ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR N-NITROSODIPHENYLAMINE

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CONTENTS

ADDENDUM for N-Nitrosodiphenylamine
Supplement to the 1993 Toxicological Profile for N-Nitrosodiphenylamine

Background Statement

This addendum to the Toxicological Profile for N-Nitrosodiphenylamine supplements the profile that was released in 1993.

Toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). CERCLA mandates that the Administrator of ATSDR prepare toxicological profiles on substances on the CERCLA Priority List of Hazardous Substances and that the profiles be revised "no less often than once every three years." CERCLA further states that the Administrator will "establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" [Title 42, Chapter 103, Subchapter I, § 9604 (i)(1)(B)].

The purpose of this addendum is to provide to the public and other federal, state, and local agencies a non-peer reviewed supplement of the scientific data that were published in the open peer-reviewed literature since the release of the profile in 1993.

Chapter numbers in this addendum coincide with the Toxicological Profile for N-Nitrosodiphenylamine (1993). This document should be used in conjunction with the profile. It does not replace it.

2. HEALTH EFFECTS

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

2.2.2 Oral Exposure

2.2.2.7 Genotoxic Effects

Westphal et al. (2001) examined the genotoxicity of N-nitrosodicylohexylamine in isolated human lymphocytes using N-nitrosodiphenylamine as a positive control in the Ames test and cytokinesis block micronucleus assay. N-nitrosodiphenylamine has a similar molecular structure to N-nitrosodicyclohexylamine and has shown carcinogenic effects at 1,000 ppm (parts per million) or 4,000 ppm in both sexes of F-344 rats and induced transitional cell carcinomas of the urinary bladder of male and female $B6C3F_1$ mice (NTP 1990, 1991). N-nitrosodiphenylamine-induced micro-colonies at concentrations above 250 µg (micrograms) in the presence of Arochlor 1254 in *Salmonella typhimurium* TA104. Similarly, McGregor (1994) used the Ames test with different strains of *Salmonella typhimurium* and found that the effects of N-nitrosodiphenylamine were inconclusive or negative, except in one study which showed micro-colony induction in *Salmonella typhimurium* TA104 in the presence of Arochlor-1254-induced S9 (McGregor, 1994).

2.3 TOXICOKINETICS

2.3.3 Metabolism

Sheweita and Mostafa (1996[a]) evaluated the effects of N-nitroso compounds, including N-nitrosodiphenylamine, on hepatic levels of glutathione (GSH), glutathione reductase (GSH-R), and glutathione S-transferase (GST) in of male Balb/C mice. N-nitrosodiphenylamine was dissolved in distilled water and administered intraperitoneally (i.p.) as a single dose of 20 mg/kg (milligrams/kilogram). N-nitrosodiphenylamine treatment induced a 50% reduction in liver GSH content, a 60% reduction in liver GST activity, and a 77% increase in GSH-R enzyme activity. The decrease in liver GSH content and decrease in liver GST enzyme activities may alter the effects of N-nitrosodiphenylamine by reducing conjugation with GSH and increasing mutagenicity.

In a similar study, different N-nitrosamines were administered to male mice to examine the effect on modification of enzyme activity in mice liver microsomes (Sheweita and Mostafa, 1996[b]). A single i.p. dose of 20 mg/kg of N-nitrosodiphenylamine was administered to one group of male mice. N-nitrosodiphenylamine decreased cytochrome P450 content by 54% and alylhydrocarbon hydroxylase (AHH) activity by 64%. N-nitrosodiphenylamine increased dimethylnitrosamine *N*-demethylase activity by 42%, NADPH-cytochrome *c* reductase activity by 57%, and cytrochrome b_5 content by 159% (Sheweita and Mostafa, 1996[b]).

1,1 Diphenylhydrazine was oxidized to N-nitrosodiphenylamine by microsomes obtained from ciliary bodies of bovine eyes (Nikaido et al., 1992). Photochemical oxidation of 1,1-diphenylhydrazine occurred in varying degrees when placed in visible light in the presence of photo-sensitizers riboflavin, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), lumiflavin, lumichrome, NAD^+, NADH, $NADP^+$, and NADPH. Superoxide dismutase and L-ascorbic acid both blocked the photochemical oxidation of 1,1 diphenylhydrazine to N-nitrosodiphenylamine. There was a large decrease in oxidation activity of 1,1 diphenylhydrazine to N-nitrosodiphenylamine in the presence of NADPH and riboflavin by superoxide dismutase, but it was unaffected by catalase, carbon monoxide and SKF525. Superoxide radicals appear to be responsible for the oxidation of 1,1-diphenylhydrazine to the N-nitrosodiphenylamine via ocular tissue microsomes and photo-sensitizers.

2.5 RELEVANCE TO PUBLIC HEALTH

An *in-vitro* study conducted by Boyd et al. (2008) examined the cytotoxic effects of N-nitrosodiphenylamine and other nitrosamines using a cell-micro-electronic sensing technique on three human cell lines (T-24 bladder, Hep-G2 liver, A549 lung carcinoma cells) and Chinese hamster ovary cells (CHO-K1). N-nitrosodiphenylamine had the highest toxicity values for each cell line and induced cell-cycle arrest in the cytotoxicity assays. *In-vitro* cytotoxicity inhibitory concentration values (IC_{50}) reported for each cell line were as follows: T-24 (0.59 mM); Hep-G2

(1.9 mM); A549 (0.75 mM); CHO-K1 (0.72mM). These results can be utilized to characterize chemical toxicity based on *in-vitro* cytotoxicity values or IC_{50} values, which are similar to *in-vivo* lethal dose (LD_{50}) toxicity ranking of chemicals.

Genotoxic Effects

N-nitrosodiphenylamine tested negative for genotoxicity in the most commonly used *in vitro* genotoxicity tests (Ames + mouse lymphoma assay + in-vitro micronucleus or chromosomal aberrations) (Kirkland, D. et al., 2005). Only 3 other compounds (n = 722) tested negative for genotoxicity in all three tests. It may be that N-nitrosodiphenylamine is an extremely weak carcinogen that does not give rise to significant genotoxic effects *in-vitro*.

2.8 INTERACTIONS WITH OTHER CHEMICALS

Vicia Faba beans (VF) and bran, commonly found in Egyptian foods, may be able to protect against the negative health effects of nitrosamines (Zakhary et al., 1994). An *in vitro* study found that the presence of VF and bran decrease the formation of N-nitrosodiphenylamine. The reduction was dependent on concentration, the form of VF or bran, and the duration of the reaction.

3. CHEMICAL AND PHYSICAL INFORMATION

No updated data.

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

No updated data.

5. POTENTIAL FOR HUMAN EXPOSURE

It is well known that the use of chlorine, chloramines and chlorine dioxide to disinfect drinking water effectively prevents waterborne diseases (Zhou et al., 2009). Epidemiological studies have reported evidence of an increased risk of bladder cancer following consumption of chlorinated water. N-nitrosodiphenylamine is an un-intended disinfection byproduct formed from the interaction of chloramines and organic materials found in water. Diphenylamine is used as an insecticide, a storage preservative for apples, and a rubber antioxidant, which is found in the environment, and is believed to be a precursor of N-nitrosodiphenylamine (Zhou et al., 2009).

N-nitrosodiphenylamine is considered a probable human carcinogen by US Environmental Protection Agency (EPA). The MCL (maximum contaminant level) for N-nitrosodiphenylamine is 7 µg/L (micrograms/Liter) or 7 ppb (parts per billion) based on a lifetime cancer risk of 1 in 1,000,000. An MCL is the highest level of a contaminant that is allowed in drinking water. Recently, Zhou et al., (2009) detected diphenylamine at 1.3 ng/L (nanograms/Liter or parts per trillion) in source water and no N-nitrosodiphenylamine was detected. However, after several hours of adding 1 mM (milliMolar) of chloramines to the source water, N-nitrosodiphenylamine was detected at 0.4 ng/L, while diphenylamine was correspondingly reduced to 0.4 ng/L. Furthermore, these investigators found that after increasing the pH of the water from 4 to 10 that N-nitrosodiphenylamine increased by 10 fold (Zhou et al., 2009). This study provided evidence that further studies are necessary to completely elucidate the mechanisms of formation of nitrosamines in chloraminated drinking water (Zhou et al., 2009).

Zhao et al. (2008) reported evidence similar to that of Zhou et al. (2009) which indicated that formation of un-intended disinfection byproducts such as N-nitrosodiphenylamine are linked to chloramination of source water, and its interaction of organic matter in the water (Zhao et al. 2008). These investigators indicated that disinfection byproducts formed in water is a difficult process, and is influenced by many variables such as temperature, pH, turbidity, and natural organic content in the source water (Zhao et al., 2008). There are ongoing research concerning un-intended disinfection byproducts such as nitrosamines in drinking water and increased cancer risk in humans. The main finding in the Zhao et al. (2008) investigation was that depending on the source water, chloramines had a potential to produce greater levels of nitrosoamines than chlorines (Zhao et al., 2008).

5.3 ENVIRONMENTAL FATE

5.3.2 Transformation and Degradation

5.3.2.2 Water

A simple inexpensive cost effective technique was used to oxidize N-nitrosamine co-contaminants in wastewater in an above ground polypropylene Baker tank (Jamall and Brown 2006). The wastewater was from post harvest treatment of fruits (e.g., apples and pears) that is used to extend the shelf life of the fruits. The study was conducted using potassium permanganate ($KMnO_4$) and Fenton's reagent (hydrogen peroxide with iron as a catalyst) for the oxidation of N-nitrosamines. Wastewater treated with 0.07 M or 0.14 M $KMnO_4$ had N-nitrosodiphenylamine levels reduced by 89% (from a mean of 52 ppm to a mean of 5.5 ppm) and 97% (52 ppm to 1.4 ppm), respectively, in comparison to control water samples. The wastewater levels of these contaminants were sampled at 1 hr and 12 hr. The differences in levels between the two times they were sampled were insignificant (Jamall and Brown, 2006).

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

N-nitrosodiphenylamine was formed as an unintended by-product after water was treated with various disinfectants (e.g. chlorine, chloramines, chlorine dioxide, advanced oxidation processes) (Zhao et al., 2006). N-nitrosodiphenylamine was detected at 4 locations in the water distribution system from 0.65 ng/L to 1.86 ng/L (i.e., parts per trillion). Disinfectant-by-products are formed when residuals of water disinfectants such as chlorine or chloramine react with naturally occurring organic materials in water (Boyd et al., 2008).

5.4.4 Other Environmental Media

N-nitrosodiphenylamine was detected in "Braeburn" apple cortex tissue samples following two months of storage in both natural air and a controlled atmosphere (1 kPa of O_2 and 3kPa of CO_2 at 0.5°C) (Mattheis and Rudell 2008). N-nitrosodiphenylamine content in control samples was higher compared with apples treated with the ethylene action inhibitor 1-methylcyclopropene. After four months of storage, N-nitrosodiphenylamine levels were no longer detectable (Mattheis and Rudell, 2008).

6. ANALYTICAL METHODS

An *in-vitro* study conducted by Boyd et al. (2008) examined the cytotoxic effects of N-nitrosodiphenylamine using a cell-micro-electronic sensing technique on three human cell lines (T-24 bladder, Hep-G2 liver, A-549 lung carcinoma cells) and Chinese hamster ovary cells (CHO-K1). Chemical toxicity was monitored by using the cell lines as the living components of the sensors of a real-time cell electronic sensing method. The sensing technique measured the change in impedance of micro-electronic wells that correlate linearly with the change in the number of cells, providing reliable information on inhibitory toxicity values. The method provided a way to profile cytotoxicity of chemicals in the same class.

Using liquid chromatography coupled to tandem mass spectrometry, common propellant powder stabilizers in organic gunshot residue, including N-nitrosodiphenylamine, were identified with high accuracy compounds and underwent solid phase extraction after being swabbed from the hands of the shooter using cotton moistened with isopropyl alcohol/water. The reported instrumentation detection limit for N-nitrosodiphenylamine was 0.27 nMol/L (nanoMolar) (Laza, D. et al., 2007).

6.2 ENVIRONMENTAL SAMPLES

Zhao et al. (2006) developed a new analytical method to detect N-nitrosamines in drinking water using a combined method of solid-phase extraction (SPE), micro-column liquid chromatography (LC), tandem mass spectrometry (LC-MS/MS), and multiple reactions monitoring (MRM). The method detection limit was 0.1 ng/L. Water samples were collected from four locations along a distribution system where surface water was treated with chlorine and UV radiation. The chromatographs and retention times for nine N-nitrosamines were identified by monitoring ion pairs and observing spiked water samples. The SPE-LC-MS/MS MRM technique was sensitive enough to detect both GC non-detectable and GC detectable N-nitrosamines. The recovery of N-nitrosodiphenylamine through SPE and LC-MS/MS was 111%. The new technique effectively indicated occurrence, distribution, and quantification of various N-nitrosamines in drinking water systems (Zhao et al., 2006).

7. REGULATIONS AND ADVISORIES

The U.S. EPA has classified N-nitrosodiphenylamine as a probable carcinogen (B2), which indicates that there is sufficient carcinogenic evidence in animals, but insufficient carcinogenic evidence in humans (EPA 1993).

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