POPPERS

COMPOUND RISK FOR HIV INFECTION

Disinhibition leading to riskier sex + Dilating blood vessels enabling entry into body + Immunosuppressive increasing susceptibility to infection if exposed to infectious agent(s)

RESEARCH BIBLIOGRAPHY

Human Studies: Poppers and Immunosuppression

Dax EM, et al. “Amyl nitrite alters human in vitro immune function.” **Immunopharmacology and Immunotoxicity** 1991;13(4):557-587. PMID: 1685501. “The changes in lymphocyte function observed in this study suggest that volatile nitrite inhalation results in a cycle of modest immunosuppression followed by gradual recovery after cessation of drug. NK (natural killer) activity was most noticeably effected and was the slowest to recover.”

Dax EM, et al. "Effects of nitrites on the immune system of humans." **Health Hazards of Nitrite Inhalants: NIDA Research Monograph 83** 1988 Mar;83:75-80. PMID: 2902516. "The level of natural killer cell activity showed an initial significant decrease, then returned to baseline levels by day 4 post inhalation." "The results showed a nitrite-induced lymphopenia resulting from a relatively short exposure." "Further, a non specific, perhaps compensatory, immunostimulation following exposure to amyl nitrite was compatible with the suggestion that the nitrites may aggravate symptoms by enhancing HIV replication. The nonspecific stimulation could mask or interfere with specific immune response to pathogens." "The results showed that exposure to amyl nitrite can induce changes in immune function even after short exposure to moderate doses. Several tests of immune function showed an "overshoot" over basal activity at 7 days following nitrite inhalation after an initial immunosuppression. A possible interpretation of the results would be that the nitrites cause a cycling of immune activity between suppressed and nonspecific stimulated levels. This situation might result in a period of immunosuppression followed by a proliferative period in which virus-containing cells propagate in the presence of a nondirected immunoresponse. In the community, nitrites are often used in an episodic manner, which may facilitate such cyclic changes.", which may facilitate such cyclic changes.”

Poppers: Immunosuppression and Gene

Expression

Fung HL & Tran DC. “Effects of inhalant nitrites on VEGF expression: A feasible link to Kaposi’s sarcoma?” **Journal of Neuroimmune Pharmacology** 2006 June;9:1-11. www.springerlink.com/content/73h7w882j6616514/fulltext.html "In a series of studies, we showed that acute and chronic in vivo exposure to isobutyl nitrite (a representative inhalant nitrite) produced significant tissue-dependent alterations in the expression of a number of cancer-and angiogenesis-related genes in mice. In particular, hepatic mRNA and protein expression of vascular endothelial growth factor (VEGF) was significantly stimulated. The in vivo growth rate of a subcutaneous VEGF-responsive tumor was also shown to be accelerated by inhalant nitrite exposure. Because the development of KS is extensively linked to VEGF and its receptors, the purported link between inhalant nitrites and KS may be explained mechanistically, at least in part, through stimulation of VEGF expression by these inhalants."

Tran DC, et al. “Effects of repeated in vivo inhalant nitrite exposure on genes expression in mouse liver and lungs.” **Nitric Oxide** 2006 June; 14(4);279-289. "In conclusion, multiple exposures to inhalant nitrite appeared to cause alterations in the expression of a number of genes relating to cancer and angiogenesis,” PMID: 16288974.

Mbulllaiteye Sam, et al. “Risk factors for human herpes virus 8 seropositivity in the AIDS Cancer Cohort Study.” **Journal of Clinical Virology** 2006 April;35(4):442-449. PMID: 16414306.

Casper C, et al. “HIV serodiscordant sex-partners and the prevalence of HHV-8 infection among HIV negative men who have sex with men: baseline data from the EXPLORE study.” **Sexually Transmitted Infections** 2006;82(3):229-235. PMID: 16731675. "Popper (amyl nitrate) use was also significantly associated with HHV-8 infection. Compared with men who never used "poppers", use less than once a week was associated with a 1.3-fold increased odds of HHV-8 infection (95% CI 0.9 to 1.9, p=0.14), 1-2 times per week 2.9-fold increase (95% CI 1.6 to 5.4, p=<0.01), and >3 days per week 3.4-fold increase (95% CI1.4 to 8.6, p=0.01). However, popper use was also significantly associated with the median number of sex partners, and performing rimming with >5 lifetime HIV unknown sex partners (p=<0.001)."

Chin-Hong PV, et al. “Age-related prevalence of anal cancer precursors in homosexual men: the EXPLORE study.” **Journal of National Cancer Institute** 2005 June 15;97(12):896-905. PMID: 15956651.

Soderberg LS, et al. “Production of macrophages IL-1beta was inhibited both at the levels of transcription and maturation caspase-1 following inhalation exposure to isobutyl nitrite.” **Toxicology Letters** 2004 Aug 30;152(1);47-56. PMID: 15294346.

Ponnappan U, et al. “Inhaled isobutyl nitrite inhibited macrophage inducible nitric oxide by blocking NFKappaB signaling and prompting degradation of inducible nitric oxide synthase-2.” **International Immunopharmacology** 2004 Aug;4(8):1075-82. PMID: 155222982.

Tran DC, et al. “Inhalant nitrite exposure alters mouse hepatic angiogenic gene expression.” **Biochem Biophys Research Communications** 2003 Oct 17;310(2):439-45. “These results demonstrate that in vivo exposure to an inhalant nitrite results in altered tissue expression of vascular endothelial growth factor (VEGF) and its receptors, suggesting that some of its toxicological effects may be mediated partly through a mechanism involving angiogenesis.” PMID: 14521929.

Casper C, et al. “Correlates of prevalent and incident KS-associated herpesvirus infection in men who have sex with men.” **Journal of Infectious Diseases** 2002 April 1; 185(7): 990-993. PMID: 11920325. "Reporting > or = 1 HIV-positive partner (OR, 5.9; 95% CI, 1.8-19.3), amyl nitrite use (OR, 7.0; 95% CI, 2.1-23.0), and lymphadenopathy in the past 6 months (OR, 7.7; 95% CI, 1.9-31.0), correlated with KSHV seroconversion." "The relationship of amyl nitrite use to KSHV seroincidence was further investigated by adding the significant univariate variables to the model, one at a time. The OR did not change after adding either HSV-2 infection or bacterial STIs to the model, but it declined from 7.0 (95% CI, 2.0-24.9) to 5.5 (95% CI, 1.4-20.8) after adding a reported history of bathhouse use. Thus, these variables did not mitigate the association between amyl nitrite use and KSHV seroconversion."

Ponnappan, et al. “Inflammatory macrophage nuclear factor-kappaB and proteasome activity are inhibited following exposure to inhaled isobutyl nitrite.” **Journal of Leukocyte Biology** 2001 April;69(4):639-644. PMID: 11310851.

Brouette T, et al. “Clinical Review of Inhalants” **The American Journal of Addictions** 2001;10:79-94. PMID: 11268830.

Martin JN, et al. “Evaluation of specific sexual practices as routes of HSHV transmission among homosexual men.” **Fourth International Workshop on KSHV and Related Agents** 2001 Aug. Oral presentation and printed abstract. University of California, Santa Cruz. "Even with adjustment for sexual practices, inhaled nitrite use during sex was associated with KSHV infection (OR=2.4, p>0.01)." Unpublished.

Pauk, et al. “Mucosal shedding of human herpesvirus 8 in men.” **New England Journal of Medicine** 2000 Nov 9;343(19):1369-77. PMID: 11070101. "Among 92 men who have sex with men who were seronegative for HIV, a history of sex with a partner who had Kaposi's sarcoma, deep kissing with an HIV-positive partner, and the use of amyl nitrite capsules ("poppers") or inhaled nitrites were independent risk factors for infection with HHV-8."

Martin J, et al. "Salivary Shedding of KSHV among homosexual men." Abstract at **3rd International KSHV Workshop** at the University of Massachusetts at Amherst, 2000 July 6-10. "In multivariate analysis among those KSHV-positive or with KS, KSHV-saliva positivity was associated with HIV infection (OR=2.2, p=0.027), and recreational use of "poppers"(OR=2.2, p=0.049)..." "If their roles are confirmed, the use of "poppers", long associated with KS per se, may be escalating the KS epidemic in homosexual men by increasing the infectiousness and/or potentiating viral activity in infected men."

Keilbassa W, et al. “Nitrite inhalation in rats elevates tissue NOS III expression and alters tyrosine nitration and phosphorylation.” **Biochemical and Biophysical Research Communications** 2000;275:335-342. PMID: 10964667.

Guo G, et al. “Acute exposure to the abused inhalant,isobutyl nitrite, reduced T cell responsiveness and spleen cellularity.” **Toxicology Letters** 2000 July 27;116(1-2):151-158. PMID: 10906432.

Shafer R, et al. “Pulmonary exposure to isobutyl nitrite reduces resistance to a respiratory infection.” Poster at **10th International Conference on Mucosal Immunology** 1999. Unpublished. "In the present study resistance to a pulmonary Listeria monocytogenes infection during subchronic pulmonary exposure to IBN was assessed. The phenotype and in vitro proliferative response to listeria antigens of the mediastinal lymph nodes (MLN) was also determined. Mice exposed to IBN had significantly more bacteria than control mice in the lungs and livers. In addition, cells from MLN did not proliferate in response to stimulation with listerial antigens. Interestingly, the percentage of CD4+ cells and CD8+ cells in the MLN were also significantly reduced in comparison to control animals. Our results demonstrate that pulmonary exposure to IBN results in increased bacterial growth in the lungs and livers of infected mice, suppresses the ability of MLN to respond to antigen-specific stimulation, and may reduce CD4+ and CD8+ T cell populations in the MLN after pulmonary infection with L. monocytogenes. These studies indicate that inhalation abuse of nitrite compounds affects pulmonary resistance to infection."

Soderberg LS. “Increased tumor growth in mice exposed to inhaled isobutyl nitrite.” **Toxicology Letters** 1999 Jan 11;104(1-2):35-41. PMID: 10048747. "To determine if exposure to nitrite inhalants could alter tumor growth, syngeneic PYB6 tumor cells were injected into groups of mice. Exposure of these mice to inhaled isobutyl nitrite increased both the tumor incidence and the tumor growth rate by almost 4-fold. Following only 5 daily exposures to the inhalant, the induction of specific T cell mediated cytotoxicity was inhibited by 36%. Similar inhalation exposures inhibited the tumoricidal activity of activated macrophages by 86%. The data suggest that exposure to abuser levels of a nitrite inhalant compromised tumor surveillance mechanisms."

James JS. “Poppers: large cancer increase and immune suppression in animal tests.” **AIDS Treatment News** 1999;317. PMID: 11366993. “ A study on mice

injected with cancer cells and then exposed to isobutyl nitrite (poppers) revealed that inhalant-treated mice developed more readily and rapidly than control mice. The control mice were also injected with cancer cells, but only breathed air. Related studies found that poppers suppress certain immune functions involved in killing tumor cells."

Wilson H. "The HIV-poppers connection." **Focus** 1999 Mar;14(4):5-6. PMID: 11366670.

Soderberg LS. "Immunomodulation of nitrite inhalants may predispose abusers to AIDS and Kaposi's sarcoma." **Journal of Neuroimmunology** 1998 Mar 15;83(1-2):157-161. PMID: 9610684.

James JS. "Poppers: more evidence of suppressed immunity." **AIDS Treatment News** 1999 Aug 20;325:8. PMID: 11366577.

Soderberg LS, et al. "Acute blood toxicity of the abused inhalant cyclohexyl nitrite." **International Journal of Immunopharmacology** 1997 May;19(5):305-10. PMID: 9439769.

Soderberg LS, et al. "Elevated TNF-and inducible nitric oxide production by alveolar macrophages after exposure to a nitrite inhalant." **Journal of Leukocyte Biology** 1996;60:459-464. PMID: 8864129.

Soderberg LS, et al. "Leukopenia and altered hematopoietic activity in mice exposed to the abused inhalant isobutyl nitrite." **Experimental Hematology** 1996 June;24(7):848-853. PMID: 8647236.

Soderberg LS, et al. "Acute inhalation exposure to isobutyl nitrite causes non specific blood cell destruction." **Experimental Hematology** 1996 April;24(5):592-596. PMID: 8605963.

National Toxicology Program "NTP Toxicology and Carcinogenesis of isobutyl nitrite (CAS No. 542-56-3) in F344 rats and B6C3F1 mice (inhalation studies)." **National Toxicology Program Technical Report Service** 1996 Jul;448:1-302. PMID: 12594527. "Under the conditions of these 2 year inhalation studies, there was clear evidence of carcinogenic activity of isobutyl nitrite in male and female F344/N rats based on the increased incidences of aveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma(combined)."

Soderberg LS, et al. "Inhaled isobutyl nitrite produced lung inflammation with increased macrophage TNF-and nitric oxide production." **Adv Exp Med Biol** 1996;402:187-189. PMID: 8787659.

Haverkos H, et al. "Measuring nitrite exposure in gay men: implications for elucidating the etiology of AIDS-related Kaposi's sarcoma." **Genetica** 1995;95(1-3):57-64. PMID: 7744258.

Ostrow D, et al. “A case control study of human immunodeficiency virus type 1 seroconversion and risk related behaviors in the Chicago MACS/CCS Cohort, 1984-1992, MACS. Copin and Change Study.” **American Journal of Epidemiology** 1995 Oct 15;142(8):875-883. PMID: 7572964.

Soderberg LS, et al. “T cell functions are impaired by inhaled isobutyl nitrite through a t-independent mechanism.” **Toxicology Letters** 1994; 70:319-329. PMID: 8284799.

Mirvish S, et al. “Mutagenicity of iso-butyl nitrite vapor in AMES test and some relevant chemical properties, including the reaction of iso-butyl nitrire with phosphate.” **Enviromental and Molecular Mutagenesis** 1993;21(3):247-252. PMID: 8462528.

Soderberg LS, et al. “Exposure to inhaled isobutyl nitrite reduces t cell blastogenesis and antibody responsiveness.” **Fundamental and Applied Toxicology** 1991;17:821-824. PMID: 1778367.

Soderberg LS, et al. “Exposure to abused inhalant, isobutyl nitrite, compromise both antibody and cell-mediated immunity.” **Adv Exp Med Biol** 1991;228:265-268. PMID: 1835258.

Dunkel V, et al. “Mutagenicity of some alkyl nitrites used as recreational drugs.” **Environmental and Molecular Mutagenesis** 1989;14:115-122. PMID: 2569972. “To evaluate further the genotoxic activity of these chemicals, six nitrites, including those commonly used by homosexuals for sexual gratification, were selected for testing in the mouse lymphoma TK+/- and Salmonella typhimurium mutagenicity assays. One chemical, n-amyl nitrite, was negative in the mouse lymphoma assay, while other five chemicals, n-butyl, isobutyl, iso-amyl, sec-butyl, and n-propl nitrite nitrite were positive. All six compounds were positive for the Salmonella assay.”

Ortiz JS, et al. “Altered T-cell helper/suppressor ratio in miDax EM et al. “Effects of nitrites on the immune system of humans.” **Health Hazard of Nitrite Inhalants: NIDA Research Monograph 83** 1988 Mar;83:59-74. PMID: 2902515.

Lewis DM, et al. “Toxicity of inhaled isobutyl nitrite in BALB/c mice: systemic and immunotoxic studies.” **NIDA Research Monograph 83** 1988 Mar;83:50-58. PMID: 3140021.

Maickel R, et al. “Fate and toxicity of butyl nitrite.” **NIDA Research Monograph 83** 1988 Mar;83:15-38. PMID: 3140019.

Haverkos H, et al. “Kaposi’s sarcoma and nitrite inhalants.” **Adv Biochem Psychopharmacology** 1988;44:165-72. PMID: 2899948.

Haverkos H, et al. “Health hazards of nitrite inhalants.” **American Journal of Medicine** 1988 Mar;84(3Pt 1):479-82. PMID: 2894765.

Mirvish SS, et al. “Indications from animal and chemical experiments of a carcinogenic role for isobutyl nitrite.” **Health Hazards of Nitrite Inhalants: NIDA Research Monograph 83** 1988 Mar;83:39-49. PMID: 2902514.

Rees DC, et al. "Acute effects of some volatile nitrites on motor performance and lethality in mice." **Neurobehavior Toxicology Teratology** 1986 Mar-Apr;8(2):139-42. PMID: 2872605.

Kaled M, et al. “Inactivation of B-12 and folate coenzymes by butyl nitrite as observed by NMR: implications on one-carbon transfer mechanism.” **Biochemical and Biophysical Research Communications** 1986;135(1):201-207.

Haverkos H, et al. “Disease manifestation among homosexual men with acquired immunodeficiency syndrome: a possible role of nitrites in Kaposi’s sarcoma.” **Sexually Transmitted Diseases** 1985 Oct-Dec;12(4):203-208. PMID: 3878602.

Gangadharam PR, et al. “Immunosuppressive action of isobutyl nitrite.” **International Congress of Immunopharmacology** 1985 May. Florence, Italy. Oral presentation. Not published. Mice were exposed to isobutyl nitrite vapors or air. Both groups were then exposed to bacteria and the mice exposed to the air only had lower illness and mortality rate. The research showed use of isobutyl nitrite resulted in "decreased numbers of lympocytes and macrophages, blood cells that are important in defending the body against infections."

Lewis DM, et al. “Subchronic inhalation toxicity of isobutyl nitrite in BALB/c mice. II. Immunotoxicity studies.” **Journal of Toxicol Environ Health** 1985;15:835-846. PMID: 3903174.

Lynch DW, et al. “Subchronic inhalation toxicity of isobutyl nitrite in BALB/c mice. I. Systemic toxicity studies.” **Journal of Toxicol Environ Health** 1985;823-833. PMID: 4057284.

Newell GR, et al. “Volatile nitrites use and adverse effects related to the current epidemic of the acquired immunodeficiency syndrome.” **Pharmacotherapy** 1984 Sep-Oct;4(5):284-91. PMID: 6150466. “These products have been found to be profoundly immunosuppressive for human lymphocytes in vitro, and their by-products when metabolized into N-nitroso compounds have been known to be highly carcinogenic in many animal species.”

Lotzova E, et al. “Depression of murine natural killer cell cytotoxicity by isobutyl nitrite.” **Cancer Immunology and Immunotherapy** 1984; 17:130-134. PMID: 6235910. "Isobutyl nitrite was NK-cell-suppressive not only after in vivo administration but, most importantly, also after inhalation." "In two different experiments, inhalation of isobutyl nitrite substantially depressed (>2fold) the NK-cell lytic potential of mice."

Jacobs RF, et al. “Cellular Immunotoxicity of amyl nitrite.” **Journal of**

Toxicology-Clinical Toxicology 1983;20(5):421-449. PMID: 6142118.

Hersh EM, et al. “Effect of the recreational agent isobutyl nitrite on human peripheral blood leukocyte and on in vitro interferon production.” **Cancer Research** 1983 March;43(3):1365-71. PMID: 6186374.

CDC. "An evaluation of the immunotoxic potential of isobutyl nitrite." **MMWR** 1983 Centers for Disease Control. pp:457-458,464. "None of the animals exposed to IBN showed any evidence of immunotoxic reactions. Methemaglobinemia was noted in animals exposed to 300 ppm of IBN, and some evidence of thymic atrophy, possibly stress -related, was found in this group.All histologic examinations have not been completed." "Nitrite inhalants do not appear to be implicated as a cause of the immunosuppression seen in AIDS, but their role as a cofactor in some of the illnesses in this syndrome has not been ruled out."

Jorgensen KA, et al. “Amyl nitrite and Kaposi’s sarcoma in homosexual men.” **New England Journal of Medicine** 1982 Sep 30;307(14):893-4. PMID: 6125888.

Marmor M, et al. “Risk factors for Kaposi’s sarcoma in homosexual men.” **Lancet** 1982 May 15;1(8281):1083-7. PMID: 6122889.

Goedert J, et al. “Amyl nitrite may alter t lymphocytes in homosexual men.” **Lancet** 1982 Feb 20;1(8269):412-16. PMID: 6121088.

Watson Sue & Murphy James. “Use of amyl nitrite may be linked to current epidemic of immunodeficiency syndrome.” Unpublished letter to Journal of American Medical Association and **The Advocate** 1982. “Groups of mice were exposed to a single capsule of amyl nitrite (Vaporole, 0.3 ml capsule, Burroughs Wellcome) in an 18 liter sealed container for 4 minutes, twice daily for 5 consecutive days beginning the day of immunization. The humoral immune response to sheep red blood cells was normal in the mice exposed to amyl nitrite. However, the cellular response to DNFB was reduced by 30-45% in mice exposed to amyl nitrite.” A copy of Watson’s letter was mailed to Committee to Monitor Poppers in San Francisco.

Quinto I. “The mutagenicity of alkyl nitrites in the Salmonella test.” **Bolletino Societa Italiana Biologia Sperimentale** 1980 Apr 30;56(8):816-20. PMID: 7004467. (translation from the Italian): “It has been shown that five alkly nitrites are mutagens by the Salmonella/microsome assay. N-propl-, n-butyl, iso-butyl and amy-nitrite are direct mutagens on TA 1535; sec-butyl-nitrite is a mutagen on TA 1535 only following metabolic activation by Aroclor-induced rat liver homogenate. Because of the know correlation between mutagenicity and carcinogenicity, we believe that amyl-nitrite and iso-butyl nitrite, which are used as human drugs, should be tested for carcinogenicity in animals; in the meanwhile, their use should be allowed only in emergencies.”

Haley TJ, et al. "Review of physiological effects of amyl, butyl, isobutyl nitrites." **Clinical Toxicology** 1980 May;16:317-329. PMID: 6105036.

Maickel RP, et al. "Acute toxicology of butyl nitrites and butyl alcohols." **Res Comm Chem Pathol Pharmacol** 1979 Oct;26(1):75-83. PMID: 515511.

Jackson, CD. "Volatile nitrites." **National Toxicology Program Working Paper** prepared by the Office of Scientific Intelligence of the National Center for Toxicological Research. Jefferson, Arkansas 72029; 1979 Aug 25;1-22.

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