ADDENDUM TO THE TOXICOLOGICAL PROFILE FOR N-NITROSODI-N-PROPYLAMINE

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ADDENDUM for N-Nitrosodi-n-propylamine
Supplement to the 1989 Toxicological Profile for N-Nitrosodi-n-propylamine

Background Statement

This addendum to the Toxicological Profile for N-Nitrosodi-n-propylamine supplements the profile that was released in 1989.

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 the Agency for Toxic Substances and Disease Registry (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 1989.

Chapter numbers in this addendum coincide with the Toxicological Profile for N-Nitrosodi-n-propylamine (1989). This document should be used in conjunction with the profile. It does not replace it.

2. HEALTH EFFECTS

2.1 INTRODUCTION

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

2.2.1 Inhalation Exposure

Reh and Fajen (1996) measured occupational nitrosamine exposures during a National Institute for Occupational Safety and Health hazard evaluation at a rubber vehicle sealing plant on February 16,1994 and on a follow-up on May 3-5, 1994. N-Nitrosodi-n-propylamine was not found at the detection limit of 0.01 $\mu g/m^3$ in salt bath line or molding/finishing operators' breathing zone air samples or general air samples.

2.2.2 Oral Exposure

2.2.2.8 Cancer Effects

Inhabitants of high- (Nan'ao County) and low- (Lufeng County) risk areas for esophageal cancer in southern China were assessed for exposure to N-nitroso compounds, including N-nitrosodi-n-propylamine (Lin et al. 2002). Samples of 24-hour diet and 12-hour overnight urine were collected from 120 healthy males (35–64 years old) from each location. Nitrosamines in urine were analyzed as performed previously (Lu et al. 1986). Total N-nitroso compound (TNOC) daily intake (4.25 ± 0.84 μmol), TNOC excretion levels (0.04 ± 0.01 μmol/12h), and daily intake of volatile nitrosamines (5.84 ± 0.07 1μmol) were significantly greater in the high-risk area than in the low-risk area. Nitrosamino acid excretion levels were not significantly different between the high- and low-risk areas, suggesting TNOC involvement in the pathology of esophageal cancer in southern China.

N-nitroso compounds known to induce kidney tumors in rats, including N-nitrosodi-n-propylamine, were assayed for DNA-damaging activity in kidney cultures of rats and humans (Robbiano et al. 1996). Nonsignificant DNA single-strand breaks were consistent when human and rat kidney cells were exposed to 10 mM of N-nitrosodi-n-propylamine for 20 hours, suggesting that N-nitrosodi-n-propylamine may be a carcinogen to human kidney cells.

Gritsiute and Barauskaite (1989) observed the incidence of tumors in two groups of male C57B6I mice intragastrically dosed twice a week for 50 weeks with either 1 or 3 mg of N-nitrosodi-n-propylamine. Tumors were observed in the esophagus, forestomach, lungs, and lymphomas. Pulmonary adenoma and forestomach papillomas were seen more frequently in the higher dosed group.

2.3 RELEVANCE TO PUBLIC HEALTH

Genotoxicity. Okochi et al. (1997) substituted S9 in the Ames test with tetrakis(pentafluorophenyl)porphyrination(III) chloride [Fe(F_5P)Cl] and tert-butyl hydroperoxide. This substitution enabled detection of nitrosamine mutagenicity from base pair substitution mutagens and frameshift mutagens without the use of enzymatic activity.

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Kushida et al. (2000) established a *Salmonella typhimurium* tester strain YG7108 2E1/OR co-expressing human CYP2E1 and human NADPH-cytochrome P450. Capacities of human CYP2E1 and CYP2A6 to metabolically activate alkyl derivatives on N-nitrosamines were compared by using the same *S. typhimurium* strain. The concentration (1.43 μM) of YG7108 2E1/OR and YG7109 2A6/OR giving a positive mutagenic result with N-nitrosodi-n-propylamine was the same for both strains. The mutagen-activating capacities of YG7108 2E1/OR and YG7109 2A6/OR with N-nitrosodi-n-propylamine were 249 and 479 nmol mutagen, respectively. The study indicates that YG7109 2A6/OR is primarily responsible for metabolic activation of N-nitrosodi-n-propylamine.

Cooper and Porter (2000) developed two dialkylnitrosamine-sensitive mutagenicity tester strains of *Salmonella typhimurium* lacking an exogenous metabolic-activating system. Human CYP2E1 and P450 reductase were expressed in *S. typhimurium* strains that lacked *ogt* and *ada* methyltansferases. N-Nitrosodi-n-propylamine was cytotoxic at concentrations above 50 μmol/plate in YG710ER and 100 μmol/plate in strains YG7104ER and TA153ER. Repairs on adducts formed by N-nitrosodi-n-propylamine depended on the activity of the *ogt* methyltransferase.

Cooper and Porter (2001) developed mutagenic *Salmonella typhimurium* tester strains (YG7104, *ogt-*; YG7108, *ogt-, ada-)* that co-express CYP2E1, rat CYP reductase, and human cytochrome b_5 to examine the effects of co-expression on nitrosamine mutagenicity. In these tester strains, a four- to five-fold increase in mutagenicity was observed in response to several nitrosamines, including N-nitrosodi-n-propylamine, compared with the response in strains composed of CYP2E1 and cytochrome reductase. The recombinant strains were 100-fold more sensitive in the presence of an S9 fraction to nitrosamines than the parent strains; a dose-dependent mutagenicity was observed with the addition of an S9 fraction. Nitrosamine mutagenicity increased when YG7101b_5ER expressed CYP2E1, P450 reductase, and cytochrome b_5 simultaneously. Cytochrome b_5 increased the mutagenicity in YG7104 (*ogt-*) cells but not as significantly in YG7101 (*ogt-, ada-*) cells. For an indeterminate reason, positioning b_5 cDNA in front of CYP2E1 and reductase cDNAs increased mutagenic response to nitrosamines.

Fujita and Kamataki(2001) investigated the capacities of human CYP (CYP1A2, CYP2C8, CYP1B1, CYP2C9, CYP2A6, CYP2C19, CYP 2D6, CYP 2E1, CYP3A4, and CYP3A5)

expressed in 11 genetically engineered strains of *Salmonella typhimurium* to metabolically activate several nitrosamines, including N-nitrosodi-n-propylamine. The minimal concentration value for activation and for mutagen-producing capacity value of CYP in activation of N-nitrosodi-n-propylamine are given in TABLE 2-1. CYP2A6 played a significant role in activating N-nitrosodi-n-propylamine.

Table 2-1. Mutagen-producing capacity of CYP in N-nitrosodi-n-propylamine (NDPA) and minimal concentration (MC) value for activation of N-alkylnitrosamines by CYP[a]

CYP	Mutagen-producing capacity of CYP in activation of NDPA	MC value for activation of NDPA by CYP
1A1	0.305[b]	57.1[c]
1A2	0.0712	425
1B1	0.0172	196
2A6	13.4	0.842
2C8	ND[d]	ND
2C9	ND	ND
2C19	ND	ND
2D6	ND	ND
2E1	1.18	64.7
3A4	0.0656	169
3A5	ND	ND

[a]Fujita and Kamataki (2001).
[b]Mutagen-producing capacity; a slope of the increase of revertant colonies against the increase of the amount of promutagen (nmol) at around MC was calculated. The slope was then divided by pmol CYP in a reaction mixture.
[c]MC (μM).
[d]ND = Not detectable.

Human b_5/CYP-competent mutagenicity tester bacteria were developed to investigate the role of b_5 in the activation of human CYP1A2, CYP 2A6, and CYP2E1 (Duarte et al. 2005). Strains BTC-b_5 1A2, BTC-b_5 2A6, and BTC- b_5 -2E1 were applied in mutagenicity assays to determine their effect on CYP-mediated bioactivation of promutagens, including N-nitrosodi-n-propylamine. The mutagenicity of N-nitrosodi-n-propylamine depended entirely on the co-expression of b_5 with CYP2A6/CYP2E1.

2.6 TOXICOKINETICS

2.6.1 Absorption

The diffusion rates of several nitrosamines, including N-nitrosodi-n-propylamine, were measured through the esophagus of male Wistar rats (9–12 weeks; 300–350 g) (Haorah et al. 1999). When measured with side-by-side diffusion apparatuses, mucosal and serosal esophageal fluxes were 18–280 times higher than the predicted flux. The serosal flux ratio (P<0.05) and mucosal flux

ratio (P<0.07) correlated with the esophageal carcinogenicity (as tested by Druckrey et al. 1967). No involvement of cytochrome P450 was determined. Skin flux increased 5–13 times after the enzymic/mechanical removal of the epidermis. The flux of N-nitrosodi-n-propylamine was 1.9–2.0 times faster through the esophagus than through the forestomach. Results indicated that N-nitrosodi-n-propylamine can be absorbed through the esophagus by passive diffusion while swallowing food or drink.

2.6.3 Metabolism

Teiber et al. (2001) investigated enzymes responsible for oxidizing N-nitroso-β-hydroxypropylpropylamine (NHPPA), the metabolite of N-nitrosodi-n-propylamine, to N-nitroso-β-oxopropylpropylamine (NOPPA) and then to a methylating agent. Human and rat cell fractions were exposed to cytochrome P450 2R1, rat NADPH-cytochrome P450 reductase, rat CYP2B1, and cytochrome b_5. Rat liver CYP2B1 and rabbit liver CYPE1 metabolized NOPPA to a methylating agent as determined by *in vitro* formation of 7-methylguanine. DNA adducts and a dose-dependent toxicity formed in human liver epithelium transfected with human 2E1 NOPPA. Results indicate that NOPPA oxidation is primarily mediated by cytochrome P450s, and CYP2E1 is capable of catalyzing both oxidations.

Bellec et al. (1997) investigated the influence of the alkyl chain length on the hydroxylation of the carbon atom of 3 nitrosamines, including N-nitrosodi-n-propylamine. Human liver samples were taken from 14 multi-organ donors (13 males and 1 female; standard deviation 37 ± 12 years). N-Nitrosodi-n-propylamine depropylation ranged from the detection limit of 6 pmol/(min×mg) to the maximum 930 pmol/(min×mg). The results indicate that CYP2A6, CYP2C9, CYP2C19, CYP2E1, and CYP3A4 all are involved in the hydroxylation of the alkyl chain of nitrosamines. As the length of the chain increased, α-hydroxylation efficiency increased. The ω-hydroxylation of the alkyl chain strongly correlated with CYP2E1 activity and liver content with N-nitrosodi-n-propylamine. The ω/α ratio of hydroxylation metabolite for N-nitrosodi-n-propylamine was approximately 0.3.

2.7 INTERACTIONS WITH OTHER CHEMICALS

Escobar-Garcia et al. (2001) studied CYP induction in rat liver by cyclohexanol (CH) and albendazole (ABZ) and compared S9 from a *Salmonella* assay with S9 from Aroclor or phenobarbital/β-napthoflavone (PB/NF)-treated rats. Male Wistar rats (200-250 g) were dosed with either CH and ABZ or Aroclor. The results of N-nitrosodi-n-propylamine mutagenic activity are given in TABLE 2-2. S9 from PB/NF- and Aroclor-dosed rats was most effective at activating N-nitrosodi-n-propylamine. The mutagenicity of N-nitrosodi-n-propylamine increased 10-fold with S9 from CH/ABZ. Results indicate that ABZ and CH complement CYP when administered simultaneously.

TABLE 2-2. Mutagenic activity in *Salmonella typhimurium* of mutagens activated by the liver S9 fraction from Aroclor-, phenobarbital/β-naphthoflavone-, and ciclohexanolalbendazole-treated rats[a]

Compound	Dose (µg/plate)	Control S9	Aroclor S9	phenobarbital/β-naphthoflavone S9	Cyclohexanol/albendazoe S9
N-Nitrosodi- n-propylamine	0[b]	95	70.3	86.7	91
	50	95.3	148.7	180.7	137.3
	100	100	188	257.7	201.7
	150	103.7	209.3	296.7	186.7
	200	104.7	247.7	398.7	213
	rev/µg[c]	**0.05**	**0.83**	**1.48**	**0.58**

[a]Escobar-Garcia et al. (2001).
[b]Each value represents the mean of histidine revertants (rev) found in three replicated plates.
[c]Slope from the linear portion of the dose-response curve.

3. CHEMICAL AND PHYSICAL INFORMATION

No updated data.

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

No updated data.

5. POTENTIAL FOR HUMAN EXPOSURE

No updated data.

6. ANALYTICAL METHODS

No updated data.

7. REGULATIONS AND ADVISORIES

No updated data.

8. REFERENCES

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