in the liver (Magee, 1956).

Only a small fraction (approximately 10 percent) of orally administered NDMA (100 nmole) was recovered in mice as NDMA after 15 minutes, which also suggested very rapid metabolism (Kawanishi *et al.*, 1983). Blocking NDMA metabolism with pyrazole resulted in the recovery of most of the NDMA dose after 60 minutes. Dutton and Heath (1956) recovered 40 percent of a subcutaneously administered dose in the expired air of rats as $^{14}CO_2$ after eight hours, which suggested metabolism of NDMA by a demethylation pathway. In female rats administered 5 mg of NDMA, 48 percent of a intragastrically administered dose was recovered as $CO_2$ after seven hours, compared to 54 percent recovery when the dose was administered by intravenous injection (Phillips *et al.*, 1975).

Two pathways of NDMA metabolism, $\alpha$-hydroxylation and denitrosation (Figure 2) have been characterized (Keefer *et al.*, 1987). Studies in the rat have indicated that around 70 percent or more of administered NDMA is metabolized via the $\alpha$-hydroxylation pathway (Burak *et al.*, 1991). Approximately 20 percent of an intravenous bolus dose of NDMA administered to male Fischer 344 rats appeared to be metabolized by the denitrosation pathway (Streeter *et al.*, 1990a).

**Figure 2. Metabolic pathway for NDMA[a]**




[a] adapted from IPSC, 2002

The α-hydroxylation metabolic pathway is initiated by the hydroxylation of NDMA at the α carbon yielding hydroxyl NDMA, an unstable intermediate. The metabolite then decomposes to yield formaldehyde and the methyldiazonium ion another unstable and reactive intermediate. The methyl group on the methyldiazonium ion reacts with nucleophiles (e.g. DNA, RNA and proteins) by transferring a methyl group and releasing $N_2$. Formaldehyde is metabolized to formate and then $CO_2$.

The other identified route of NDMA metabolism is denitrosation (Keefer *et al.*, 1987; Lorr *et al.*, 1982; Tu and Yang, 1985; Streeter *et al.*, 1990a). NDMA is first metabolized to an unstable imine intermediate and nitric oxide (NO·). The imine ion decomposes to formaldehyde and methylamine while the nitric oxide combines with oxygen to form nitrite ($NO_2^-$). Both pathways involve a cytochrome P450 dependent mixed-function oxidase system and both pathways appear to be catalyzed mainly by the same cytochrome P450 isoform, CYP2E1 (Yoo *et al.*, 1990; Lorr *et al.*, 1982; Wade *et al.*, 1987; Yang *et al.*, 1985, 1990). While CYP2E1 has been identified as the enzyme mainly responsible for NDMA metabolism at low concentrations (Thomas *et al.*, 1987, Wrighton *et al.*, 1986; Yoo *et al.*, 1990), other cytochrome P450 isoforms also appear to

be capable of metabolizing NDMA but principally at higher concentrations (Yamazaki *et al.*, 1992; Yang *et al.*, 1985; Yang *et al.*, 1990). Reconstituted purified CYP2E1 from male New Zealand rabbits, Sprague Dawley or Long Evans rats, or human liver was an effective catalyst of NDMA metabolism to formaldehyde *in vitro* (Yang *et al.*, 1985; Tu and Yang, 1985; Levin *et al.*, 1986; Yamazaki *et al.*, 1992).

Metabolism of NDMA by both pathways was markedly increased in hepatic microsomes from rats treated with inducers of CYP2E1 such as isopropanol, ethanol or pyrazole, compared to control (Lorr *et al.*, 1982). Competitive inhibitors of CYP2E1 (acetone, dioxane, ethanol, isopropanol, pyrazole) reduced NDMA metabolism through each pathway by about the same amount (Lorr *et al.*, 1982; Tu and Yang, 1985). Using an antibody to CYP2E1, both α-hydroxylation and the denitrosation metabolism of NDMA in microsomes was markedly reduced (Yang *et al.*, 1991; Yoo *et al.*, 1990; Dicker and Cederbaum, 1991).

Metabolism of NDMA in humans and rodents appears to be very similar, both qualitatively and quantitatively. Measured NDMA demethylase Km and Vmax values *in vitro* were similar in the human and the rat liver. The human enzyme cross-reacted with an antibody prepared against the rat liver enzyme (Yoo *et al.*, 1990). Most (68-78 percent) of the NDMA demethylase activity in human microsomes was inhibited by antibodies prepared against the rat enzyme (Wrighton *et al.*, 1986).

Comparative *in vitro* metabolism of NDMA in liver slices revealed the hamster with the highest rate of metabolism, with the rat somewhat greater than the human and the monkey and trout with substantially lower rates of metabolism (as measured by $CO_2$ generation and adduct formation) (Bartsch and Montesano, 1984). Inducers of NDMA metabolism in the rat (ethanol, isoniazid) also appeared to induce human hepatic NDMA demethylase metabolism (Wrighton *et al.*, 1986).

While NDMA appears to be metabolized by two pathways, the α-hydroxylation pathway is believed to be responsible for formation of the active metabolite(s) that is responsible for the observed carcinogenicity and genotoxicity of NDMA (Magee and Barnes, 1967; Preussmann and Stewart, 1984; Lai and Arcos, 1980) but not necessarily its hepatotoxic activity (Archer *et al.*, 1994; Chin *et al.*, 1993). However, Lee and associates linked hepatotoxicity with α-hydroxylation metabolism of NDMA (Lee *et al.*, 1996).

The level of NDMA in the blood following oral administration is primarily controlled by amount metabolized in the liver (virtually all of the dose is absorbed from the gut). The fraction of an orally administered dose of NDMA that occurred in blood (compared to I.V. administration) has been investigated in a number of different species (Table 3). The fraction of the oral dose (the area under the blood concentration time plot) relative to what occurred following I.V. administration (where the entire dose would have initially occurred in the blood) was markedly different in different species. Approximately 10 percent of the oral administered dose was accounted for in the blood of the rat and hamster, while at least 50 percent of the dose was observed in the monkey, dog or pig (Gombar *et al.*, 1987, 1990; Streeter *et al.*, 1990b). This suggests that metabolism was saturated in the larger species while most of the oral dose administered was immediately metabolized in the liver (first pass clearance) in the rat and hamster. No comparable studies were identified in humans.

**Table 3. Fraction of Oral Dose Accounted for in the Blood[a]**

| Species | Fraction of oral dose in blood (%) |
|---|---|
| Hamster | 11 |
| Rat | 8 |
| Monkey | 49 |
| Dog | 93 |
| Pig | 67 |

[a]Area under curve (oral administration)/ Area under curve (i.v. administration)

The concomitant (or prior) administration of ethanol, a competitive inhibitor of NDMA metabolism in the liver, with NDMA resulted in marked increases in the AUC (blood) and decrease in blood clearance in the mouse and monkey (Anderson *et al.*, 1992, 1994). Higher tissue levels were also observed when ethanol was co-administered with NDMA in the mouse (Anderson *et al.*, 1986, 1994).

Consistent with these findings was the observation that co-administration of ethanol with NDMA to the monkey resulted in increased methylation in ovary, esophagus, bladder, spleen and cerebellum, but no increase in methylation in liver, compared to when NDMA was administered alone (Anderson *et al.*, 1996). Increased methylation in the non-hepatic tissues appears to have occurred because ethanol inhibited NDMA metabolism in the liver. Similar findings were observed in the rat (Swann *et al.*, 1984). Increased tumors in extrahepatic organs in mice also occurred when ethanol was co-administered with NDMA, compared to NDMA alone (Anderson, 1988, 1992; Griciute *et al.*, 1987).

A number of agents and interventions can markedly influence the metabolism of NDMA and other nitrosamines. Protein reduced diets and competitive inhibitors such as ethanol, methanol and disulfiram that reduce NDMA metabolism in the liver have been demonstrated to reduce hepatotoxicity and the incidence of liver tumors (Swann and McLean, 1971; Swann *et al.*, 1980; Anderson *et al.*, 1994). When hepatic metabolism is reduced by the inhibitors, the amount of extrahepatic metabolism increases along with increased carcinogenicity in the kidney and other tissues (depending on the nitrosamine) (reviewed by Barsch and Montesano, 1984)). Other agents or interventions such as fasting and ethanol administration to rats induce NDMA metabolism (Tu and Yang, 1983). In humans, microsomes obtained from the livers of alcoholics appear to metabolize NDMA to formaldehyde or nitrite two to three fold faster than in non-alcoholics (Amelizad *et al.*, 1989).

The metabolism of NDMA by demethylation generates the methyldiazonium ion that reacts with DNA, RNA and proteins. $O^6$-methyl guanine-DNA transferase (MGMT), is an enzyme that repairs $O^6$-methyl guanine and other $O^6$-alkyl guanine DNA adducts (Saffhill *et al.*, 1985; Lindahl *et al.*, 1988; Pegg, 1990; Swenberg *et al.*, 1982). The enzyme transfers the methyl group from guanine to a cysteine residue on the transferase (itself), which results in the permanent inactivation of the enzyme (Pegg, 1990; Lindahl *et al.*, 1988; Pegg and Byers, 1992). The level of MGMT activity is quite variable in

different tissues and species (Hall *et al.*, 1985, 1990; Gerson *et al.*, 1986). Shiraishi and coworkers observed a high homology of amino acid sequences of the human and mouse liver MGMT enzyme (Shiraishi *et al.*, 1992).

### *Excretion*

Because of its rapid metabolism, little NDMA is expected to be excreted unchanged. No unchanged NDMA that was intravenously administered to hamsters was detected in the urine (Streeter *et al.*, 1990b). Little NDMA was recovered in the excreta, which also indicated that this compound was rapidly metabolized (Magee, 1956). Little of the administered dose was recovered in the urine and the removal of the kidney had little effect on the amount of NDMA recovered.

## TOXICOLOGY

### *Toxicological Effects in Animals*

Since the early observation that human exposure to NDMA resulted in cirrhosis of the liver, most toxicity studies of NDMA in animals have focused on the liver.

#### Acute Toxicity

The oral $LD_{50}$ of NDMA in the rat was reported as 37 mg/kg (RTECS, 2002) and 40 mg/kg (ATSDR, 1989). The oral $LD_{50}$ in hamster was 28 mg/kg, the 4-hour inhalation $LC_{50}$ was 78 ppm in rats and 57 ppm in mice (RTECS, 2002; ATSDR, 1989).

#### Subchronic Toxicity

Subchronic exposure of rats mice, hamsters, cats, dogs, rabbits, guinea pigs and other animals to NDMA resulted in a variety of adverse effects (e.g. toxicity in the lung, liver, kidney). The predominant effect was hepatotoxicity in a number of species with varying susceptibilities (Maduagwu and Bassir, 1980). Hepatotoxicity was characterized by necrosis and hemorrhage, fibrosis, ascites depending on dose, route and length of exposure (George and Chandrakasan, 2000; George *et al.*, 2001; Desjardins *et al.*, 1992; Jezequel *et al.*, 1987). Decreased survival in these animals is usually linked to severe hepatotoxicity. Gastrointestinal hemorrhage was observed in rats administered 10 mg/kg of NDMA in the diet for 34-37 days (Barnes and Magee, 1954).

#### Genetic Toxicity

Because NDMA has become a prototypical compound for carcinogenic agents that are believed to act by reacting with DNA, the genotoxicity of NDMA has been extensively studied in a host of *in vitro* or *in vivo* bioassays. Genotoxic effects have been reported, as indicated by increased frequency of sister chromatid exchange, unscheduled DNA

synthesis, chromosomal aberrations, micronuclei, DNA strand breaks and the formation of adducts. ATSDR in 1989 summarized some of the available *in vivo* and *in vitro* studies regarding the genotoxicity of NDMA (ATSDR, 1989). Tables 4 and 5 are adopted from the ATSDR summary (ATSDR, 1989). IPSC in 2002 provides a more recent summary of genotoxic studies that buttresses the conclusion that NDMA is genotoxic in most species, cell types and test systems (IPSC, 2002). A comprehensive list of "mutation data" and references can be found in Registry of Toxic Effects of Chemical Substances (RTECS, 2002).

**Table 4. *In Vitro* NDMA Genotoxicity Findings [a]**

| Endpoint | Species/Test System | Results | |
|---|---|---|---|
| | | With Activation | Without Activation |
| Gene mutation | *Salmonella typhimurium* | + | + |
| | *Escherichia coli* | + | |
| | *Saccharomyces cerevisiae* | + | |
| | Chinese hamster V79 and ovary cells | + | - |
| | Mouse lymphoma L5178Y cells | + | - |
| DNA fragmentation | Rat hepatocytes | | + |
| | Human hepatocytes | | |
| Chromosomal aberrations | Chinese hamster lung cells | + | + |
| | Rat ascites hepatoma (AH66B) and rat esophageal (R1, R3) tumor cells | | + |
| Sister-chromatid exchange | Rat esophageal tumor, ascites hepatoma | | + |
| | Human lymphocytes | + | - |
| | Human fibroblasts | + | |
| | Chinese hamster ovary cells | + | - |
| | Chinese hamster V79 cells | + | - |
| | Chinese hamster primary lung cells | + | - |
| DNA Damage | Rat hepatocytes | | + |
| DNA repair/synthesis | Rat hepatocytes | + | + |
| | Human lymphoblasts | + | |
| | Mice hepatocytes | | + |
| | Hamster hepatocytes | | + |
| | Rat pancreatic cells | | - |

[a] Adapted from ATSDR (1989)

Two notable studies observed genotoxicity in the offspring of animals exposed to NDMA (Chhabra *et al.*, 1995, Diaz Gomez *et al.*, 1986). NDMA was administered (intragastric; 1.0 mg/kg or 0.1 mg/kg) to two pregnant Patas monkeys on gestation day 147 (Chhabra *et al.*, 1995). The animals were anesthetized and the fetuses were removed 2 hours after NDMA administration and DNA was isolated from various maternal and fetal tissues. $O^6$-methylguanine adducts were detected in all fetal tissues sampled, with highest levels in the liver and placenta. Interestingly, the administration of ethanol reduced adduct levels in the fetal liver and placenta and roughly doubled adduct levels detected in other fetal tissues such as the spleen, kidney, and lung.

Oral administration of NDMA to nursing Sprague-Dawley rat dams (1 or 10 mg/kg) resulted in detectable levels of liver and kidney DNA and RNA adducts in their 14 day old offspring 24 hours post treatment (Diaz Gomez *et al.*, 1986).

**Table 5. *In Vivo* NDMA Genotoxicity Findings [a]**

| Endpoint | Species/Test System | Results |
|---|---|---|
| DNA methylation | Rat, mouse, hamster and/or gerbil liver | + |
| | Human liver | + |
| DNA fragmentation | Rat liver and kidney elution | + |
| | Mouse liver and kidney elution | + |
| +DNA synthesis and repair | Fetal mouse kidney and liver | + |
| | Mouse testes | + |
| | Rat liver | + |
| | Rat respiratory cells | + |
| | Rat spermatocytes | - |
| Sex-linked recessive lethal mutations | Drosophila melanogaster | + |
| Sperm abnormalities | Mouse | - |
| Sister chromatid exchange | Chinese hamster bone marrow | +/- |
| | Mouse bone marrow | + |
| Chromosome aberrations | Hamster embryo fibroblasts | + |
| Micronucleus | Rat bone marrow | +/- |
| | Rat hepatocytes | + |
| | Mouse bone marrow | + |
| | Hamster embryonic fibroblasts | + |

[a] Adapted from ATSDR (1989)

**Developmental and Reproductive Toxicity**

Few studies were located on the reproductive or developmental effects of NDMA. The available data are inadequate to assess the reproductive or developmental toxicity of

NDMA. Female CD-1 mice were administered 0.1 ppm NDMA in drinking water for 75 days prior to mating, throughout pregnancy and during lactation (Anderson *et al.*, 1978 as reported by IPSC, 2002). The number of stillbirth and newborn deaths increased two-fold compared to control. No other effects were reported and no abnormal histopathology in the fetuses was evident that accounted for the deaths.

Intraperitoneal administration of a very high dose of NDMA (37 mg/kg) to pregnant female mice on gestation day 16 or 19 resulted in the death of all fetuses (Anderson *et al.*, 1989). No information regarding maternal toxicity was provided and the dose used was reported to be in the range of the $LD_{50}$ in mice. In the same study, a dose of 7.4 mg/kg administered to the pregnant mice on either day was reported to be "tolerated," and resulted in a significant increase in the number of female offspring with liver tumors when administered on day 19 (Anderson *et al.*, 1989). Several studies reviewed by ATSDR in which large doses of NDMA were administered during pregnancy (by gavage or in the diet) on unspecified day(s) resulted in increased fetal mortality (ATSDR, 1989). Little information regarding maternal toxicity was reported in these studies.

**Immunotoxicity**

Immunosuppressive properties of NDMA have been reviewed by Haggerty and Holsapple, 1990. Potential effects of NDMA on immune function were first investigated because many carcinogens are also known to suppress the immune system. Holsapple and coworkers (Holsapple *et al.*, 1984), Duke and associates (Duke *et al.*, 1985), Desjardins *et al.*, 1992, Johnson *et al.*, 1987, Thomas *et al.*, 1985, and Jeong and Lee, 1998 observed that NDMA suppressed various measures of humoral immunity. For example, exposure of female $B6C3F_1$ mice for 14 days to NDMA (1.5, 3.0 or 5.0 mg/kg) by intraperitoneal injection suppressed IgM antibody-forming cell response to sheep red blood cells (on day four) in a dose–dependent manner (Holsapple *et al.*, 1984). In the same study, spleenocyte proliferation in response to lipopolysaccharide was also suppressed by NDMA administration. Other measures of immune dysfunction including reduced host resistance to infectious agents (reduced response to streptococci and influenza challenge) following NDMA administration also indicate effects on humoral immunity (Thomas *et al.*, 1985).

Effects on cell-mediated immunity are more equivocal (Holsapple *et al.*, 1985; Thomas *et al.*, 1985). Exposure of female $B6C3F_1$ mice for 14 days to NDMA (1.5, 3.0 or 5.0 mg/kg) by intraperitoneal injection resulted in depressed T-lymphocyte function as measured by T-cell proliferation in response to T-cell mitogens (Holsapple *et al.*, 1985). NDMA exposure also resulted in a dose-related reduction in mixed lymphocyte response to DBA-2 lymphocytes. However, NDMA-exposed animals exhibited an increased resistance to challenge with *Listeria monocytogenes* bacteria (Holsapple *et al.*, 1985). This was also observed in mice treated with cyclophosphamide. NDMA administration to adult female mice by intraperitoneal injection resulted in increased host resistance to administered melanoma cells (as indicated by a decreased incidence and number of lung nodules in animals injected with $B_{16}F_{10}$ melanoma) (Duke *et al.*, 1985). These somewhat surprising findings of increased cell-mediated immunity following NDMA administration

may be the result of increased numbers or activity of monocytes or macrophages (and perhaps natural killer cell activity).

**Carcinogenicity**

The carcinogenic activity of NDMA in animals was first observed by Magee and Barnes (1956), in the form of liver tumors following oral administration of NDMA to rats. Nineteen of 20 rats fed 50 mg/kg NDMA in their diet developed hepatic tumors within 40 weeks. Numerous investigators have confirmed these findings in the liver and other tissues, employing various routes of administration in a number of species. These studies have been reviewed by IARC (1972, 1978), ATSDR (1989), Lijinsky (1983), and IPSC (2002). A comprehensive list of "tumorigenic data" and references can be found in the Registry of Toxic Effects of Chemical Substances (RTECS, 2002).

In addition to NDMA, other nitrosamines have been shown to produce cancer in several species, using various routes of administration and exposure protocols (reviewed by EPA, 1980; ATSDR, 1989; Lijinsky, 1983). Interestingly, the tumor sites were often different than those caused by NDMA. The liver is not a common target for most nitrosamines (Lijinsky, 1983). For example, while chronic oral exposure to NDMA yielded mainly liver tumors, the administration of N-nitrosodiethylamine yielded esophageal tumors at low doses, with liver tumors only at high doses (Lijinsky, 1983).

Different species and strains respond differently to NDMA (Lijinsky, 1983, Yan *et al.*, 1998). Oral exposure to NDMA yielded lung and liver tumors in the mouse, liver and kidney tumors in the rat, and liver and nasal cavity tumors in the Syrian Hamster (Lijinsky, 1983; Tomatis *et al.*, 1964). Intraperitoneal administration of NDMA to mice yielded lung tumors but no liver tumors (Clapp and Toya, 1970). In the hamster, oral administration of NDMA resulted in elevated hepatocellular carcinomas and cholangiocarcinomas but no lung tumors (Tomatis *et al.*, 1964). These observations were part of investigations on the carcinogenic mechanisms of nitrosamines, in attempts to understand the etiology of cancer. The following discussion will focus on the findings in rats or mice, in which there are superior cancer bioassays.

<u>Inhalation exposure</u>

When NDMA was administered by inhalation to BALB/c mice (0.005 or 0.2 mg/m$^3$ for 17 months) or Wistar rats (0.005 or 0.2 mg/m$^3$ for 25 months), tumors were observed in the lung, liver, and kidney (Moiseev and Benemansky, 1975 as reported in IARC, 1978). Marked increases in tumors of the nasal cavity were observed in female Sprague-Dawley rats administered NDMA by inhalation, 0.04, 0.2 or 1.0 ppm, four times a week for 207 days (Klein *et al.*, 1991).

Tumors of the nasal cavity were observed in 4 of 6 BD rats exposed by inhalation to NDMA twice weekly at a concentration which resulted in an equivalent dose of 4 mg/kg, and 8 of 12 rats at half that concentration (Druckrey, 1967 as reported in IARC, 1978).

<u>Oral exposure</u>

Oral administration of NDMA resulted in tumors of the liver and kidney in the mouse; liver, bile duct and kidney in the rat; liver and bile duct in the hamster; and liver in the

rabbit and guinea pig (IARC, 1978; Preussmann and Stewart, 1984; Peto *et al.*, 1991a,b). Magee and Barnes (1956) reported that the dietary administration of 50 mg/kg NDMA resulted in hepatic tumors in 19 of 20 rats by 40 weeks. However, when male rats were administered NDMA in their diet at doses of 100 or 200 mg/kg for one to four weeks, or when only one 30 mg/kg dose was administered, kidney tumors occurred, but no hepatic tumors were observed (Magee and Barnes, 1959, 1962).

In a very large cancer bioassay, groups of 60 male or female Colworth-Wistar rats were administered NDMA in their drinking water, beginning at six weeks of age (Peto *et al.*, 1991a; Peto *et al.*, 1991b). The control group contained 240 male or female animals. Sixteen dose groups (Tables 6-9) were included in this study and the exposure continued for the animals' lifetime (no scheduled terminal sacrifices). Body weights and water intakes were recorded weekly. Because there was no correspondence between NDMA concentration and water intake, daily dosage was estimated based on an average of measured water consumption across all dose groups in male or female rats (41 and 72 mL/kg-day respectively).

Survival in the eight lowest dose groups was excellent (up to 3.5 years, median survival for males was 33 months, median survival for females was 30 months). The prolonged survival allowed tumors to develop that probably would not have been detected in a standard two-year bioassay. However, early mortality in the highest eight dose groups, mostly due to liver tumors, would be expected to markedly reduce the detection of tumors at other sites.

The animals were sacrificed and necropsied if moribund or exhibiting palpable liver alterations. In each animal, tumors that were present were judged to be directly or indirectly responsible for the animal's death, or were considered to be incidental to the cause of death. This determination was vital to the statistical treatment of the study findings.

The investigators provided summaries of the occurrence of tumors in various treatment groups. Specific tumor findings were not reported for individual animals. Attempts to obtain such records were unsuccessful, as the records are no longer available, according to one of the investigators (personal communication with R Peto). The study results are shown in Tables 6-9. Tumors were reported in both males and females in four cell types in the liver, the hepatocyte, bile duct, mesenchyme and Kupffer cell (Tables 6 and 7). The authors also summarized hepatic tumor-bearing animals (Tables 8 and 9).

The investigators reported that nearly all of the bile duct neoplasms were benign while nearly all neoplasms of the liver cell were malignant. While the tumors were classified as mostly benign, they were lethal. The investigators reported that at the intermediate dose "…so many animals die prematurely of bile duct neoplasm that few have time to die of hepatocelllar neoplasms." In humans, a majority of primary tumors of the biliary tract are carcinomas (De Groen *et al.*, 1999).

**Table 6. Hepatic Tumors by Subtype in Male Rats Administered NDMA**

| Exposure group | NDMA in drinking water (mg/L) | Estimated intake (mg/kg-day)[a] | Animals with hepatic tumors (tumor bearing animals)[b] | | | |
|---|---|---|---|---|---|---|
| | | | Liver cell | Bile duct | Mesenchyme | Kupffer Cell |
| 1 | 0 | 0 | 10 | 3 | 0 | 0 |
| 2 | 0.033 | 0.001 | 4 | 2 | 0 | 0 |
| 3 | 0.066 | 0.003 | 3 | 3 | 0 | 1 |
| 4 | 0.132 | 0.005 | 2 | 2 | 1 | 0 |
| 5 | 0.264 | 0.011 | 4 | 2 | 1 | 0 |
| 6 | 0.528 | 0.022 | 4 | 1 | 0 | 0 |
| 7 | 1.056 | 0.044 | 5 | 1 | 1 | 2 |
| 8 | 1.584 | 0.065 | 8 | 4 | 0 | 0 |
| 9 | 2.112 | 0.087 | 7 | 7 | 7 | 0 |
| 10 | 2.640 | 0.109 | 13 | 13 | 5 | 3 |
| 11 | 3.168 | 0.131 | 14 | 12 | 12 | 0 |
| 12 | 4.224 | 0.174 | 19 | 12 | 10 | 0 |
| 13 | 5.280 | 0.218 | 27 | 16 | 3 | 2 |
| 14 | 6.336 | 0.261 | 32 | 18 | 7 | 0 |
| 15 | 8.448 | 0.348 | 44 | 8 | 4 | 0 |
| 16 | 16.896 | 0.697 | 46 | 0 | 10 | 0 |

[a] Intakes estimated by authors (Peto *et al.*, 1991a).
[b] From Table 7 (Peto *et al.*, 1991a).

**Table 7. Hepatic Tumors by Subtype in Female Rats Administered NDMA**

| Exposure group | NDMA in drinking-water (mg/L) | Estimated intake (mg/kg-day)[a] | Animals with hepatic tumors (tumor bearing animals)[b] | | | |
|---|---|---|---|---|---|---|
| | | | Liver cell | Bile duct | Mesenchyme | Kupffer Cell |
| 1 | 0 | 0 | 11 | 4 | 0 | 1 |
| 2 | 0.033 | 0.002 | 2 | 1 | 1 | 0 |
| 3 | 0.066 | 0.005 | 2 | 4 | 0 | 0 |
| 4 | 0.132 | 0.010 | 4 | 1 | 0 | 0 |
| 5 | 0.264 | 0.019 | 2 | 4 | 1 | 0 |
| 6 | 0.528 | 0.038 | 6 | 4 | 1 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | 1.056 | 0.076 | 6 | 9 | 2 | 1 |
| 8 | 1.584 | 0.115 | 3 | 39 | 0 | 0 |
| 9 | 2.112 | 0.153 | 7 | 33 | 3 | 1 |
| 10 | 2.640 | 0.191 | 7 | 44 | 1 | 0 |
| 11 | 3.168 | 0.229 | 4 | 48 | 3 | 0 |
| 12 | 4.224 | 0.306 | 7 | 46 | 2 | 0 |
| 13 | 5.280 | 0.382 | 13 | 44 | 0 | 0 |
| 14 | 6.336 | 0.459 | 20 | 38 | 2 | 0 |
| 15 | 8.448 | 0.612 | 40 | 10 | 5 | 0 |
| 16 | 16.896 | 1.224 | 41 | 1 | 12 | 0 |

[a] Intakes estimated by authors (Peto *et al.*, 1991a).
[b] From Table 7 (Peto *et al.*, 1991a).

**Table 8. Hepatic Tumors in Male Rats Administered NDMA**

| Exposure group | NDMA in drinking-water (mg/L) | Estimated intake (mg/kg-day)[a] | Animals with hepatic tumors (Tumor Bearing Animals)[b] |
|---|---|---|---|
| 1 | 0 | 0 | 13 |
| 2 | 0.033 | 0.001 | 5 |
| 3 | 0.066 | 0.003 | 7 |
| 4 | 0.132 | 0.005 | 5 |
| 5 | 0.264 | 0.011 | 6 |
| 6 | 0.528 | 0.022 | 5 |
| 7 | 1.056 | 0.044 | 9 |
| 8 | 1.584 | 0.065 | 12 |
| 9 | 2.112 | 0.087 | 19 |
| 10 | 2.640 | 0.109 | 35 |
| 11 | 3.168 | 0.131 | 38 |
| 12 | 4.224 | 0.174 | 41 |
| 13 | 5.280 | 0.218 | 48 |
| 14 | 6.336 | 0.261 | 56 |
| 15 | 8.448 | 0.348 | 56 |
| 16 | 16.896 | 0.697 | 59 |

[a] Intakes estimated by authors (Peto *et al.*, 1991a).
[b] From Table 8 (Peto *et al.*, 1991a).

**Table 9. Hepatic Tumors in Female Rats Administered NDMA**

| Exposure group | NDMA in drinking-water (mg/L) | Estimated intake (mg/kg-day)[a] | Animals with hepatic tumors (Tumor Bearing Animals)[b] |
|---|---|---|---|
| 1 | 0 | 0 | 16 |
| 2 | 0.033 | 0.002 | 4 |
| 3 | 0.066 | 0.005 | 6 |
| 4 | 0.132 | 0.010 | 5 |
| 5 | 0.264 | 0.019 | 7 |
| 6 | 0.528 | 0.038 | 12 |
| 7 | 1.056 | 0.076 | 18 |
| 8 | 1.584 | 0.115 | 42 |
| 9 | 2.112 | 0.153 | 43 |
| 10 | 2.640 | 0.191 | 51 |
| 11 | 3.168 | 0.229 | 55 |
| 12 | 4.224 | 0.306 | 56 |
| 13 | 5.280 | 0.382 | 58 |
| 14 | 6.336 | 0.459 | 59 |
| 15 | 8.448 | 0.612 | 57 |
| 16 | 16.896 | 1.224 | 58 |

[a] Intakes estimated by authors (Peto *et al.*, 1991a).
[b] From Table 8 (Peto *et al.*, 1991a).

Survival curves for the various dose groups provided by the investigators revealed that tumors in the lower dose groups appeared quite late in the affected animals' lifetime. Had the animals been sacrificed at two years, many of the tumors in the low dose groups may have not been detected. Also notable was that in the higher dose groups the tumors appeared much earlier. Thus, the dose of administered NDMA appeared to govern both the incidence of hepatic tumors and when they occurred.

Given that the appearance of tumors (how many and at what time they appeared) was governed by the NDMA dose, the investigators performed a time-to-tumor analysis. The incidences of liver tumors (all sites) were found to be related to both dose and time in the following manner:

Male rats $CI = 37.43(d + 0.1)^6 t^7$

Female rats $CI = 51.45(d + 0.1)^6 t^7$

Where:

CI = cumulative index (incidence)

d = dose (mg/kg-day)

t = time (yr)

This extensive study was chosen for derivation of the cancer potency and development of the proposed PHG.

In another study, two generations of female BALB/c mice were administered NDMA (3 ppm) in their drinking water for up to 80 weeks (Terracini *et al*., 1973). Exposure to NDMA began when the mice were weaned at 4-5 weeks of age. Lung tumors were observed in 44 of 62 NDMA-treated mice compared to 20 of 62 control mice in the first generation, and 44 of 66 treated mice compared to 15 of 69 control mice in the second generation.

Female Fischer 344 rats were administered NDMA (5.5 or 13 mg/L) in their drinking water, 5 days a week for 7 months (Lijinsky and Reuber, 1984). The rats were then maintained for the duration of their lifetime, as no terminal sacrifice was undertaken. Mortality occurred earlier in rats treated with NDMA. Liver tumors were observed in 2 of 20 control rats, in 14 or 20 at the low dose, and 17 of 20 rats at the high dose.

Male and female Wistar rats were provided with 0, 0.1, 1.0, or 10 ppm NDMA in their diet for 96 weeks (Arai *et al.*, 1979). Hepatocellular carcinomas were observed in 3 of 17 females receiving 1.0 ppm and 2 of 9 receiving 10 ppm of NDMA, with 1 of 17 and 3 of 9 females displaying hemangioendothelioma in the liver at the aforementioned doses. Three of 17 males receiving 10 ppm NDMA displayed hemangioendothelioma. No tumors were detected in control rats.

NDMA was administered in the diet of female Porton rats (0, 2, 5, 10, 20 or 50 ppm) for up to 120 weeks, although most animals were sacrificed by 120 weeks (Terracini *et al.*, 1967). A marked increase in liver tumors was observed in the female rats administered NDMA, which appeared to be dose related (Table 10). No kidney tumors were observed in these animals.

**Table 10. Liver Tumors in Female Porton Rats Administered NDMA[1]**

| Concentration in diet (ppm) | Liver tumors weeks 0-60 | Liver tumors weeks 61-120 |
|---|---|---|
| 0 | 0 of 4 | 0 of 25 |
| 2 | 0 of 5 | 0 of 13 |
| 5 | 0 of 8 | 7 of 69 |
| 10 | -- | 2 of 5 |
| 20 | 5 of 10 | 10 of 13 |
| 50 | 10 of 12 | -- |

[1]Terracini *et al.*, 1967.

Other routes of exposure

NDMA was administered subcutaneously to male or female Chinese hamsters once weekly at doses of 3.4, 1.8 or 0.9 mg/kg. Mean survival was 29 to 36 weeks in treated animals and 82-87 weeks in controls. Most of the treated animals developed liver tumors, but there were very few tumors at other sites (Reznik *et al.*, 1976). The administration of a single dose of NDMA by the intraperitoneal route (5, 10, 15 mg/kg) to male mice resulted in 9/18, 16/19 and 4/5 mice with lung tumors, respectively, compared to 25/52 in the control group (Clapp, 1973).

Wistar rats were subcutaneously administered 0.125, 10, 20 or 30 mg/kg NDMA on day one, seven, 21 or 70 (Campbell *et al.*, 1974). Significant increases in kidney tumors were observed in animals injected on day one or day seven at the lower doses, while only the highest dose yielded significant number of kidney tumors on day 21 and 70. No kidney tumors were detected in control animals.

Weekly subcutaneous or intraperitoneal injections of 0.15 mg of NDMA to various strains of mice for 1 to 25 weeks resulted in very high incidences of liver (hemangioendothelial sarcomas) and lung tumors (Kuwahara *et al.*, 1972; Otsuka and Kuwahara, 1971). No control groups were utilized in these studies but the investigators reported that these strains of mice had very low incidence of spontaneous tumors.

Intraperitoneal injection of neonatal C57BL/6 mice (selected because of their relative insensitivity to carcinogens) with 10.5 or 26.2 mg/kg (total dose) of NDMA on day eight and day fifteen resulted in a statistically significant increase in liver tumors at 15 months (Dass *et al.*, 1998). Significant increases in lung tumors were not observed. Thrice weekly intraperitoneal injection of male C3H mice with 3 mg/kg NDMA for seven weeks resulted in 37 of 38 mice with lung tumors, compared to 7 of 28 in animals receiving the vehicle (Devereux *et al.*, 1991). The number of hepatic tumors in treated mice did not appear to be significantly different from that in the control group.

Prenatal/perinatal exposure

Female Strain A mice were administered 10 μg/L NDMA in drinking water from four weeks prior to mating, throughout pregnancy and lactation (Anderson *et al.*, 1979). After weaning, the offspring were then exposed to 10 μg/L NDMA in their drinking water until they were 22 weeks of age. Eight of 25 treated male mice compared to 1 of 23 control mice displayed lung tumors, which was reported to be statistically significant. No treatment-related tumors were observed in the female offspring. Significant increases in tumors were not observed in the liver.

Single or repeated injection of 12.5 to 75 mg/kg NDMA to mice during the final days of pregnancy resulted in lung adenomas and hepatomas in the offspring (Smetanin, 1971 as reported by IARC, 1978). Intraperitoneal injection of male Fischer 344 rats with 0-50 mg/kg NDMA three days post-weaning yielded a dose-dependent increase in kidney tumors (Driver *et al.*, 1987).

Tomatis (1973) observed that the administration of NDMA during the final days of pregnancy induced tumors in rats and mice (as reported by U.S. EPA, 1980). The effects

were observed following oral, subcutaneous, intraperitoneal, or intravenous administration but were not observed when the compound was administered earlier in the pregnancy.

**Mechanism**

Metabolism

Extensive research has been focused on the mechanism(s) of tumors induced in animals by NDMA and other nitrosamines. Magee and Hultin (1962) and Magee and Farber (1962) demonstrated *in vitro* and *in vivo* alkylation of nucleic acids and proteins by NDMA and other nitrosamines in the rat and mouse. Subsequently, a number of investigators have linked the appearance of methylated DNA adducts derived from NDMA with the appearance of tumors. Both the ability of a tissue to metabolize NDMA and thereby generate DNA adducts, and the ability of the tissue to repair DNA adducts appear to play important roles in the mechanism of NDMA carcinogenicity.

NDMA metabolism can occur in a number of tissues but occurs primarily in the liver, particularly following oral exposure. Small oral doses of NDMA are essentially removed from the blood by the liver in the first pass, because of its rapid metabolism. Thus, when small doses of NDMA were administered orally to rats, nucleic acid alkylation was detected largely in the liver (Diaz Gomez *et al.*, 1977). The oral administration of higher doses of NDMA (or other nitrosamines) resulted in the methylation of DNA in extrahepatic tissues such as the esophagus or the kidney (Diaz Gomez *et al.*, 1977; Swann *et al.*, 1980; Pegg and Hui, 1978). The occurrence of tumors in extrahepatic tissues following the oral administration of a single large dose of NDMA appears to be related to the saturation of first pass clearance by the liver.

When a single large dose of NDMA was administered by intraperitoneal injection to male rats, tumors were detected in the kidney, particularly in rats provided a protein-deficient diet, but only one rat developed a hepatic tumor (Hard and Butler, 1970). A protein-deficient diet in rats resulted in a marked decrease in *in vitro* NDMA metabolism in the liver slices but not in kidney slices (Swann and McLean, 1971). Furthermore, blood levels of NDMA remained elevated in protein-deprived rats. The methylation of guanine in DNA and RNA increased in the kidney of rats provided a protein-deficient diet compared to a normal diet, while guanine methylation decreased in the liver (Swann and McLean, 1971). As expected from the above findings, the administration of NDMA to rats maintained on a protein–deficient diet resulted in high (100 percent) incidence of renal tumors, while comparably treated rats maintained on a conventional diet exhibited only a 35 percent incidence of renal tumors (Hard and Butler, 1970).

Pretreatment of animals with phenobarbital, which suppressed hepatic NDMA demethylation (Lee *et al.*, 1989), resulted in a reduction of liver tumors (Guengerich, 1988). In contrast, the administration of ethanol, an agent that induced hepatic NDMA metabolism, (Lee *et al*., 1989) increased rat liver microsomal CYP2E1 content and preneoplastic lesions in the rat liver (Tsutsumi *et al*., 1993). In addition to inducing NDMA demethylase, ethanol is a competitive inhibitor of NDMA metabolism. Tissue levels (blood, liver, kidney, lung and brain) of NDMA in mice administered 50 ppm

NDMA in drinking water were substantially increased when ethanol was administered (Anderson *et al.*, 1986). The administration of ethanol in combination with NDMA resulted in tumors in extrahepatic tissues such as the lung and kidney (Anderson *et al.*, 1988, 1992). Reduced numbers of hepatomas were observed in animals administered NDMA in combination with ethanol, compared to NDMA alone (Griciute *et al.*, 1987).

DNA Adduct Formation

Diaz Gomez *et al.* (1977) observed that formation of the DNA adduct N-7-methyl guanine was proportional to NDMA dose in the liver and kidney (at higher doses) in female rats orally administered NDMA. $O^{6-}$Methylguanine DNA adducts that accumulated over 28 days were linearly related to NDMA dose in the rat liver (Souliotis *et al.*, 1995). The administration of 0.25-2.7 ppm NDMA in drinking water to female Wistar rats resulted in a linear increase with dose of N-7-methyl guanine adducts in the liver at 28 days (Souliotis *et al.*, 2002). The findings of these studies suggest that the metabolism of NDMA in the liver was not saturated in the dose range used by Peto *et al.* (1991a,b).

DNA methylation was not observed to begin in the kidney until higher doses of NDMA were administered (orally), whereupon methylation in the kidney was proportional to dose (Diaz Gomez *et al.*, 1977). When small doses of NDMA were administered by intraperitoneal injection, bypassing first pass clearance by the liver, alkylation was observed in both the liver and kidney (Pegg and Hui, 1978; Diaz Gomez *et al.*, 1977). The administration of NDMA to Patas monkeys by gavage yielded levels of $O^6$-methyl guanine DNA adducts four hours post-administration in various tissues (e.g. kidney, esophagus, stomach, brain) that were similar to what was observed in the liver. This is unlike what is typically observed in rodents, in which adducts occurred primarily in the liver (Anderson *et al.*, 1996). The administration of ethanol prior to NDMA resulted in a marked increase in adducts in the esophagus, pancreas, colon, bladder, brain and uterus, while having little effect on adduct levels in the monkeys' liver (Anderson *et al.*, 1996).

Exposure to NDMA of a human fibroblast cell line transfected with cDNA for rat CYP2E1 resulted in decreased cell viability; and time- and dose-dependent increased methylation of DNA, RNA and proteins (Lin *et al.*, 1998). The viability of the same cell line that was not transfected and displayed no demethylase activity was not altered by NDMA treatment, nor were its macromolecules methylated by the addition of NDMA to the cell culture. Inhibitors of P450 2E1 such as ethanol and 4-methyl pyrazole prevented the alkylation of macromolecules and prevented the NDMA-mediated toxicity in the transfected cells.

The induction of CYP2E1 activity by pretreatment of rats with pyridine (as measured by p-nitrophenol hydroxylase activity) resulted in a marked increase in the methylation of calf thymus DNA by NDMA in microsomes isolated from hepatocytes from the rats (Shu and Hollenberg, 1997). An inhibitor of CYP2E1 activity, diethyldithiocarbamate (DDC), markedly reduced the methylation of calf thymus DNA *in vitro* by NDMA in microsomes obtained from control rats or rats treated with pyridine. Interestingly, pretreatment of rats with phenobarbital, which had little effect on CYP2E1 activity, reduced DNA methylation by NDMA in microsomes prepared from hepatocytes from the treated rats. DDC yielded little inhibition of the low levels of calf thymus DNA methylation by

NDMA in microsomes from rats treated with phenobarbital, suggesting that the metabolism of NDMA involved other cytochrome P450 isoforms. Experiments in intact cultured hepatocytes yielded results similar to these microsomal studies (Shu and Hollenberg, 1997).

While adduct formation occurs at a number of DNA sites, certain sites are preferred. The administration of NDMA to rat and other animals resulted in formation of DNA adducts primarily at the N-7 position (70 percent of the total) and $O^6$ position (7 percent of the total) of guanine (Beranek, 1990). Adducts have also been detected at the N-3 position of adenine (3 percent of the total) (Beranek, 1990) and $O^4$-methylthymine (0.1 percent of the total) (Hall *et al.*, 1990). While the majority of adducts occur at the N-7 guanine position, the adducts at the $O^6$ position of guanine have been associated with the mutagenic and carcinogenic response (Loveless, 1969; Archer, 1989; Pegg and Byers, 1992). The $O^6$ alkylation leads to a GC-AT transition during DNA replication (Swann, 1990; Pegg, 1990).

An $O^6$-methylguanine mutation in a specific codon in the *k-ras* oncogene was observed in lung tumors from mice treated with NDMA (Belinsky *et al.*, 1989). Spontaneous lung tumors from control mice were not characterized by this mutation. Mutations in an activated *k-ras* oncogene were observed in lung tumors in C3H and A/J strains of mice treated with NDMA (Devereux *et al.*, 1991; Belinsky *et al.*, 1989).

DNA Repair

$O^6$-methylguanine-DNA transferase (MGMT), is an enzyme that repairs $O^6$-methyl guanine and other $O^6$-alkyl guanine DNA adducts (Saffhill *et al.*, 1985; Lindahl *et al.*, 1988; Pegg, 1990; Swenberg *et al.*, 1982). The enzyme transfers the methyl group from guanine to a cysteine residue on the transferase (itself), which results in the permanent inactivation of the enzyme (Pegg, 1990; Lindahl *et al.*, 1988; Pegg and Byers, 1992). The level of MGMT activity in tissues and species is quite variable (Hall *et al.*, 1985; Hall *et al.*, 1990; Gerson *et al.*, 1986).

Gerson *et al.* (1986) measured MGMT activity (normalized to protein and DNA) in human, rat, and mouse tissues. The liver had the highest levels of MGMT in all three species; the levels in human tissues were substantially higher than that observed in the rodents. These findings suggest that the mouse and rat may be more sensitive to the carcinogenic effects of NDMA.

Differences in the basal level on MGMT, how much of the enzyme is depleted, and ability of NDMA to induce MGMT synthesis may explain the sensitivity of various species, tissues and cell types to the toxicity and carcinogenicity induced by NDMA (and other nitrosamines). Dolan *et al.* (1990) demonstrated significant increases in cell toxicity using agents that inhibited DNA repair by MGMT. A number of studies have linked the ability of the tissue to repair $O^6$-methylguanine DNA adducts (or alkylguanine adducts for other nitrosamines) to the level or activity of MGMT in the tissue or a particular cell type within the tissue (Swenberg *et al.*, 1982; Pegg and Byers, 1992; Pegg, 1990; Magee *et al.*, 1975; Nicoll *et al.*, 1975; Pegg, 1977; Kamendulis and Corcoran, 1994 ; Swenberg *et al.*, 1982).

Following the administration of sufficiently large dose(s) of NDMA to overcome first pass clearance, alkylation of DNA occurred in both the liver and kidney but the adducts appeared to be more persistent in the kidney (Nicoll *et al.*, 1975; Fan *et al.*, 1991). The occurrence of tumors in the kidney but not in the liver in the rat following the administration of a single large dose of NDMA appears to be the consequence of the rat liver having a much higher level of MGMT activity than the kidney (Barsch and Montesano, 1984). Renal tumors probably occurred in the kidney because of the inability of the kidney but not the liver to rapidly repair DNA methylated by a single large dose of NDMA (Magee *et al.*, 1975; Nicoll *et al.*, 1975; Pegg, 1977).

In the female Syrian Golden hamster, a single dose of 21 mg/kg of NDMA by gavage resulted in a 35 percent incidence of liver tumors but no kidney tumors (Tomatis and Cefis, 1967). The hamster and rat liver are characterized by similar constitutional levels of MGMT but there is a marked difference in the recovery of the enzyme following NDMA administration. There is a very slow recovery in the hamster after 10 mg/kg or 25 mg/kg of NDMA. The much more rapid repair of DNA adducts in the liver of rats compared to the hamster may account for the occurrence of tumors only in the hamster when a single large dose of NDMA was administered (Hall *et al.*, 1990).

Transgenic C3H male or female mice with high levels of liver MGMT activity had markedly reduced carcinogenic response in the liver (tumor bearing animals, carcinoma bearing animals) following NDMA administration compared to control (Nakatsuru *et al.*, 1993). Transgenic mice with high levels of human MGMT had markedly lower levels of lymphomas induced by N-methyl-N-nitrosourea, another carcinogen that appears to act by methylating $O^6$-guanine (Dumenco *et al.*, 1993). MGMT-deficient female mice (knockout mice) given one 5 mg/kg dose of NDMA by intraperitoneal injection had increased numbers of liver or lung tumors compared to control (wild type mice) (Iwakuma *et al.*, 1997). No information regarding kidney tumors was provided.

However, other studies indicate that the ability (or lack thereof) of liver cells to repair DNA damage does not appear to be responsible for the increase in tumors in the rat liver. MGMT levels in the rat liver were not depleted, but appear to have increased when NDMA exposure continued for at least 120 days (Souliotis *et al.*, 2002). MGMT levels also appeared to increase with dose in the rat liver after 180 days of exposure to NDMA (Souliotis *et al.*, 2002). In an earlier study, MGMT levels seemed to slightly increase with dose while $O^6$-methylguanine DNA adduct levels were proportional to dose in the liver of rats chronically exposed to NDMA in their drinking water (at doses similar to those employed by Peto) (Souliotis *et al.*, 1995).

Neither the depletion of MGMT nor a marked increase in DNA adducts at higher doses appeared to explain the marked increase in tumors observed in rats at higher doses of NDMA in studies by Peto and coworkers (Peto *et al.*, 1991a,b). The observation that the pattern of DNA adduct accumulation (linear with dose) in rats (Souliotis *et al.*, 1995) was not consistent with the increase in liver tumors with dose (sublinear) in the Peto study (Peto *et al.*, 1991a.b) suggests that other factors are involved in governing the pattern of the carcinogenic response.

Using immunohistochemical staining analysis, Takahashi *et al.* (1996) observed that the basal level of MGMT mRNA was quite low in the rat hepatocytes while other cell types

in the liver (e.g., bile duct cells, fibrous tissue, and vascular endothelial cells) had elevated basal MGMT mRNA levels. Following NDMA administration, a marked increase in MGMT mRNA levels occurred in centrilobular hepatocytes, while there was no evidence of an induction of mRNA in other cell types (bile duct cells, fibrous tissue and vascular endothelial cells). The levels of MGMT mRNA in the other cell types did remain elevated.

Dose-dependent increases in $O^6$-methylguanine adducts were observed in hepatocytes, particularly centrilobular hepatocytes, while only low levels of adducts were detected in the other cell types (Takahashi *et al.*, 1996). The adducts also appeared to persist longer in the hepatocytes. The investigators suggested that other factors appear to be modulating the carcinogenic process in the hepatocytes, after noting that tumors occur in hepatocytes, the only cells where MGMT mRNA levels were induced. However, the levels of MGMT mRNA in other tissues were already elevated, perhaps preventing adduct formation in these tissues, while the induced level of MGMT mRNA may have been inadequate to repair the increase adduct formation that occurred in hepatocytes due to high levels of NDMA metabolism by CYP2E1. Interestingly, high levels of MGMT mRNA were detected in bile duct cells, which were not induced by the administration of NDMA. Peto and coworkers observed a high incidence of tumors in this cell type in their study (Peto *et al.*, 1991b; Peto *et al.*, 1991a).

The Souliotis *et al*. (2002) observation of modest increases in hepatic cell proliferation in female rats at high dose levels of NDMA administered in drinking water (0.2-2.64 ppm) suggests another possible mechanism for the pattern of carcinogenic response in the rat liver observed by Peto and coworkers (Peto *et al.*, 1991b; Peto *et al.*, 1991a). These investigators employed a dose range that was consistent with that employed in the Peto study. While the labeling index increased only at higher doses of NDMA (which was consistent with the dose-response relationship observed in the Peto *et al.* (1991a,b) study), adduct accumulation was proportional to dose, which was not consistent with the dose and tumor response observed by Peto and coworkers. Depletion of MGMT also did not appear to explain the increase in tumors at higher doses in the Peto study, as MGMT activity appeared to be higher in animals receiving the higher NDMA doses in this study.

Craddock (1975) only observed hepatocellular carcinoma in animals receiving partial hepatectomy following the treatment of female rats with a single high dose of NDMA, which also suggests a role for cellular proliferation. Lindamood *et al*. (1984) investigated DNA alkylation and cell proliferation in the liver of C3H and C57BL mice and F-344 rats administered NDMA in their drinking water. These investigators observed increased alkylation of DNA with dose of NDMA (0, 10, 30 and 100 ppm in their drinking water) in both the rat and mice, but only observed evidence of cell proliferation (increases in labeled thymidine in DNA) in the high dose group of the C3H mice (30 ppm) after 16 day of exposure to NDMA. No evidence of cell proliferation was observed in any dose group in the F-344 rat or the C57BL mouse (Lindamood *et al.*, 1984).

## *Toxicological Effects in Humans*

### Acute Toxicity/Subchronic Toxicity

Toxic effects of NDMA on liver of workers were reported as long ago as 1937 (Freund, 1937, as reported in U.S. EPA, 1980). The development of liver cirrhosis in two laboratory workers following the introduction of NDMA to the laboratory triggered the study of NDMA toxicity (Barnes and Magee, 1954).

### Carcinogenicity

IARC reviewed the data regarding human cancer studies in 1972 and 1978 and concluded that "no case reports or epidemiological studies were available to the Working Group" (IARC, 1978). The International Programme on Chemical Safety's (IPCS) INCHEM service recently summarized the epidemiology studies that investigated the link between nitrosamines exposure (NDMA in particular) and cancer in humans (IPSC, 2002). The findings included the following statement: "Although the database is rather limited, data from epidemiological studies are at least suggestive of an association between exposure to NDMA and several forms of cancer (i.e., gastric and lung), with some consistency of evidence for gastric cancer and for exposure–response for lung cancer, the latter in studies in which matching or control for confounders was most extensive. Although estimated intakes in these investigations were based on dietary recall, and although confounding factors such as alcohol were not accounted for, the data fulfill, at least in part, some of the traditional criteria for causality of an association between ingestion of NDMA and cancer."

Case-control studies investigated the link between NDMA exposure and stomach, esophageal or lung cancer. Because nitrate and nitrite can be converted *in vivo* to nitrosamines, some studies have also investigated the link between exposure to these compounds and cancer in humans. Estimates of intake of NDMA, nitrate or nitrite, that were at best uncertain, were based on dietary recall and estimated concentrations of nitrosamines in dietary items. While certain confounders such as smoking were considered, others, such as other constituents in the diet, were generally not considered.

Two case control studies indicated a positive relationship between oral exposure to NDMA (Pobel *et al.*, 1995) or nitrosamines (Gonzalez *et al.*, 1994 ) and the occurrence of gastric cancer. One study did not find a relationship between NDMA exposure and stomach cancer (Risch *et al.*, 1985) while another study did link exposure to NDMA with cancer in the oral cavity, esophagus and larynx (Rogers *et al.*, 1995). De Stefani and coworkers (De Stefani *et al.*, 1996) and Goodman and associates (Goodman *et al.*, 1992) linked exposure of NDMA in the diet with increased risk of lung cancer .

Knekt and coworkers conducted a prospective study, which investigated the link between dietary NDMA, nitrate and nitrite intake, and gastrointestinal cancers (Knekt *et al.*, 1999). Detailed food consumption data were collected from approximately 10,000 participants. Beer consumption was also noted. A significant positive correlation was observed between exposure to NDMA and colorectal cancer. There was also a

significant association between the intake of smoked and salted fish but not cured meat and meat products, and colorectal cancer. No positive association between nitrate or nitrite consumption and cancer was observed. No statistically significant association between beer consumption and gastrointestinal cancers was observed in this study.

Other studies have looked at exposure to workers, particularly rubber workers, to nitrosamines (Sorahan *et al.*, 1989; Delzell *et al.*, 1981; Straif *et al.*, 2000). Rubber workers have a very high documented workplace exposure to nitrosamines. In a study of rubber workers, Straif and coworkers reported a link between inhalation exposure to nitrosamines and cancers of the esophagus, oral cavity and pharynx (Straif *et al.*, 2000).

**Examination of the Evidence of Carcinogenic Activity of NDMA**

Animal Studies

NDMA has been evaluated in numerous animal bioassays using various routes of exposure and experimental protocols. Significant increases in tumors were observed in male and female, mature and immature animals and in essentially all species that were evaluated (mice, rats, hamsters, rabbits, guinea-pigs). Carcinogenic activity was observed following oral or inhalation exposure to NDMA as well as following intraperitoneal or subcutaneous injection of animals. Significant increases in tumors have been observed when as little as one dose was administered or when NDMA was administered for the lifetime of the animal.

Human Studies

A series of epidemiological studies have linked exposure to NDMA or other nitrosamines with cancer in the gastrointestinal tract. The estimates of exposure are highly uncertain and other constituents in the diet could be responsible for the higher incidence of cancer in these studies. One study suggested an increase in lung cancer associated with dietary exposure to NDMA. Estimates of exposure are uncertain in these studies, so potency estimates based on these studies would be very tenuous.

Genotoxicity

NDMA displayed genotoxicity in a host of *in vitro* and *in vivo* assays. NDMA displayed positive results (e.g. micronuclei, sister chromatid exchange) *in vivo,* in rodents administered NDMA in a variety of tissues: hepatocytes, bone marrow lymphocytes, esophageal and kidney cells. DNA mutations (adducts) have been detected in numerous studies in which animals were administered NDMA.

NDMA displayed genotoxicity (e.g., unscheduled DNA synthesis, sister chromatid exchange, gene mutations) *in vitro* with or without metabolic activation in bacteria, human, and rodent cells. Adduct formation has been detected *in vitro* in tissue slices and in reconstituted microsomal cytochrome P450 systems incubated with DNA.

Mechanism

Considerable effort has been focused on understanding the mechanism of action of NDMA-induced carcinogenesis. Tumors typically occur only in tissues that have the ability to metabolize NDMA. The occurrence of tumors appears to be related to

occurrence of significant levels of cytochrome P450 (principally CYP2E1) within the cell and the ability of cells to metabolize NDMA by the alpha hydroxylation pathway. The metabolism of NDMA results in the formation of an unstable metabolite (the methyldiazonium ion) that reacts with DNA to form DNA adducts. The formation of $O^6$methyl-guanine DNA adducts from NDMA metabolism has been linked to the appearance of tumors. Repair of DNA by MGMT (or lack of repair) also appears to have a key role in the development of cancer. Other factors also appear to modulate NDMA ability to produce tumors in experimental animals.

Conclusion

Given the strong evidence of carcinogenic activity in animals, suggestive evidence in humans, substantial evidence of genotoxicity, and considerable knowledge regarding the mechanism of carcinogenicity of NDMA, it is prudent to conclude that NDMA in drinking water is a carcinogenic risk and, therefore, develop a PHG based on this toxic endpoint.

**Sensitive Populations**

Studies in animals have demonstrated an increase in tumors in the mouse administered ethanol in combination with NDMA, compared to NDMA alone (Anderson *et al.*, 1992). Markedly increased levels of adducts have been detected in extrahepatic tissues in Patas monkeys administered ethanol prior to NDMA (Anderson *et al.*, 1996). Smoking, an important source of exposure to nitrosamines such as NDMA, in combination with ethanol, has been associated with an increased incidence of cancer in humans (NTP, 2000).

The consumption of alcoholic beverages has been linked to an increase in cancer in humans (IARC, 1978; Mirvish, 1995; NTP, 2000; OEHHA, 2004). The consumption of alcoholic beverages in combination with smoking has also been linked to a higher risk of cancer (NTP, 2000). However, the evidence specifically linking exposure to ethanol with an increase in tumors in experimental animals is lacking (NTP, 2000). There is also a lack of genotoxic and mechanistic findings that link exposure to ethanol to cancer. Consequently, Swann and coworkers (Driver and Swann, 1987) and Anderson and associates (Anderson *et al.*, 1995) proposed that ethanol's carcinogenic actions might be related to its ability to induce and/or inhibit nitrosamine metabolism. Many other carcinogenic agents could also be affected by ethanol influence on metabolism in the liver.

Given these findings, individuals exposed to NDMA who consume alcoholic beverages may be at greater risk of cancer. But a number of other substances also stimulate metabolism of xenobiotics. Therefore, individuals that consume alcohol are not considered as a uniquely sensitive population.

## DOSE-RESPONSE ASSESSMENT

### *Noncarcinogenic Effects*

Because of the well-known carcinogenic activity of NDMA, few investigators have conducted animal studies aimed at evaluating other toxic endpoints. NDMA has been observed to produce severe hepatotoxicity resulting in cirrhosis at high doses. Other less severe toxic endpoints in the liver have not been adequately characterized. Effects on the immune system in animals at high NDMA doses have also been demonstrated. Due to the lack of information regarding NDMA toxicity at lower doses, the development of a dose-response relationship for NDMA for non-carcinogenic effects is problematic.

### *Carcinogenic Effects*

The findings of recent studies in humans are suggestive of a link between exposure to NDMA and an increase in gastrointestinal or lung tumors. In these studies, exposure to NDMA is poorly characterized. In addition, most of these studies are characterized by exposure to other nitrosamines or other carcinogens as well. The limitations of these studies complicate attempts to develop a dose-response relationship between NDMA exposure and incidence of cancer in humans. Therefore, the findings of animal studies will be employed to develop a dose-response relationship for NDMA.

A number of cancer bioassays in animals have linked exposure of NDMA to a statistically significant increase in tumors. The study of Peto and coworkers is judged the best study to develop a dose-response relationship between exposure to NDMA and the occurrence of liver tumors in the rat (Peto *et al.*, 1991a,b). In their study, Peto and associates employed 16 dose groups of both male and female rats. The animals were allowed to survive to the “natural” end of their lifetime (there was no terminal sacrifice at two years). Limitations of this study are few. The investigators focused on tumors in the liver so tumors that may have occurred at other sites did not appear to be carefully assessed. The investigators conducted a time-to-tumor analysis based on all tumor sites in the liver. The risk associated with tumors at each site is needed. The investigators did not publish individual animal results and efforts to obtain these data were unsuccessful (personal communication with R Peto). Without individual animal data, a time-to-tumor analysis for individual tumor sites cannot be undertaken.

Two approaches to develop a dose-response relationship for NDMA will be performed. Both approaches will rely on models to obtain the dose associated with a 10 percent increased incidence of tumors above background.. A ten percent incidence of tumor or roughly five tumors per 48 rats is a statistically significant increase in tumors above control in this study (based on Fisher’s Exact test) although it is not considered a notable increase in tumors. A ten percent increase in tumors is considered to be in the observable range (borderline observable) in this study.

The first approach will rely on the time-to tumor-analysis conducted by the investigators. A second approach will employ various models to develop dose-response relationships for tumors at specific sites. Once the dose associated with a ten percent increased

incidence of tumors is determined, a simple linear extrapolation is employed to estimate the cancer potency of NDMA.

Time-to-Tumor Model

The findings of Peto and coworker revealed a statistically significant increase in liver tumors in both male and female rats, with females being more sensitive than males. The findings of their study also suggest that the development of a dose-response relationship based solely on the incidence of tumors at a given site may underestimate risk (by not considering other sources of mortality that could influence the incidence of tumors at a specific site). The early mortality in the animals (which was mainly due to tumors), particularly at higher doses, would be expected to result in less time for tumors to develop and therefore fewer tumors may have been detected than if the animals survived longer. Furthermore, almost all of the animals developed tumors in the higher dose groups. The incidence of tumors did not change with dose in the higher dose groups, because essentially all animals had tumors. However, the tumors appeared earlier in the higher dose groups.

Given these findings, a dose-response relationships based on a time-to-tumor analysis would appear to be sensible because the model accounts for the effect of dose on the survival in the animals and how survival alters the incidence of tumors. Also the model considers the effect of dose on when the tumors appeared. Peto and coworkers' time-to-tumor analysis of liver tumors yielded the following relationship between the cumulative incidence (CI) of tumors at all sites in the liver, and dose and time in the female rat:

$$CI = 51.45(d + 0.1)^6 t^7$$

where:

d = dose (mg/kg-day)

t = time (yr)

Solving for the dose associated with 10 percent incidence of liver tumors at two years, the standard lifetime of a rat, yielded:

d = 0.06 mg/kg-day in the female rat

The equivalent dose in humans can be calculated as the ratio of rat to human body weight to the ¾ power:

$$dose_{human} = dose_{rat}\ (0.06\ mg/kg\text{-}day) \times (0.25\ kg/70\ kg)^{1/4} = 0.0145\ mg/kg\text{-}day$$

A linear dose-response relationship is derived using the dose associated with a 10 percent incidence of tumors, and zero response at zero exposure (the origin). The slope of the linear relationship, the oral cancer potency, is:

$$\text{Oral potency} = \frac{(0.1 - 0)}{(0.0145 \text{ mg/kg-day} - 0)} = 6.9 \text{ (mg/kg-day)}^{-1}$$

The dose associated with $10^{-6}$ risk is therefore:

$10^{-6}$ / 6.9 (mg/kg-day)$^{-1}$ = 1.4 x$10^{-7}$ mg/kg-day

Alternative Approach

While Peto and coworkers did not publish their data on the effects of NDMA on individual animals, they did provide a complete summary of the incidence of tumors at various sites in the liver. These data can be employed to develop dose-response relationships at specific tumor sites, although this analysis of cancer potency requires the use of models that do not consider when the animal died, what caused the death, or when the tumors occurred.

The administration of NDMA to rats resulted in a marked increase in hepatocellular tumors and tumors of the bile duct (Table 7). Given that the incidence of bile duct tumors was much higher than that of hepatocellular tumors at lower doses, this dose-response analysis focused on the bile duct tumors for a low-dose extrapolation. The incidences of bile duct tumors were essentially 100 percent in the higher dose groups (groups 8-11) (Table 7). At the highest doses (groups 14-16), the incidence of bile duct tumors were markedly lower due to early deaths from liver cell tumors. Therefore, the higher dose groups were not particularly useful for developing a dose-response relationship at lower doses. In the lowest dose groups (groups 1-6), there was no evidence of a statistically significant increase in tumors. Thus, the dose response relationship was constrained to include at least one dose group where there was a statistically significant and notable increase in bile duct tumors. In addition, it was deemed desirable to include the maximum number of the dose groups (maximizing the amount of data used) to derive a dose response-relationship when it was prudent to do so (i.e., when it improved the fit for the dose-response extrapolation.).

The increases in bile duct tumors above the rates in controls were evaluated using the Fisher's Exact test and a Mantel-Haenszel test for trend components of the Tox-Risk Software (Crump *et al*., 2000). Because the study included 16 dose groups, an $\alpha$ of 0.05 was adjusted (for the Fisher's Exact test) to address multiple comparisons using a Bonferroni correction ($\alpha$/16 or 0.003). The increase in tumors was considered statistically significant when the addition of a dose group resulted in both the Fisher's Exact test and the Mantel-Haenszel test for trend indicating statistical significance, and when the increase in tumors was judged to be notable. When seven or more doses were evaluated (or when doses greater than 0.038 mg/kg/day were included in the statistical analysis), the observed increases in bile duct tumors were statistically significant and notable. This finding constrained the dose-response relationship to a minimum of seven dose groups.

Dose response relationships were investigated for seven or more doses in the Peto *et al*. study (1991a,b) using various models in the U.S. EPA BMDS software version 1.2.1 (U.S. EPA, 2000). When the lowest seven dose groups were evaluated, all of the models

yielded acceptable fits (p >0.1) (Table 11). When eight doses were evaluated, only one of the models, the linearized multistage model, yielded an acceptable fit to incidence of bile duct tumors (p > 0.1) (Table 11). Using the lowest eight dose groups, the multistage model yielded an acceptable fit with a lower 95 percent confidence limit on the dose associated with 10 percent incidence of bile duct tumors of 0.032 mg/kg/day. The linearized multistage model did not yield an acceptable fit using the nine or ten lowest dose groups.

**Table 11. Goodness of Fit of the Modeled[a] Dose-Response Relationships Associated with Bile Duct Tumors. Seven vs. Eight Lowest Dose Levels**

| **Model** | **Seven Doses p-value[b]** | **Eight Doses p-value[b]** |
|---|---|---|
| Gamma | 0.4 | 0.06 |
| Quantal linear | 0.4 | 0 |
| Logistic | 0.2 | 0.02 |
| Log logistic | 0.4 | 0.04 |
| Probit | 0.2 | 0.01 |
| Log probit | 0.1 | 0.03 |
| Quantal quadratic | 0.1 | 0.001 |
| Linearized Multistage | 0.25 | 0.25 |
| Weibull | 0.4 | 0.04 |

[a]Models from U.S. EPA Benchmark Dose Software.
[b]p- value of Chi-Square goodness of fit test.

The equivalent dose in humans is the ratio of rat to human body weight to the ¾ power:

$$\text{dose}_{\text{human}} = \text{dose}_{\text{rat}}\ (0.032\ \text{mg/kg-day}) \times (0.25\ \text{kg}/70\ \text{kg})^{1/4} = 0.0078\ \text{mg/kg-day}$$

A linear dose-response relationship is derived using the dose associated with a 10 percent incidence of tumors, and zero response at zero exposure (the origin). The slope of the linear relationship, the oral cancer potency, is:

$$\text{Oral potency} = \frac{(0.1 - 0)}{(0.0078\ \text{mg/kg-day} - 0)} = 12.8\ (\text{mg/kg-day})^{-1}$$

The dose associated with $10^{-6}$ risk is therefore:

$$10^{-6} / 12.8\ (\text{mg/kg-day})^{-1} = 7.8 \times 10^{-8}\ \text{mg/kg-day}$$

Strengths and weaknesses of the two approaches

Time to Tumor Model - The findings of Peto and coworkers revealed that the dose-response relationship did not appear to be linear in the observable range. In the observable range, an increase in dose resulted in a marked increase in the incidence of tumors and a decrease in the time to appearance of the tumors. At higher doses, the incidences of tumors were near 100 percent, and the time when the tumors occurred continued to decrease. The time-to-tumor analysis conducted by the investigators utilized all dose groups, accounting for both the incidence of tumors and when the tumors occurred. Competing mortality, principally from tumors at other locations, was also accounted for by the time-to-tumor model. The time-to-tumor model predicted a relationship between dose, the incidence of liver tumors, and when they appeared that appeared to be consistent with the apparent non-linearity of the dose-response relationship in the range of observation. Unfortunately, the modeling was based on combined tumors at four liver cell sites (although in reality significant numbers of tumors occurred only in two cell types, hepatocytes and bile duct). Typically, tumors would be segregated by tissue type in the liver and a dose-response relationship would be developed for each cell type. The risk associated with tumors at a specific site, while preferred, cannot be obtained from these data using a time-to-tumor model because the results in individual animals are not available. Another limitation of developing a dose-response relationship using a time-to-tumor model is that the dose-response calculation provided by the investigators could not be confirmed, as the data were not available. In addition, a lower bound estimate of the dose associated with a ten percent incidence of tumors could not be determined.

Linearized Multistage Model - The dose response relationship derived using the linearized multistage model is based on tumors of the bile duct and not total liver tumors, which is methodologically preferable. A dose-response relationship for tumors at individual sites could be obtained because the investigators published complete summary tables of the incidence of tumors at each of the four liver sites (although not the times when they occurred). Another advantage of using the multistage model is that the calculation of the dose-response relationship can be independently confirmed. Limitations of this approach are important. The dose response relation uses only a portion of the data (eight of 16 dose groups) because the model does not appear to describe the shape of the dose-response relationship throughout the observable range, particularly at higher doses (dose levels nine to sixteen). The relationship does not consider the effect of dose on when the tumors occurred or sources of competing mortality. On the other hand, the potency determined from these bile duct tumors is somewhat greater than that derived from the time-to-tumor analysis of Peto *et al*., and might for that reason be considered the preferable value (more health-protective).

Because the time-to-tumor analysis does not describe a lower bound of dose associated with a 10 percent increase in bile duct tumors, we have chosen to use the results of the linearized multistage method to derive the PHG. We conclude that the two approaches are supportive of each other with a difference of less than 2-fold.

## CALCULATION OF THE PHG

### *Non-Carcinogenic Effects*

A protective level has not been developed for non-cancer effects due to the lack of adequate toxicological studies that investigated non-carcinogenic toxic endpoints. The high cancer potency and unequivocal nature of this chemical as a carcinogen would make a non-cancer health-protective value of very limited relevance.

### *Carcinogenic Effects*

The health-protective concentration for NDMA for drinking water is based on the ingestion route only. The Henry's Law constant for NDMA is $1.99 \times 10^{-6}$ atm-m$^3$/mole at 37 $^{o}$C, indicating that almost no NDMA will partition into the air during showering. The NDMA skin permeability constant, Kp, of 0.000265 cm/hr is very low, and therefore dermal exposure in the shower would not contribute significantly to the overall exposure.

Two approaches were employed to obtain a dose response relationship for NDMA, a time–to tumor model provided by Peto and coworkers and an alternative approach based on a multistage model of the incidence of tumors in the bile duct. The dose-response relationship based on the linearized multistage model was judged preferable because it provided a lower confidence bound on the dose associated with 10 percent incidence of bile duct tumors. A health-protective concentration, C, is therefore calculated as follows:

$$C = \frac{10^{-6} \times BW\ (kg)}{P_{oral}\ (mg/kg\text{-}day)^{-1} \times L_{ingest}/day}$$

where:

BW = body weight (a default of 70 kg);

$P_{oral}$ = oral cancer potency = 6.9 (mg/kg-day)$^{-1}$;

$L_{ingest}$/day = daily amount of water ingested (2 L/day).

Thus,

$$C = \frac{10^{-6} \times 70\ kg}{12.8\ (mg/kg\text{-}day)^{-1}) \times 2\ L/day} = 3 \times 10^{-6}\ mg/L = 0.003\ \mu g/L\ (ppb)$$

The proposed PHG for NDMA is therefore set at $3 \times 10^{-6}$ mg/L, or 0.003 ppb, representing a lifetime upper-bound cancer risk of 1 in 1 million. Other toxic effects associated with NDMA were observed at very high exposure levels. The proposed PHG for NDMA is

presumed protective against these other toxic effects, although non-cancer effects are poorly characterized for this chemical.

There is evidence that agents such as ethanol that can alter metabolism of chemicals in human liver, can thereby influence the rate of NDMA metabolism and hence its toxicity. The data in humans are sparse but there is some evidence to indicate that individuals who consume large amounts of alcohol may have higher levels of NDMA demethylase; they could therefore be considered at somewhat higher risk of adverse effects of NDMA. A quantitative assessment of this potential effect is not feasible. This phenomenon should not be considered unique to NDMA. Many xenobiotics are metabolized by enzymes that are subject to induction and therefore the toxicity of these chemicals can be altered by exposure to other chemicals.

Given the lack of specific information on the existence of any other subpopulations that may be sensitive to the toxic effects of NDMA, the PHG of 0.003 ppb is considered protective of lifetime exposure to NDMA. Levels of NDMA in drinking water associated with a lifetime theoretical extra cancer risk of $10^{-4}$ or $10^{-5}$ are 0.3 and 0.03 ppb, respectively.

## RISK CHARACTERIZATION

The proposed PHG for NDMA is based on risk associated with the lifetime ingestion of drinking water. Various sources of uncertainty regarding the development of health-protective criteria for the oral route are discussed.

Hazard Identification - There is overwhelming evidence that exposure to NDMA resulted in increased incidence of cancer in animals. An increase in tumors associated with NDMA exposure has been observed in a variety of animal species, in both males and females, by both oral and inhalation exposure routes, in immature and mature animals, and using a number of experimental protocols. Studies of human exposure are much more limited and are suggestive but certainly not conclusive that human exposure to NDMA results in an increase in cancer.

Considerable effort has been directed at understanding the carcinogenic process associated with NDMA, particularly in rats and mice. Metabolism to an active metabolite appears to be a prerequisite. Studies have demonstrated that DNA is methylated as the result of NDMA metabolism and the formation of adducts, particularly at the $O^6$-guanine position, is involved in the carcinogenic process. These studies have also revealed that repair or the lack of repair of the $O^6$-methyl guanine adducts by the enzyme MGMT is also involved in the carcinogenic process.

While there are very few studies that have specifically investigated the carcinogenic effect of NDMA in humans, NDMA metabolism in animals and humans is very similar. The cytochrome P450 isoform CYP2E1 that generates the active metabolites in rodents is detected in the human liver and reacts with an antibody prepared against the rat enzyme. The generation of adducts from NDMA *in vitro* has been detected in liver slices from human livers. In addition, adducts have been detected in human liver DNA following a NDMA poisoning episode (Herron and Shank, 1980). MGMT, the enzyme responsible

for the repair of $O^6$-methyl guanine DNA adducts, has been detected in the liver of humans. Therefore, it is likely that the findings of animal studies of an increase in risk associated with exposure to NDMA is relevant to human exposure to the chemical.

Dose Response – A number of animal studies could have been employed to develop a dose-response relationship for NDMA. The study of Peto and associates was judged the best study and therefore was employed to describe a dose-response relationship for oral exposure to NDMA. The results of this study were provided in tabular form (tumor incidence at four separate sites), although individual animal results are no longer available (personal communication with RP). Thus a dose-response relationship for tumors at individual sites could be obtained including a lower confidence bound on dose, because the investigators published complete summary tables of the incidence of tumors at each of the four liver sites. Neither the linearized multistage model nor any of the models in the BMD software use all 16 dose groups because the models did not appear to describe the shape of the dose-response relationship throughout the observable range, particularly at higher doses (dose levels nine to sixteen). The linearized multistage model also did not consider the effect of dose on when the tumors occurred nor sources of competing mortality at a given tumor site that could influence the incidence of tumors at that site (animals may have died from tumors at other sites or early mortality could have prevented a tumor from developing in the animal at the given site).

While the Peto *et al*. (1991a,b) study is well-conducted, there is always uncertainty associated with employing the results of an animal study to estimate risk in humans. Metabolism of NDMA appears to be similar in rats and humans, mediated by the same enzyme, CYP2E1. However, there are some differences in the metabolism of NDMA in rodents and humans. Humans appear to have higher basal levels of MGMT than the rat, suggesting differences in susceptibility. However, this enzyme is inducible and increased levels of MGMT would be expected to have occurred in rodents in the cancer bioassays, because NDMA induces MGMT. Another indication of a possible difference in metabolism is indicated by adducts occurring in extra-hepatic tissue in the monkey, while occurring mainly in the liver in the rat.

Exposure Assessment – An upper-bound estimate of drinking water consumption (2 L/day) and a default 70 kg adult body weight were employed to develop the health-based criteria for oral exposure to NDMA. Given its very low volatility and skin permeability, little NDMA would be expected to partition into the air and be inhaled during showering, or be absorbed by the skin. Therefore, the PHG is based only on the oral route of exposure.

Sources of NDMA other than drinking water are possible. For estimation of cancer risk, it has been customary not to incorporate an estimate of relative source contribution (RSC), and therefore RSC is not utilized here. The rationale for excluding explicit consideration of other sources is that the cancer risk assessment is based on extra risk (in addition to other exposure sources), and that cancer risk estimation methods are conservative enough already. For non-cancer effects, relative source contribution must be explicitly considered in order to judge whether total exposures exceed a dose-effect threshold. This is not germane to the linear extrapolation cancer risk estimation method utilized in derivation of the PHG.

Risk Characterization - The various sources of uncertainty attendant in the hazard identification, dose response, and exposure assessment are reflected in the estimates of risk. While the study of Peto and associates, which was performed in animals, was judged to be a superior toxicological study, better estimates of human risk could theoretically be obtained from human studies. Available epidemiological studies are inadequate, but future studies could be employed to better characterize the human impact of this chemical.

## OTHER REGULATORY STANDARDS

The U.S. EPA recommends that levels (Ambient Water Criterion for humans) in lakes and streams should be limited to 0.0014 ppb of N-nitrosodimethylamine at a $10^{-6}$ risk level, to prevent possible health effects from drinking water or eating fish contaminated with N-nitrosodimethylamine (U.S. EPA, 1980).

The U.S. EPA requires that spills or accidental releases of 10 pounds or more of N-nitrosodimethylamine be reported to the U.S. EPA (2001). The California Notification Level (previously known as Action Level) for NDMA is 0.01 μg/L. Notification levels are defined by the California Department of Health Services as "health-based advisory levels established by DHS for chemicals in drinking water that lack maximum contaminant levels" (DHS, 2005).

IARC (1978) evaluated the evidence for carcinogenicity and determined "there is sufficient evidence of a carcinogenic effect of N-nitrosodimethylamine in many experimental animal species. Similarities in its metabolism by human rodent tissues have been demonstrated. Although no epidemiological data were available (and efforts should be directed toward this end), N-nitrosodimethylamine should be regarded for practical purposes as if it were carcinogenic to humans."

NDMA was added to the list of chemicals known to the State to cause cancer [Title 22, California Code of Regulations, Section 12000] as of October 1, 1987, pursuant to California's Safe Drinking Water and Toxic Enforcement Act of 1986. The no-significant-risk-level for NDMA is 0.04 μg/day (22 CCR 12705b).

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