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Health Hazards of Nitrite Inhalants

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Editors:

Harry W. Haverkos, M.D.
Divlsion of Clinical Research
National Institute on Drug Abuse

John A. Dougherty, Ph.D.
Veterans Administration Medical Center
Lexington, KY

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Health Hazards of Nitrite Inhalants

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Preface

The National Institute on Drug Abuse (NIDA) is concerned about the acquired immunodeficiency syndrome (AIDS) for two reasons. First, intravenous drug abusers constitute approximately 25 percent of reported AIDS patients in the United States. Human immunodeficiency virus (HIV) infection is transmitted among intravenous drug abusers primarily when contaminated needles and other paraphernalia used to inject drugs are shared. Infected drug abusers are capable of transmitting HIV to their sexual partners during sexual contact and to their unborn children during pregnancy. Second, the use of drugs may expedite disease progression by further decreasing immune function or by acting as the vehicle of transmission of other infectious agents, such as hepatitis B virus, which may also be immunosuppressive. Drugs of abuse may promote the development of malignancies. Nitrite inhalants, commonly used by homosexual men, have been associated with Kaposi's sarcoma (KS) in AIDS. The mechanism of action of nitrites as a cofactor in KS, if any, has yet to be elucidated.

On March 31, 1987, NIDA sponsored a technical review entitled "The Extent of Use and Health Hazards of Nitrite Inhalants." Approximately 25 scientists attended the meeting in Rockville, MD. The purpose of the workshop was to review the status of research regarding nitrite inhalants and their potential public health implications and to set directions for future studies. In part, this meeting was called to provide up-to-date information about nitrite inhalants for NIDA to respond to a congressional request for information as part of the Anti-Drug Abuse Act of 1986.

This monograph is a collection of presentations delivered at the meeting. The chapters are organized to present the history of nitrite use; the basic biochemical, pharmacologic, and toxicologic effects of various forms of alkyl nitrites; the effects of nitrite use on the

immune system; and epidemiological findings associating nitrite use with KS in AIDS. The purpose of this introduction is to provide an overview of issues and controversies raised at the meeting that are covered in more detail in individual chapters.

There are many controversial issues surrounding nitrites, not the least of which are their current regulatory status. As Guy Newell discusses in his historical presentation, amyl nitrite is a prescription drug, but butyl nitrite, with effects very similar if not identical to the amyl congener, is not considered to be a drug because it is marketed as a “room odorizer.” Proposals to regulate the sale of butyl nitrites have been periodically considered by various agencies of the Federal Government.

A series of metabolic and toxicity studies of four nitrite butyl esters in mice was presented by Roger Maickel. The lethality of these compounds was related to rapid hydrolysis to nitrite ions with the subsequent oxidation of hemoglobin to methemoglobin. However, methemoglobin formation cannot account for all toxic effects. While there were wide differences in toxicity, the relative toxic potencies of the four butyl nitrite isomers found in “room odorizers" were maintained under a variety of experimental conditions and routes of administration.

The acute toxicity of nitrites in animals and man was reviewed by Ronald Wood. Skin and tracheobronchial irritations (especially about the nose and lips), burns from accidental ignition, headaches, hypotension, cyanosis, methemoglobinemia, intoxication, and the development of habitual use patterns are possible adverse effects of nitrite inhalation.

Much of the discussion at the meeting focused on two possible pharmacologic mechanisms by which nitrites may be involved in the genesis of KS in AIDS: carcinogenicity and immunosuppression.

Although dependent on an unproven and controversial mechanism, nitrites have been hypothesized to interact with organic amines and amides in vivo to form significant amounts of highly carcinogenic N-nitrosamines. Direct data on the carcinogenicity of nitrites in animals are sparse. However, the in vitro studies of lipid peroxidation and the finding of route-dependent in vivo formation of nitrosamines from amyl nitrite and methylaniline in mice, reported by

Sidney Mirvish, suggest that the possibility warrants serious investigation. Interestingly, the areas where absorbed concentrations of volatile nitrites would be expected to be highest--the skin surrounding the nose and in the nasal/pulmonary mucosa-are also reported to be the areas in which KS occurs in persons with AIDS. This association logically leads to the hypothesis that there is a causal relationship between nitrites and KS, perhaps mediated by the formation of N-nitroso compounds. How HIV infection initiates or promotes this process is not clear.

Besides participating in the formation of carcinogenic metabolites, alkyl nitrites may increase the likelihood of KS by altering immune function. Dan Lewis, Jesse Ortiz, and Elizabeth Dax studied the effects of nitrites on immunologic function using variations on two basic strategies: first, examination of effects on immunologic components (lymphocyte numbers, thymus weight, etc.) and second, measurement of effects of nitrite pretreatment on the responsiveness of immune system components to challenge with various adjuvants, mitogens, and antigens. Given the complexity of the immune systems in mice and humans, the possible variations in nitrite exposure parameters, the large number of dependent variables and sampling times to choose from, and the diversity of analytic methods available, one might have predicted in advance the divergent results obtained. Lewis reported no significant detrimental effects on the immune systems of mice from exposures to 300 parts per million isobutyl nitrite vapor for 13 weeks. However, the other two researchers showed different patterns of decreases in T-lymphocyte numbers and changes in immune functions after 21 weeks of intranasal amyl nitrite in mice (Ortiz, this volume) or after 13 sessions of amyl nitrite inhalation in human volunteers (Dax, this volume). It is apparent that there is much to learn before the relevance of nitrites to disease processes, such as KS, is understood.

Much has been written about the extent of nitrite use among homosexual men. In addition, household and high school surveys have quantitated use of nitrites among adolescents and young adults, but these surveys have not distinguished use by sexual orientation. In this monograph two surveys of nitrite inhalant use by drug abusers are presented. Richard Schwartz presents data collected from adolescents at a residential drug treatment community in suburban Virginia. Schwartz also assesses the rates of acute toxicity attributed to nitrites among adolescent abusers. Robert Lange presents

data concerning nitrite use among intravenous drug abusers in treatment from six regions of the United States and among homosexual men in Baltimore. Lange suggests that nitrite use is decreasing among homosexual men because of the fear of an association with AIDS. Nitrite use among intravenous drug abusers is not as extensive as among homosexual men and is not apparently changing.

The unique epidemiology of KS in AIDS suggests that a cofactor is necessary to explain its pathogenesis. Harry Haverkos reviewed the existing epidemiologic studies of nitrite use and KS in homosexual men and found inconclusive results; nitrite use is associated with KS in some studies but not in others. In discussing the variables that may be responsible for the varied results, he ruled out bloodborne infectious agents and focused attention on drug use and sexually transmitted microbial agents as the most likely places to look for the KS cofactor. His discussion points out the difficulties of interpreting questionnaire data when sample sizes are small and methods, populations, and questions vary. Tighter control over survey conditions and standardized methods would make these studies more efficient, but may be impractical to achieve.

An open discussion moderated by Dr. Newell followed the presentations at the workshop. More epidemiologic and laboratory studies are needed to assess the role, if any, of nitrite inhalants as a cofactor in AIDS-related KS. There is a need to develop questionnaires for studies that access nitrite exposure over one's lifetime, analogous to pack-years in cigarette usage. Despite the epidemiologic associations with KS, butyl nitrite has never been tested as a carcinogen. Such studies should be conducted. Studies of animal models infected with retroviruses and challenged with large quantities of nitrites before, during, and/or after retrovirus infection would be useful.

More research is needed to determine the dose-response curve of nitrite inhalation in humans. Many individuals use other drugs, such as alcohol, marijuana, and/or cocaine, with nitrites. What are the effects of these drugs in combination?

Nitrite inhalants are important drugs of abuse in the United States. Their association with KS and AIDS raises an important scientific question about possible synergistic reactions between viruses and chemicals in the development of cancer. It is our hope that this

monograph will stimulate interest in nitrite inhalant research and attract investigators who can conduct the multidisciplinary research necessary to address the scientific questions raised at this NIDA technical review.

Harry W. Haverkos, M.D.
Clinical Medicine Branch
Division of Clinical Research
National Institute on Drug Abuse
Rockville, MD

John A. Dougherty, Ph.D.
Veterans Administration Medical Center
Lexington, KY

Nitrite Inhalants: Historical Perspective

Guy R. Newell, Margaret R. Spitz and Michael B. Wilson

INTRODUCTION

Early studies of Kaposi's sarcoma (KS), manifesting as part of the acquired immune deficiency syndrome (AIDS), showed that many people with KS had used volatile nitrites. In the body, nitrites may be converted to N-nitroso compounds, which are known to be mutagens, teratogens, and strong carcinogens. In addition, studies found nitrites to have potent immunosuppressive properties. These facts led us to consider volatile nitrites as possible etiologic cofactors in AIDS-related KS.

THERAPEUTIC USE OF NITRITES

In 1859, the vasodilatory effect that follows inhalation of amyl nitrite was described. This led to its first therapeutic use in 1867 as relief from angina pectoris (Brunton 1867). The original form of the drug was glass ampules enclosed in mesh: they were called pearls. When crushed between the fingers, they made a popping sound; hence, the colloquialism "poppers" evolved. Butyl nitrite was also investigated in the late 1880s. Although it has the same properties as amyl nitrite, it was never used clinically (Brunton 1897). Isobutyl nitrite is a third member of this family of compounds, and the vasodilatory effects of these three aliphatic nitrites-amyl, butyl, and isobutyl-were found to be similar (Haley 1980). Nitrites exert their vasodilatory effects by relaxing the muscles in blood vessel walls (Nickerson 1975). Vasodilation of the cerebral blood vessels causes an increase in intracranial pressure, producing a euphoric effect or "high." These aliphatic nitrites were also reported to enhance sexual performance (Everett 1975; Hollister 1975).

TOXICITY OF VOLATILE NITRITES

In spite of having a wide range of human toxicities, amyl nitrite was considered safe for therapeutic use (table 1). The Food and Drug Administration (FDA) eliminated the prescription requirement in September 1960. The pharmaceutical industry first presented testimony regarding significant abuse of amyl nitrite among young adults (Lubell 1964). Following that testimony in 1969, the FDA reinstated the prescription requirement. However, abuse of this drug continued in spite of the FDA's regulation. The National Institute on Drug Abuse noted 13 emergency room admissions resulting from nitrite side effects in 1976, and it investigated 84 related complaints that year (Reed 1979).

CARCINOGENICITY OF N-NITROSO COMPOUNDS

Nitrite, present as a natural impurity in salt, was a key ingredient in curing meats for thousands of years. Many foods are naturally rich in nitrates, which are converted to nitrites by bacteria. Nitrite can combine with other chemicals called amides or amines to form a family of compounds called nitrosamines. These substances as a group are among the most potent carcinogens known. Nitrosamines are not permitted to be knowingly added to food at any level, although it is likely that some foods contain small amounts of this carcinogenic substance. In 1978, the FDA set the amount of allowable nitrite to be added to bacon at 120 ppm and required that bacon be free of preformed nitrosamines at 10 ppb, the lowest amount detectable by available technology.

Magee and Barnes first reported the carcinogenicity of N-nistrosodimethylamine (NDMA) in 1956 (Magee and Barnes 1956). In all, 290 N-nitroso compounds have been tested for carcinogenicity, and 252 (87 percent) of these have shown such activity (Bogovski and Bogovski 1981). These compounds are active carcinogens in 39 different animal species; no species tested is resistant to their carcinogenic effects (Preussmann 1983). Experiments on the comparative metabolism of N-nitrosamines using animal and human tissue provide convincing evidence of similar activating metabolic pathways (Bogovski and Bogovski 1981). Thus, humans are most likely to be susceptible to the carcinogenic effects of these compounds. Organic nitrites, including amyl, butyl, and isobutyl nitrite, are mutagenic according to the Ames test with or without metabolic activation: N-nitroso compounds have the ability to induce

TABLE 1. *Toxicity of inhaled volatile nitrites*

System or Illness	Effects
Central Nervous System	Light-headedness, weakness, nausea, ataxia, delirium, headache (short-lived "nitrite headaches" and prolonged pulsatile headaches), syncope (after profound vasodilation), tolerance, sedation, and anesthesia. (Last two effects reported for animals exposed to high concentrations.)
Cardiovascular System	Profound hypotension and cutaneous flushing followed by reflex vasoconstriction and tachycardia, and transient electrocardiographic changes (inverted T waves and depressed ST segments).
Hematologic System	Methemoglobinemia (documented after oral and parenteral administration and repeatedly mentioned as a possibility after inhalation; not reported in association with hedonistic use of these substances), and normocytic normochromic anemia after exposure in an industrial setting.
Ocular Organs	Increased intraocular pressure and supraorbital pain.
Pulmonary Organs	Irritation (reported for animals exposed to high concentrations).
Cancer	Inhaled nitrites could interact freely with endogenous trivalent nitrogen compounds to produce nitrosamines that are known to be carcinogens.

SOURCE: Adapted from Sigell et al. 1978, Copyright 1978, the American Psychiatric Association.

cancer after only one dose (Jorgensen 1982). These are among the most potent known chemical carcinogens in animals.

IMMUNOSUPPRESSIVE EFFECTS OF VOLATILE NITRITES

In 1982, Goedert et al. found that the helper:suppressor ratio of peripheral blood lymphocytes was lower among users of volatile nitrite than among nonusers. This was the first indication that these compounds had the potential to suppress the immune system in humans. However, the possibility that poppers were only a surrogate marker for other factors that were highly correlated with popper use was not ruled out.

We demonstrated the immunosuppressive effects of nitrites on human peripheral blood leukocytes in vitro (Hersh et al. 1983). Lymphocyte blastogenesis, cell-mediated cytotoxicity, and monocyte adherence were all Suppressed by concentrations of isobutyl nitrite that were noncytotoxic in terms of cell viability. Isobutyl nitrite inhibited the induction of a- and b-interferon, which also might contribute to susceptibility to infectious diseases and manifestation of cancers in users of these substances. These in vitro effects of isobutyl nitrite were evident after only 2 hours of exposure of human lymphocytes in culture, suggesting that prolonged exposure may not be necessary for immunosuppressive and carcinogenic effects to occur. Cellular cytotoxiclty was demonstrated (Jacobs et al. 1983) as was depression of murine natural killer cells (Lotzova et al. 1984). We suggested that continued use of these substances might interfere with attempts to correct the immune system by other agents and, therefore, that their use should be condemned.

NITRITES AS RECREATIONAL DRUGS

Butyl and isobutyl nitrites do not fit the definition of a food, drug, or cosmetic as specified by the Federal Food, Drug, and Cosmetic Act from which the FDA derives its regulatory authority. Therefore, they are not subject to regulation by the FDA Because amyl nitrite was no longer available after 1969 without a prescription, there was a proliferation of different brands of butyl and isobutyl nitrite during the early 1970s. Most commercial preparations contained some of each type, along with unspecified impurities (Ostrow 1982; Hersh et al. 1983).

In the 1970s. reports of nitrite use for augmentation of sexual experiences along with descriptions of their aphrodisiac properties

began (Louria 1970; Pearlman and Adams 1970; Everett 1972; Gay and Sheppard 1972; Dimijian 1978; Hollister 1975). lsraelstam et al. (1978) reported that amyl nitrite was known to have been used recreationally as early as the 1960s. They report on one man in San Francisco who tried amyl nitrite in 1963. However, the popper craze really only began during the years 1974 to 1977. By 1979, over 5 million people in the United States used these drugs more than once a week (Mayer 1983).

Sigell et al, (1978) reported that the sale of nonprescription volatile nitrites, commonly referred to as poppers, was a large business. An estimated total of $50 million a year was made from the sales of more than 100,000 bottles a week in just one city. Further, they claimed that, prior to 1978, male homosexuals indulged in the use of volatile nitrites more than any other group. A guide to homosexual lovemaking asserts that the use of amyl nitrite has “passed into every comer of gay life.” Nitrites were used primarily during sexual activity because they reduce social and sexual inhibitions, heighten sexual arousal, relax the anal sphincter, and are thought to prolong orgasm (Lubell 1964, Sigell 1978; lsraelstam et al. 1978). The chronology of volatile nitrite availability and use as it could relate to AIDS-related KS was described (Newell et al. 1984; Newell et al. 1985b) and is given in table 2.

NITRITE USE REPORTED IN THREE CASE-CONTROL STUDIES

In December 1981, we began accepting referrals of individuals with possible AIDS either with or without KS and opportunistic infections (OI). One hundred thirty-five lifestyle questionnaires were completed by the first 145 referrals. At one extreme, 31 patients had either KS or OI as severe manifestations of AIDS; at the other extreme, 29 referrals were symptom-free and served as controls (Newell et al. 1985b). In spite of the small numbers in our study, we found a statistically significant increased risk of 5.5 (95 percent confidence interval 1.1-28.7) for KS/OI patients who had used volatile nitrites compared with symptom-free (SF) controls (Newell et al. 1985b). In addition, there was a smooth dose-response gradient of 1.0 for those who never used nitrites, 4.0 for those who occasionally used, and 6.3 for those who used them frequently (table 3). Predictably, there was considerable confounding among several risk factors. In particular, use of nitrites was confounded by such activities as marijuana use, attending bathhouses, and active fisting (insertion of fist into the rectum). The estimated risk of developing KS for each factor was

TABLE 2. *Chronology of volatile nitrite availability and use*

Year	Availability and Use
1859	Flushing of skin with amyl nitrite described
1867	Therapeutic use of amyl nitrite for angina pectoris
1880s	Butyl nitrite investigated but not used clinically
1960	Amyl nitrite prescription requirement eliminated by FDA
1963	First report of recreational use
1960s	Recreational use common among young adults
1969	Amyl nitrite prescription requirement reinstated by FDA
1970	Street brands of butyl and isobutyl nitrite used; JAMA reports use for sexual augmentation
1974	Popper craze said to have begun
1976	$50 million sales per year in one city
1977	Nitrites permeate gay life
1978	Three cases of KS/PCP found in retrospect
1979	Over 5 million people used>once per week 19 cases of KS/PCP found in retrospect
1980	56 cases of KS/PCP reported
1981	Nitrite use suspected associated with KS/PCP

SOURCE: Adapted from Newell et al. 1985a, Copyright 1985, the American Journal of Medicine.

high after taking the others into account. For example, stratification of nitrite use by prior history of syphilis (table 4) resulted in odds ratios of 1.0, 3.0, and 5.0 (never, occasional, and frequent use,

respectively) among the 28 subjects with no prior history of syphilis as well as among the 32 subjects with a prior history of syphilis. This suggested that use of volatile nitrites was a significant risk factor for developing KS, independent of a prior history of syphilis. We also found that increasing use of nitrites paralleled sexual activity (table 5).

Marmor et al. (1982) compared 20 KS patients with 40 homosexual controls drawn from a physician's practice and matched them for race and age. The major predisposing risk factors for development of KS among the patients were amyl nitrite use and number of sex partners per month. Marmor found that development of KS was associated with the use of amyl nitrite but not with the use of butyl nitrite. We believe that butyl nitrite was the predominant form of nitrite used, but amyl nitrite was endorsed because of the way the question regarding nitrite use was phrased, as Marmor and associates suggested. As noted above, the effects of amyl, butyl, and isobutyl nitrites are the same (Haley 1980). One of Marmor's patients reported using nitrites only once, 3 years prior to diagnosis of his KS.

The Centers for Disease Control (CDC) study (Jaffe et al. 1983) compared 50 KS or OI patients with 120 homosexual controls selected from either a venereal disease clinic (78 controls), or a physician's practice (42 controls). In the CDC study, nitrite use was extensive (95 to 96 percent) among patients and both control groups. However, lifetime usage was significantly greater among patients than among either of the control groups. In all three studies, controls were drawn from physician practices or clinics, and so did not necessarily represent the male homosexual population at large. However, since the controls were probably in poorer health than individuals in the ideal referent population would be, risk estimates would be biased in favor of unity. Thus, any differences found would be minimum risk estimates. This source of bias was discussed in each paper.

Comparable findings from the three studies are shown in table 6. In all studies, use of nitrites by cases was more prevalent than use among the controls. If one discards the one individual reported by Marmor et al. (1982), who only used nitrites once, the percent of use among the cases in the three studies is 95, 96, and 94 percent. In any epidemic, it is not unusual for some patients not to give a clear history of exposure. The comparable prevalence of exposure among the control groups used by Jaffe et al. (1983) does not militate against nitrites as a potential contributing factor for development of

TABLE 3. *Nitrite use as a risk factor for Kaposi's sarcoma/ opportunistic infections (KS/OI)*

Nitrite Use	KS/OI Number	KS/OI Percent	Controls Number	Controls Percent	Odds Ratio	95 Percent Confid. Interval
Never	2	6.5	8	27.6	1.0	-
Occasional	7	22.5	7	24.1	4.0	0.6 - 26.0
Frequent	22	71.0	14	48.3	6.3	1.2 - 34.0
Overall Odds Ratio					5.5	1.1 - 28.7

NOTE: Never=once only or once per year to allow for the experimenter; occasional=once per 6 months to once per month; frequent=once per month or more.

SOURCE: Newell et al. 1985b, Copyright 1985. Oxford University Press.

TABLE 4. *Nitrite use and history of syphilis*

History of Syphilis	Frequency of Nitrite Use	KS/OI	SF	Odds Ratio
No	Never	1	6	1.0
	Occasional	2	4	3.0
	Frequent	7	8	5.3
Yes	Never	1	2	1.0
	Occasional	5	3	3.3
	Frequent	15	6	5.0

NOTE: Never=once only or once per year to allow for the experimenter: occasional=once per 6 months to once per month: frequent=once per month or more Often

TABLE 5. *Nitrite use by sexual activity*

Nitrite Use	Average Number Partners per Week	Average Number Different Partners per Week
Never	2.3	1.7
Occasional	3.1	2.0
Frequent	4.0	2.6

NOTE: Never=once only or once per year to allow for the experimenter; occasional=once per 6 months to once per month: frequent once per month or more Often.

the disease. As noted above, the lifetime use was significantly greater among cases than among both control groups. Also, Marmor et al. (1982) noted that passive exposure at homosexual discotheques was reported by many subjects. Laboratory studies showed that one brief exposure to human lymphocytes in culture was sufficient to cause immunologic damage (Hersh et al. 1983).

TABLE 6. *Prevalence of nitrite use among three case-control studies*

Study	Cases		Controls	
	Number Used	Percent	Number Used	Percent
Marmor et al. 1982	20	100	40	68
Jaffe et al. 1983	50	96	120	96.95
Newell et al. 1985	31	94	29	72

COHORT EFFECT OF NITRITE EXPOSURE

Fifty percent of all patients in these studies with AIDS-related KS/OI were between 30 and 39 years of age, and 90 percent of all patients were men between the ages of 20 and 49. The cohort of men 30 to 39 years old (mid-age 35) in 1980 would have been 20 to 29 years old

(mid-age 25) in 1970 when volatile nitrites became available on the open market. Further analyses of our data showed a dose-response gradient among patients in the 30 to 39 age group, but not in the other two age groups (table 7). The numbers of subjects in the younger and older age groups were small.

TABLE 7. *Odds ratios for nitrite use by age at referral*

Nitrite Use	Age at Referral 20-29	30-39	40-49
Never	1.0	1.0	1.0
Occasional	6.0	4.0	1.0
Frequent	2.8	9.3	3.0

NOTE: Never=once only or once per year to allow for the experimenter; occasional=once per 6 months to once per month: frequent=once per month or more often.

Nevertheless, the increased risk clustered in the predicted age group. This cohort would have reached mid-age of 30 years during 1975 when nitrite use was reported to be very common among male homosexuals. The age of the cohort of men who were developing KS/OI is related to the history of nitrite availability and use shown in table 8.

Four logical times for initial exposure to nitrites were: (1) 1965, the central year from 1960 to 1969 during which amyl nitrite was available without a prescription; (2) 1970, when street-variety nitrites became available; (3) 1974, when the literature began reporting its use among homosexual males; and (4) 1977, by which time its use was repotted to be very common. Onset of the KS epidemic was reported in mid-1981. We believe that 1965 is too soon for significant exposure to have occurred, thus the lag time of 16 years is probably too long. At the other extreme, the 4 years between 1977 and 1981 may be too short for the development of cancer, although it is possible if the carcinogen were potent enough or the exposure were highly concentrated. Metabolic products of inhaled nitrites may satisfy both of these criteria. The bulk of the literature suggests

that nitrite use had saturated the gay male population by 1974. Thus, a better estimate for onset of exposure would be a peak in 1974 followed by the epidemic reported in 1981, a span of 7 years.

TABLE 8. *Time intervals from exposure to nitrites to first report of AIDS epidemic*

Year	Availability and Use of Nitrites	Onset of Epidemic Reported 1981
1965	Amyl nitrite available 1960- 1969	16 years
1970	Street-variety "poppers" available	11 years
1974	"Popper" craze widely recognized	7 years
1977	Nitrites reported "in every corner of gay life"	4 years

SUMMARY

There are important reasons for considering nitrite inhalation as a factor in the development of AIDS-related KS in young male homosexuals. These are (1) the pharmacologic properties of amyl, butyl, and isobutyl nitrites, which are toxic; (2) the mutagenic, teratogenic, and carcinogenic products resulting from metabolism of N-nitroso compounds; (3) the potent carcinogenicity of N-nitroso compounds in 39 different animal species: and (4) the deleterious effects of volatile nitrites on human lymphocytes both in vitro and in vivo .

Specifically related to this epidemic, there are additional reasons for pursuing the connection between nitrite inhalation and development of KS. These include: (1) the timing of the production and sales of volatile nitrites for use as recreational drugs and the subsequent outbreak of the AIDS epidemic (7 to 10 years); (2) the extensive use of nitrites among male homosexuals; (3) the virtual universal history of nitrite use by young male homosexuals in whom KS has developed

during the past 3 years; and (4) the age group in which KS is developing is consistent with a cohort initially exposed 7 to 10 years ago.

CONCLUSION

We conclude that nitrite use may contribute to the development of AIDS-related KS among male homosexuals. Immunosuppression may allow expression of human immunodeficiency virus that was previously suppressed. The interaction of nitrites with other identified risk factors is yet to be elucidated.

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AUTHORS

Guy R. Newell, M.D.
Margaret R. Spitz, M.D., M.P.H.
Michael B. Wilson, M.Ed. (Deceased)

Department of Cancer Prevention and Control
The University of Texas
M.D. Anderson Hospital
1515 Holcombe Boulevard
Houston, TX 77030

The Fate and Toxicity of Butyl Nitrites

Roger P. Maickel

INTRODUCTION

Volatile organic nitrites have evolved over the past quarter century as popular street drugs, inhaled to after consciousness and intensify sexual experiences (Sigell et al. 1978). Originally, the specific compound used for this purpose was amyl nitrite, used in the treatments of angina and poisoning by hydrogen sulfide or hydrogen cyanide (Stine et al. 1978). This agent, which is primarily iso-pentyl nitrite, was removed from the category of prescription drugs by the Federal Drug Administration (FDA) in 1960; however, by 1968, abusive use had become so common that it was returned to prescription status (Israelstam et al. 1978).

Subsequently, the ready availability of butyl nitrites, used in a variety of commercial applications (intermediates for perfumes, antifreeze preparations, etc.), made these compounds an attractive substitute. The pharmacology of the butyl nitrites is similar to that of amyl nitrite; indeed, they were once considered as alternative agents for the therapy of angina (Brunton 1897). They are presently available from a variety of sources (pornography shops, “head” shops, mail order catalogues) and can be obtained under a wide variety of suggestive trade names (table 1). Nickerson et al. (1979) discussed the pharmacology and uses of these compounds; a related report discusses their abusive use and some biomedical effects (Pryor et al. 1980).

NATURE OF BUTYL NITRATES

The term “butyl nitrites” refers to four compounds, the nitrous acid esters of n-butyl, isobutyl, sec-butyl, and tert-butyl alcohols. One of

TABLE 1. *Current trade names for butyl nitrites*

Aroma of Men	Heart On
Ban Apple Gas	Highball
Bang	Jac Aroma
Bolt	Liquid Increase
Bullet	Looker Room
Climax	Mama Poppers
Crypt Tonight	Oz
Cum	RUSH
Discorama	Satan's Scent
Hardware	Toilet Water

the problems in dealing with these substances is the fact that commercially available samples are generally not pure. In fact, analysis of such materials in this laboratory indicated that the labeled component was actually from 44 to 96 percent of the sample (table 2). A sample of "RUSH," sold as a "room odorizer" by Gemini Books, Lafayette, IN, was found to be primarily isobutyl nitrite (IBN). The compounds are rather unstable unless stored at low temperatures and

TABLE 2. *GLC analysis of commercials available butyl nitrites*

Compound	Authentic Compound		Maior Containment	
	Percent	R.T.* (minutes)	Percent	R.T.* (minutes)
lsobutyl nitrate (lBN)[a]	63	4.7	26	6.6
n-butyl nitrite (NBN)[a]	79	5.5	13	6.1
sec-butyi nitrite (SBN)[a]	44	4.0	56	5.6
tert-butyl nitrite (TBN)[a]	96	4.6	4	3.0
RUSH[b]	63	4.7	17	6.6

*Retention time.

[a]Pfaltz and Bauer, Inc., Stamford, CT.

[b]Pacific Western Distributing Corp.. San Frandsco, CA.

SOURCE: GLC analysis as reported by Maickel and McFadden (1979).

in the absence of light, air, and water (Noyes 1943); the primary breakdown products are the corresponding alcohols. When prepared in the laboratory by the method of Noyes (1943), IBN, n-butyl nitrite (NBN), and set-butyl nitrite (SBN) can be produced with >99 percent purity: tert-butyl nitrite (TBN) requires a simple distillation process to achieve a similar level of purity (Maickel and McFadden 1979).

STABILITY STUDIES

All butyl nitrites are believed to be rapidly hydrolyzed in vivo, yielding the corresponding alcohols and nitrite ions (Sutton 1963). Some idea of the rapidity of in vivo degradation of these compounds may be observed in mice exposed to the butyl nitrites through inhalation of concentrations sufficient to cause death within 10 to 20 minutes (i.e., 3,000, 2,000, 4,000, and 50,000 ppm of IBN, NBN, SBN, and TBN, respectively). No detectable levels (less than 1 .0 ug/ml) could be found of IBN, NBN, or SBN. Under these conditions, the blood level of TBN was 16.7 ± 3.2 (SEM) u g/ml.

Since plasma levels of the butyl nitrites were so low even when mice were exposed to lethal doses, it was felt that the stabilii of these compounds should be assessed under various test conditions. Accordingly, a study was performed of the stability of the various butyl nitrites in 0.1 M phosphate buffer (pH=7.4) at 37 °C. The results (table 3) showed that rapid decomposition of these compounds occurred under such conditions. Three of the compounds (IBN, NBN, and SBN) had monophasic decay curves with half-lives of 14.0, 15.2, and 21.8 minutes, respectively, while TBN showed a blphasic decay curve with a half-life of 5.6 minutes for the first phase, and 16.2 minutes for the second phase. All four compounds decayed according to first order kinetics.

When similar studies were performed using mouse plasma (rather than phosphate buffer) under similar conditions, even more rapid degradation was observed (table 4). All four compounds showed a rapid initial decay phase (with a half-life of 2.8 to 3.7 seconds) for the first 10 to 20 seconds, followed by a second, slower phase (with a half-life of 75 to 219 minutes) that extended out to >3.0 hours. The transition from first to second phases occurred at a concentration of approximately 0.1 mg/ml for all four butyl nitrites and the decay curves were first order in both phases.

Finally, similar studies were performed to examine the stability of the butyl nitrites when incubated with mouse whole blood at 37 °C

TABLE 3. *Stability of butyl nitrites in phosphate buffer (pH = 7.4) at 37 °C*

Compound	Half-Life Minutes) Phase I	Phase II
IBN	14.0±0.7	–
NBN	15.2±1.8	–
SBN	21 .8±1.9	–
TBN[a]	5.6±0.4	16.2±2.7

[a]Phase I/Phase II transition at approximately 12 minutes.

NOTE: Butyl nitrites (1 mg/ml) were added to 0.1 M phosphate buffer (pH-7.4) in septum-stoppered vials in a shaking incubatur at 37 °C. Samples were withdrawn at 2 to 5 minute intervals for GLC analysis as described by McFadden et al. 1981. Data were analyzed by feast-squares regress&n analysis; values are reported as mean ± SEM (n=5).

TABLE 1. *Stability of butyl nitrites in mouse plasma at 37 °C*

Compound	Half-Life (Minutes) Phase I	Phase II
SBN[a]	2.79±0.16	219±65
TBN[a]	2.82±0.23	127±28
NBN"	3.41±0.70	162±50
IBN[a]	3.66±0.26	75±21

[a]Phase I/Phase II transition at approximately 15 seconds.

NOTE: Butyl nitrites (1 mg/ml) were added to mouse plasma in septum-stoppered vials in a shaking incubator at 37 °C. Samples were withdrawn at 5-second to 10-minute intervals for GLC analysis as described by McFadden et at. (1981). Data ware analyzed by least-squares regression analysis: values are reported as mean ± SEM (n=6-7).

(table 5). Under these conditions, the rates of decay for all four compounds were extremely rapid. NBN and IBN decayed in a mono-phasic fashion with half-life values <2 seconds, while SBN and TBN showed biphasic decay curves with initial phase half-life values of <3 seconds and second phase decay values of 6.1 and 20 minutes, respectively. Thus, the breakdown of butyl nitrites was relatively

TABLE 5. *Stability of butyl nitrites in mouse whole blood at 37 °C*

Compound	Half-Life Minutes) Phase I	Phase II
NBN	<1.0	
IBN	1.60±0.05	
SBN[a]	1.91±0.06	6.1±1.6
TBN[a]	2.72±0.26	20.2±2.3

[a]Phase I/Phase II transition at approximately 15 seconds.

NOTE: Butyl nitrites (1 mg/ml) were added to mouse whole blood in septum-stoppered vials in a shaking incubator at 37 °C. Samples were withdrawn at 5 to 10 second intervals for GLC analysis as described by McFadden at al. (1981). Data were analyzed by least-squares regression analysis; values are reported as time ± SEM (n=4).

rapid in aqueous conditions and markedly enhanced in the presence of mouse plasma and even more so in the presence of whole blood.

Osterloh and Goldfield (1984) have shown that NBN is rapidly converted to n-butanol, nitrite and nitrate in water; the rapidity of breakdown increased as the pH was lowered from 9 to 4. In whole blood, NBN was rapidly converted to butanol and nitrate with formation of methemoglobin; in plasma, these authors found NBN to be relatively stable.

TOXICITY

The resultant effects of the presence of erythrocytes, coupled with earlier observations that IP dosage of butyl nitrites (but not the corresponding butyl alcohols) to mice resulted in discoloration of the blood (Maickel and McFadden 1979), suggested that methemoglobinemia was a significant factor in the toxicity of the butyl nitrites. Accordingly, a series of studies were performed to examine the ability of butyl nitrites to produce methemoglobin in hemolyzed mouse erythrocytes. The results of these studies are presented in table 6, showing NBN as the most potent compound in this regard, closely followed by IBN, the most commonly available butyl nitrite in

TABLE 6. *Butyl nitrite-induced methemoglobin formation in mouse hemolyzed erythrocytes in vitro*

Compound	MH^{50} (95% Confidence Linmits)	Relative Poetency
NBN	131 (180-99)	1.00
IBN	174 (211-148)	0.75
SBN	202 (295-149)	0.65
TBN	5072 (6508-4089)	0.03

NOTE: MH values are the concentration (g butyl nitrites whole blood) necessary to oxidize 50 percent of the Hb to metHb. Values and 95 percent confidence limits ware datermined by the dose-response analysis of Goldstein (1964).

SOURCE: McFadden et al. 1981, Copyright 1981, Purdue University, Department of Pharmacology and Toxicity.

commercially (street drug) available preparations. TBN was less potent by more than an order of magnitude.

Similar data were obtained from studies in which methemoglobin levels were determined in mice exposed to lethal concentrations of the four butyl nitrites in dynamic inhalation chambers. In such a test situation, death occurred in 10 to 20 minutes at the exposure concentrations used. As seen in table 7, TBN is much less potent than the other three butyl nitrites, with required lethal concentrations at an order of magnitude greater. Even at this concentration, a significantly lower level of methemoglobin was produced by TBN as compared to the levels produced by NBN, IBN, and SBN.

One interesting aspect of these compounds is the relatively consistent lethal potency when given by various routes. Table 8 displays a summary of this data as derived from LD, determinations. NBN was the most potent compound, regardless of route of administration, with IBN next in potency in every case. With IP dosage, SBN and TBN were virtually identical, while with PO dosage, TBN was a bit more potent than SBN. On inhalation, TBN was clearly the least toxic substance by almost an order of magnitude. Similar results with NBN have been repotted by Osterloh and Goldfield (1985).

TABLE 7. *Levels of methemoglobin* in *mice exposed to lethal concentrations of butyl nitrites*

Compound	Exposure Concentration (ppm)	Percent Methemoglobin
NBN	2,000	97.4 ± 2.4
IBN	3,000	03.5 ± 1.7
SBN	4.000	83.1 ± 3.0
TBN	50,000	83.2 ± 2.2

NOTE: Concentrations of butyl nitrites were determined by GLC as reported by McFadden and Maickel (1981). Methemoglobin levels were determined as described by Frankel et al. (1970); values are mean ± SEM (n=4-5).

SOURCE: McFadden et al. 1981, Copyright 1981, Purdue University, Department of Pharmacology and Toxicology.

Additional studies have also been performed to see if various treatments could reduce or prevent the toxicity of these compounds. Since most commercially available preparations contain IBN, and several of these commercial preparations have been implicated in human poisonings via the oral route (Dixon et al. 1981), IBN was used as the test agent. The lethality of a PO dose of 400 mg/kg of IBN could be significantly reduced by pretreating mice with methylene blue (50 mg/kg, IP) or toluidine blue (50 mg/kg, IP), but not with ascorbic acid (200 mg/kg, IP) or sodium selenite (3 mg/kg, IP). In contrast, mice given 300 mg/kg, PO of IBN, showed no significant antidotal effects in response to 100 percent oxygen, ascorbic acid, methylene blue, or sodium selenite (McFadden and Maickel 1982). These data would suggest that methemoglobinemia produced by butyl nitrites plays a significant role in the lethality caused by these compounds.

Further, in mice pretreated with methylene blue (50 mg/kg, IP) 15 minutes prior to a 45-minute period of exposure to 500 ppm of butyl nitrites, methylene blue completely prevented methemoglobinemia produced by NBN, IBN, and SBN. Under these conditions, TBN did not produce any significant methemoglobinemia (McFadden et al. 1961). Similarly, methylene blue pretreatment significantly increased the median lethal time values for survival when mice were exposed to higher concentrations of all four butyl nitrites (table 9). In this regard, Klonne et al. (1987) have reported single exposure LC50

TABLE 8. *Comparative potency (lethality) of butyl nitrites administered by different routes*

Compound	IP Dosage (mg/kg) LD_{50}	R.P.	PO Dosage (mg/kg) LD_{50}	R.P.	Inhalation (ppm) LC_{50}	R.P.
NBN	158	1.00	180	1.00	567	1.00
IBN	184	0.86	279	0.65	1,033	0.55
SBN	592	0.27	428	0.42	1,753	0.32
TBN	613	0.26	336	0.54	10,852	0.05

NOTE: IP deaths @ 0.5 hr.; PO deaths @ 2.0 hr.; Inhalation deaths @ 1.0 hr.; R.P.=relative potancy where most potent compound=1.00. Data on IP dosage from Maickel and McFadden (1979); on PO dosage from McFadden and Maickel (1982); and on inhalation from McFadden et al. (1981).

TABLE 9. *Effects of methylene blue pretreatment on survival of mice exposed to butyl nitrites*

Compound		LD_{50} (Minutes) Control	LD_{50} (Minutes) Methylene Blue	Ratio (Methylene Blue to Control)	Methylene (ppm)
IBN	1500	20.5	232.1	11.3	
NBN	1000	24.0	187.0	7.8	
SBN	2000	43.0	261.5	6.1	
TBN	50000	32.7	68.6	2.1	

NOTE: Mice were dosed with 50 mg/kg, IP, methylene blue 15 minutes prior to butyl nitrite exposure. LT_{50} values were determined by probit analysis.

SOURCE: McFadden et al. 1981, Copyright 1981, Purdue University, Department of Pharmacology and Toxicology.

values in rats for: ethyl, methyl, n-propyl, n-butyl, isopentyl, and isobutyl nitrites to be 160, 176, 300, 420, 716, and 777 ppm, respectively.

OTHER STUDIES

Finally, several studies have been performed to examine the subchronic toxicology of butyl nitrites in mice by inhalation (McFadden and Maickel 1985). Mice were exposed to IBN (400 ppm), NBN (300 ppm), SBN (500 ppm), or TBN (1000 ppm) for 7 hours daily for 60 days. Under these conditions, survival rates were 80 to 95 percent. During the first 30 days of exposure, treated animals had significantly lower body weights than untreated controls; during the second 30 days of exposure, the body weights of mice exposed to IBN, NBN, or SBN returned to control values, while those of mice exposed to TBN continued to be significantly lower than those of controls. A summary of organ weights of these mice is shown in table 10. Liver weight was significantly increased only in the IBN-treated mice, kidney weight in those treated with IBN and SBN, and lung weight in all except the mice exposed to TBN. In contrast, spleen weights were significantly elevated in mice exposed to all four butyl nitrites, presumably reflecting the adverse effects of butyl nitrites on erythrocytes.

Selected enzymes were also determined in these mice. Plasma isocitric dehydrogenase was significantly depressed in mice exposed to TBN, liver microsomal glucose-6-phosphatase was significantly depressed in mice exposed to IBN, and liver microsomal cytochrome P-450 was significantly depressed in mice exposed to NBN (McFadden and Maickel 1985).

SUMMARY

In summary, all four butyl nitrites were found to be moderately toxic compounds in mice, especially when given PO, TBN was the least toxic and also the least potent in producing methemoglobinemia.

TABLE 10. *Effects of 60 days exposure to butyl nitrites on organ weights of mice*

Compound	Proportion of Body Weight (Butyl Nitrite/Control)			
	Kidney	Liver	Lung	Spleen
IBN	1.15[a]	1.14[a]	1.74[a]	2.57[a]
NBN	1.04	0.98	1.33[a]	1.82[a]
SBN	1.12[a]	0.96	1.11	1.27[a]
TBN	0.97	0.96	1.60[a]	1.31[a]

[a]significantly greater than control.

NOTE: Mice were treated as described in McFadden and Maickel (1985).

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AUTHOR

Roger P. Maickel, Ph.D.
Department of Pharmacology and Toxicology
School of Pharmacy and Pharmacal Sciences
Purdue University
West Lafayette, IN 47907

The Acute Toxicity of Nitrite Inhalants

Ronald W. Wood

INTRODUCTION

Alkyl nitrites produce a variety of effects when inhaled. Except in the context of anginal pain or as an antidote to cyanide poisoning, these effects cannot be construed as having therapeutic utility. A number of demonstrable risks accompany the inhalation of these materials, and this chapter will review the acute hazards associated with the nonmedical use of these chemicals. Aside from the spectrum of effects desired by the user, there are less desirable "side effects" as well as frank injuries associated with the use of these products, including skin and tracheobronchial irritation; bum injuries; acute toxicity mediated by hypokinetic anoxia, methemoglobinemia, and associated disorders of blood and blood-forming organs; and the induction of a substance abuse disorder.

DESIRED ACUTE EFFECTS AND UNDESIRED SIDE EFFECTS

Inhalation of high concentrations of nitrites relaxes smooth muscle: the consequent intense peripheral vasodilation produces flushing, a fall in blood pressure, and a reflex increase in heart rate to maintain perfusion of vital organs (Haley 1980). These effects are accompanied by feelings of warmth, rapid pulse, and throbbing sensations. Volatile nitrites are frequently used as an adjunct to sexual behavior, because of their smooth muscle effects, nitrites can reduce sphincter tone and alter tumescence. The vasodilation is accompanied by heat loss and a subsequent chill. Headache, nausea, and fainting are common sequelae of nitrite inhalation.

Skin and Tracheobronchial Irritation

Skin contact with commercial products containing butyl nitrite can produce a crusty lesion at the site. Repetitive use of the material can lead to a proliferation of these lesions around the nose and lip (Fisher et al. 1981; Fisher 1984; Romaguera and Grimalt 1982) and has been reported around the penis, the scrotum, and elsewhere (Bos et al. 1985). The latter report suggests that a true allergic response to these materials may occur.

The irritating properties of these materials are not confined to the skin and have manifested themselves in tracheobronchitis with erythema of sufficient severity to require hospitalization, and with complaints of cough, fever, mild hemoptysis, and exertional dyspnea (Covalla et al. 1981). Subchronic toxicity evaluations support concern about lung injury (McFadden and Maickel1985; Lynch et al. 1985).

Burn Injuries

Aikyl nitrites are flammable and explosive. At least one bum injury has been reported through the National injury Information Clearing House (NEISS data base) of the Consumer Product Safety Commission (CPSC), following the use of a room odorizer product near a candle. “This incident involved a 26- yearold male who opened a small bottle on a stand when it ignited, flamed up, and burned him. During this accident, the victim spilled some of the gymnasium room odorizer onto parts of the living room furnishings. As a result, part of an ottoman and small sections of the carpet were damaged. It is believed that the liquid gymnasium room odorizer ignited, as its vapors came into contact with a lighted cigarette in a nearby ash tray. The victim received bums on the left side of his nose and an area of his left cheek. The victim was also singed on his right arm, forehead and some parts of his hair” (CPSC memo from Schmettzer to Perez, July 11, 1977).

The materials are labeled with a warning of this hazard, which Is not to be underestimated; small quantities have been responsible for refrigerator explosions in laboratories, and larger quantities have been implicated in the largest fire in San Francisco since the earthquake of 1998 (Turner 1981). Despite these hazards, many of the products packaged in small vials with shrink-fit plastic comply with U.S. Postal Service regulations for the shipment of small quantities of flammable and explosive materials.

Acute Toxicity

Whether inhaled or swallowed, nitrites can produce anoxic states. The administration of nitrites by any route can produce profound methemoglobinemia. Tissue anoxemia can result from methemoglobinemia (Darling and Roughton 1942; Lester and Greenburg 1944). Ascorbic acid and methylene blue may be effective prophylactic agents for nitrite poisoning if administered promptly, but laboratory studies offer little support for this in the case of isobutyl nitrite ingestion (McFadden and Maickel 1982). Prolonged administration of the materials may lead to several disorders of the Mood and blood-forming organs, including Heinz-body hemolytic anemia and splenomegaly (Romeril and Concannon 1981); increased spleen weight has been observed in mice chronically exposed to butyl nitrites (McFadden and Maickel 1985). The administration of nitrites can also produce hypokinetic anoxia, an oxygen starvation of vital organs secondary to sustained profound peripheral vasodilation, pooling of Mood in the extremities, and impaired vascular return (Wilkins et al. 1937).

A number of human anecdotes report on the acute toxicity of volatile nitrite “room odorizer” products and provide correlative observations that substantiate concern aroused by experimental work with laboratory animals. Severe methemoglobinemia can result from deliberate inhalation of room odorizer products (Home et al. 1979; Shesser et al. 1981; Guss et al. 1985). Ingestion can produce a more rapid and malignant methemoglobinemia than can inhalation and can be lethal (Dixon et al. 1981; Shesser et al. 1980; Shesser et al. 1981; Smith et al. 1980; Wason et al. 1980).

The acute toxicity of the alkyl nitrites has received attention in the laboratory and is relevant to the regulation of these materials in interstate commerce, including packaging and labeling requirements. A summary of these experimental findings is presented in table 1. Room odorizer products are currently considered to be “toxic” by inhalation or ingestion, although the oral toxicity of the material has not been evaluated adequately according to the CPSC. The materials display an unusually steep lethality function so that, in the effective range, small increments in dose produce large changes in the number of resultant deaths (Wood and Cox 1981; Klonne et al. 1987). In addition, there is a very narrow margin of safety between behaviorally effective and lethal concentrations (Rees et al. 1986).

Users estimate they can derive 40 “doses” from a typical room odorizer, yielding approximately 0.2 ml per self-administration (Israelstam et al. 1978). This estimate is neither a dose nor an exposure concentration, but a rate of loss of a volatile material from an open container. There have been no studies that describe either the absorbed dose or the exposure concentrations that are effective in producing smooth muscle relaxation, alterations in cardiovascular function, behavioral impairment, or self-administration under the brief exposure conditions typical of self-administration. However, Pryor et al. (1989) did expose rats to isobutyl nitrite for 15 to 60 seconds, at concentrations that increased gradually in the exposure chamber. The LC50s expressed as a peak concentration ranged from 4.5 to 4.8 percent. The slope of the lethality function was influenced by the rate of change of concentration. A constant concentration-time product relationship was not obtained in this study, or in that of Klonne et al. (1987), suggesting that the dramatic acute effects of the agent may alter the pharmacokinetics and exaggerate the toxicity of these agents.

For some of the aliphatic nitrites, the alcohol from which they are synthesized may contribute significantly to their toxicity when injected or ingested. In addition, the alkyl nitrites are metabolized to nitrite ions and the corresponding alcohol, which may lead to delayed deaths or hepatotoxicity. n-Butyl alcohol has an oral median lethal dose (LD_{50}) for the male rat of 790 mg/kg (Purchase 1969), in contrast to 13,600 mg/kg for ethanol (Smyth et al. 1941). In an investigation of several highly pure butyi nitrites and their alcohols given to mice intraperitoneally, set- and tert-butyl nitrites were found to have significant delayed toxicity; the toxicity of the alcohols by the same route was also delayed, and the LD_{50} ranged from 254 (n-butyl) to 544 mg/kg (isobutyl) 7 days after administration (Maickel and McFadden 1979).

SUBSTANCE ABUSE DISORDERS

There is clear evidence that volatile nitrites are used as drugs of abuse. National surveys indicate that high school seniors and adults not only have used alkyl nitrites as drugs, but also that 7.9 to 11.1 percent of high school seniors from 1979 to 1985 reported having tried these drugs in their lifetime (Johnston et al. 1986). The incidence of deliberate use by homosexual men has been greater and is discussed in more detail shortly. Lowry (1980) conservatively estimated that 250 million recreational doses a year were consumed in the United States (Lowry 1982).

Volatile nitrites are persistently self-administered by people. Israelstam et al. (1978) interviewed 150 users of isobutyl nitrite and reported that users who administered nitrites did so from three to six times per "occasion"; the number of occasions ranged from only once to four times a week. The duration that this frequency of self-administration was maintained was unspecified. Goedert et al. (1982), in an attempt to determine if volatile nitrites alter immune function, studied 17 men; 10 were described as regular users, inhaling nitrites from 1 to 20 times per month; 4 had used nitrites for longer than 6 years. Romeril and Concannon (1981) presented two cases, each of which reported 20 sniffs per occasion, two to three times per week, for either 3 or 24 months. Fisher et al. (1981) reported on two men who stated that they were "in the habit" of inhaling butyl nitrite and continued to do so during the 6-week period that they were seen by a physician.

According to the nosological scheme of the Diagnostic and Statistical Manual (DSM Ill) of the American Psychiatric Association, one definitional criterion of "substance abuse" is a duration of pathological use for at least 1 month; several of the cases described above meet this criterion. Other than the duration of use, definitional criteria for "pathological use" vary with the substance abused but may include episodes of complication due to substance intoxication, e.g., alcoholic blackouts, opioid overdose; need for daily use of the substance for adequate functioning; and continuation of substance use despite a serious physical disorder that the individual knows is exacerbated by use of the substance. Examples from the literature fulfilling these criteria follow.

Compications Due to Substance Intoxication

Shesser et al. (1981) report a hospital admission following several hours of continuous inhalation of an isobutyl nitrite preparation. The patient was alert and combative, and the parents sought to have the patient admitted to the hospital because of mental status change and cyanosis. The patient admitted to having had several drinks of alcohol. Treatment of the patient with oxygen and intravenous methylene blue and ascorbic acid resulted in alleviation of the cyanosis; with the patient becoming calmer and reoriented. Covalla et al. (1981) report a hospital admission for severe tracheobronchitis following the.patient's inhalation, with a friend, of two bottles of LockerRoom in a week's time. Hi friend developed a similar but less severe illness. The cases in the following paragraph also constitute complications of substance intoxication.

Continuation of Substance Use Despite a Serious Physical Disorder Exacerbated by Use of the Substance

Home et al. (1979) report emergency room admission on two separate occasions of a 25year-old man after he inhaled butyl nitrite. The occurrence of clinically significant methemoglobinemia was not sufficient to deter self-administration in this individual. In a survey of 255 experienced users (Lowry 1979), 10 percent had experienced nasal irritation at least once, and 5 percent had experienced nausea or temporary loss of erection. These negatlve effects were associated with "overuse" (emphasis Lowry's). Fisher et al. (1981) report several cases of facial dermatitis due to butyl nitrite inhalation. Two of the three cases reported that they were "in the habit of inhaling butyl nitrite," and continued to do so during the 6-week period in which the dermatitis was evident. The skin lesions cleared when nitrite use was terminated.

Need for Daily Use of the Substance for Adequate Functioning

Sigell et al. (1978) interviewed an unspecified number of users, some of whom claimed that they were no longer able to perform sexually without the use of these drugs. Everett (1975) has made a similar assertion.

CONCLUSION

Research funded by the National Institute on Drug Abuse (NIDA) has demonstrated that drugs abused repetitively by humans are self-administered by animals; drugs that are abused sporadically by humans (most hallucinogens), or not at all (major tranquilizers), are not taken by laboratory animals. Thus, there is a pharmacologic sine qua non for the ability of drugs to maintain self-administration: in the absence of intrinsic abuse potential, humans will not persistently abuse a drug. Although there have been no laboratory investigations using animal self administration preparations, volatile nitrites have abuse potential because they would not generate persistent human self-administration in its absence.

The extrapharmacologic determinants of substance abuse are of great importance; in this case, the pattern of distribution, availability, and promotion of materials in commerce play predominant roles in elevating the abuse liability of the volatile nitrites. The pattern of distribution of volatile nitrites is typical of a free market with access to the mails and the right to advertise. The interruption of this

pattern of distribution would reduce the abuse liability of these agents.

TABLE 1. *Acute toxicity of akyl nitrites*

	Compound	Species	LD_{50}/LC_{50}	Reference
Oral				
	n-Butyl	Rat	83 mg/kg (79.5-86.5)	Wood and Cox 1981 Federal Hazardous Substances Act protocol Ethanol vehicle (50 mg/kg="highly toxic")
	n-Butyl	Mouse	180 mg/kg (0-288)	McFadden and Maickel 1982 (2-hour LD_{50})
	sec-Butyl		428 mg/kg (358-469)	
	tert-Butyl		336 mg/kg (292-447)	
	Isobutyl		279 mg/kg (0-610)	
	n-Butyl	Mouse	171 mg/kg (27-249)	McFadden and Maickel 1982 (7-day LD_{50})
	sec-Butyl		423 mg/kg (393-456)	
	tert-Butyl		308 mg/kg (220-426)	
	Isobutyl		205 mg/kg (5-311)	
Intraperitoneal				
	Amyl	Mouse	130 mg/kg (111-152)	Dewey et al. 1973
	n-Butyl	Mouse	158 mg/kg (127-197)	Mcfadden and Maickel 1979 (30-minute LD_{50})
	sec-Butyl		592 mg/kg (476-734)	
	tert-Butyl		625 mg/kg (520-750)	
	Isobutyl		169 mg/kg (139-199)	
Intravenous				
	Amyl	Mouse	51 mg/kg (38-68)	Dewey et al. 1973 (24-hours LD_{50})

TABLE 1. (Continued)

Compound	Species	LD_{50}/LC_{50}	Reference
Inhalation			
Isbutyl	Mouse	1346 ppm (1219-1473)	Rees et al. 1986 (30-minute exposures)
n-butyl		949 ppm (897-1001)	
Isoamyl		1430 ppm (1302-1559)	
Isobutyl	Mouse	1033 ppm (843-1234)	McFadden et al. 1981 (60-minute exposures)
n-butyl		567 ppm (531-625)	
sec-Butyl		1753 ppm (1552-1964)	
tert-Butyl		10852 ppm (626-15408)	
Methyl	Rat	176 ppm (169-183)	Klonne et al. 1987 (4-hour exposures)
Ethyl		160 ppm (151-169)	
n-Propyl		300 ppm (293-308)	
n-Butyl		4210 ppm (410-431)	
Isobutyl		777 ppm (747-809)	
Isopentyl (Isoamyl)		716 ppm (702-731)	
n-Butyl	Rat	1470 ppm (1226-1823)	CPSC memo fro Perez to Preuss, September 24, 1979
Isobutyl		1000 ppm (815-1255)	Federal Hazardous Substances Act protocol
Isoamyl	Rat	1118 ppm (797-1493)	(1-hour exposures)

Note: LD_{50}=Median lethal dose (lethal to 50 percent of test subjects).
LC_{50}=Lethal concentration, 50 percent.

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AUTHOR

Ronald W. Wood, Ph.D.
Research Associate Professor
Department of Environmental Medicine
New York University Medical Center
550 First Avenue
New York, NY 10016

Indications From Animal and Chemical Experiments of a Carcinogenic Role for Iso-Butyl Nitrite

Sidney S. Mirvish, Martin O. Ramm, and David M. Babcook

INTRODUCTION

We have been studying the in vivo formation of carcinogenic nitrosamines and nitrosamides (which together constitute the highly carcinogenic N-nitroso compounds) after exposure of laboratory animals to the atmospheric pollutant nitrogen dioxide (NO_2). In the course of those experiments, we found that the nitrite ester of cholesterol participated in the formation of nitrosamines. This finding is consistent with suggestions that simple nitrite esters may form similar carcinogenic compounds in man. In this chapter we describe experiments evaluating the carcinogenic potential of amyl nitrite and isobutyl nitrite (IBN), a major component of abused room odorizers, and speculate on the possible relationship between nitrite ester abuse and the development of Kaposi's sarcoma (KS) in patients with Acquired Immunodeficiency Syndrome (AIDS). We first summarize our data on NO_2 and describe the nitrite ester studies, and then discuss the possible connections between KS and nitrite ester abuse.

NITROSAMINE FORMATION DUE TO EXPOSURE TO NO^2

In chemical systems, NO_2 reacts with amines to form nitrosamines. On this basis we studied whether the same reactions could occur *in vivo.* We exposed rats to NO_2 in air (50 ppm for 4 hours), gavaged them with the secondary amine morpholine, and later analyzed the carcasses for the corresponding nitrosamine, N-nitrosomorpholine (NMOR). The final stage of the analysis involved gas chromatography of the NMOR, with detection by Thermal Energy Analysis (GC-TEA), a system that can detect as little as 0.1 ng nitrosamine. While no NMOR was detected, the carcasses did contain a "nitrosating agent"

that reacted with morpholine in dichloromethane or ether solution to produce NMOR *in vitro* (Mirvish et al. 1981). All subsequent studies were done on Swiss mice. The nitrosating agent was soluble in lipids and occurred only in the skin; most of it persisted in the body for 24 hours after the NO_2 exposure (Mirvish et al. 1983). The nitrosating agent was shown to be produced by direct contact of NO_2 with the skin. Later, these findings provided some ideas about the possible route of exposure to ISN vapor.

To identify the nitrosating agent derived from NO_2 we examined the nature of the precursor in skin lipids that reacted with NO_2 to produce this agent. About 40 percent of the precursor was associated with the triglyceride fraction and 60 percent was identified as cholesterol (Mirvish et al. 1986). These findings were discovered by separation of the lipids on thin-layer and high-performance liquid chromatography, with analysts of each fraction for a nitrosating agent precursor. Cholesterol reacted with NO_2 to produce the known nitrite ester, cholesteryl-3-B-nitrite. Synthetic cholesteryl nitrite reacted with morpholine by the scheme shown below to give 13 percent yields of NMOR. We showed that the cholesterol-derived nitrosating agent in mouse skin was almost certainly cholesteryl nitrite. These findings agreed with previous studies showing that nitrite esters (including IBN) react fairly readily with secondary amines to form nitrosamines (Doyle et al. 1983; Dabora et al. 1984; Loeppky et al. 1984).

$$RONO + R'_2NH \rightarrow ROH + R'_2NNO$$

Thus far, we have described *in* vitro reactions of the $N0_2$derived nitrosating agent to form nitrosamines. To be significant, these reactions must also be shown to occur *in vivo.* To test for this, we applied one of the secondary amines, morpholine or N-methylaniline, to the skin of mice 20 hours after they were exposed to 50 ppm NO_2 in air for 4 hours. After another 0.5 to 2.0 hours, the mice were killed and the skins were analyzed for NMOR or N-nitroso-N-methylaniline (NMA) by GC-TEA. We did not observe significant NMOR, but did detect NMA, with maximum values of 18±7 nmol/mouse (mean ± SD) for 6 mice killed 40 minutes after the amine was applied (Mirvish and Ramm 1987). In a similar experiment, the mice were shaved 30 minutes after methylaniline was painted, and the hair and shaved skin were analyzed separately for NMA. The hair contained 87 percent of the NMA. This demonstrates the importance of direct access of NO_2 to the skin lipids, which should be more facile for lipids in hair than for those in the skin itself. We attribute the

formation of NMA, but not NMOR, mainly to the lipid solubility of N-methylaniline, which enables this amine to react with the skin nitrosating agent (Morpholine is not extractable by lipids at neutral pH.) The analyses included checks to ensure that the nitrosamines were not produced during the workup.

Lipid Peroxidation By Cholesteryl Nitrite

Nitrite esters (but not nitrate esters) absorb ultraviolet light between 320 and 380 nm, which helps explain why nitrite esters are decomposed by light or heat to yield alkoxy radicals and nitric oxide (Smith 1988):

$$RON0 \xrightarrow{\text{light}} RO + NO$$

This reaction proceeds readily, with a free energy of 37 kcal/mol (Gingras and Waters 1954). For this reason, methyl nitrite is used to initiate radical reactions in studies on atmospheric pollution (Magalhaes and Chalk 1986). It struck us that such a reaction of cholesteryl nitrite could be biologically significant, because radical production is closely linked with the promotion stage of carcinogenesis (Kensler and Taffe 1986). Lipid peroxidation is a measure of radical production In the lipid phase of tissues and also shows associations with tumor promotion. For these reasons, we tested whether cholesteryl nitrite could catalyze the peroxidation of methyl linoleate, used as a model for unsaturated lipids (Mirvish et al. 1987).

One gram of methyl linoleate, with and without the addiiion of 50 mg cholesteryl nitrite, was allowed to autoxidize in a sealed dessicator (20 cm in diameter) for up to 7 days. To measure autoxidation, we determined conjugated dienes end compounds (malondialdehyde plus endoperoxides) that react with thiobarbituric acid (TBA) (Buege and Aust 1978).

Table 1 shows that cholesteryl nitrite significantly increased the production of both conjugated dienes and TBA-reacting substances. In a preliminary experiment, painting a cholesteryl nitrite solution (12.5 mg/250 l acetone) once on the skins of mice produced a significant increase after 1 to 2 days in TBA-reacting material in the skin lipids (4.5 ± 0.2 (four mice, mean SE) compared to 0.1 ± 0 (two mice) in mice painted with acetone alone). As explained earlier,

TABLE 1. *Effect of the nitrite esters cholesteryl nitrite and IBN on autoxidation of methyl linoleate*[a]

Nitrite ester	Exposure time (days)	Number of exposures	Yield of peroxidized material (nmol/mg methyl linoleate, mean ± SD) Conjugated diene test: Ester absent	Conjugated diene test: Ester present	TBA test: Ester absent	TBA test: Ester present
Cholesteryl nitrite	0	3	14 ± 2.4	21 ± 2.4[b]	0.4 ± 0.3	6.1 ± 0.3[c]
	1	2	40 ± 24	635 ± 3.5[c]	0.8 ± 0.1	14 ± 3.4[c]
	3	2	101 ± 98	931 ± 0[c]	4.2 ± 1.8	23 ± 7.8[b]
	7	1	94	714	1.6	38
IBN	0	2	16 ± 2.1	16 ± 2.1	0.6 ± 0.4	0.6 ± 0.4
	4-5	2	98 ± 43	98 ± 3.5	0.8 ± 0.4	54 ± 22[c]
	7	2	286 ± 117	150 ± 18	3.5 ± 1.7	55 ± 30[b]

[a]IBN results are based on weight of methyl linoleate at the beginning of the experiment. because some IBN distilled into the linoleate. Cholesteryl nitrite results are based on weights at the time of measurement.

[b]*Results were significantly greater than those for corresponding samples without addition of nittrite ester, with $p<0.05$b or <0.01c.

these results suggest that cholesteryl nitrite could promote carcinogenesis in mouse skin.

In Vivo Formation of the Nitrosamine NMA from Amyl Nitrite

This and the following section describe our studies on amyl nitrite and IBN, which were based on our findings about cholesteryl nitrite. The studies in this section demonstrated that amyl nitrite can react to produce a nitrosamine in vivo (Mirvish et al., in press; Mirvish and Ramm 1987).

As in the studies on skin NSA, N-methylaniline was chosen as the test amine, mainly because it is lipid-extractable from water at neutral pH and, hence, could react with the lipid-soluble nitrite ester, amyl nitrite. Adult male Swiss mice were treated with methylaniline and amyl nitrite, with each agent delivered to a different site in the body. In the main experiment, methylaniline (250 mg/ml water/kg body weight) was injected IP and, 30 minutes later, amyl nitrite (40 mg/ml corn oil/kg body weight) was gavaged. After another 30 or 60 minutes, the mice were killed and the carcasses were analyzed for NMA, using the methods described earlier. For six mice killed after 30 minutes, we obtained 480 ± 310 nmol NMA/mouse (mean ± SD). Six mice killed after 60 minutes yielded 380 ± 100 nmol NMA/mouse. The results at 30 minutes were 18 times higher than those for NMA produced from the $N0_2$derived skin nitrosating agent.

In a different procedure, methylaniline in water was gavaged, amyl nitrite in corn oil was injected IP 30 minutes later, and the mice were killed after another 30 minutes. In four mice, the NMA yield was only 6 ± 6 nmol/mouse. When amyl nitrite, but not methylaniline, was administered to four mice as in our main experiment, and an amount of methylaniline similar to that injected IP was added to each homogenate before the workup, NMA yield was only 5 ± 3 nmol/mouse, indicating that most NMA in the main experiment was produced in vivo and not during the workup.

Four mice were treated as in the first experiment, with 30 minutes elapsing between dosage with amyl nitrite and sacrifice, and certain tissues were analyzed for NMA. These yielded 630 ± 140 (stomach contents), 90 ± 2 (stomach wall), 9 ± 3 (liver), 5 ± 3 (intestines plus contents), and 0.5 ± 0.3 (Mood) nmol NMAI/mouse. Hence, most NMA was produced at the site of delivery of the amyl nitrite. These results demonstrate that simple nitrite esters can produce large yields of nitrosamine in vivo, if a lipid-soluble amine is supplied. In

humans, exposure to volatile nitrite esters could produce carcinogenic N-nitroso compounds from endogenous or exogenous amines, or, perhaps, from N-substituted amides (which can be more lipid-soluble than most amines).

In Vitro Enhancement of Lipid Peroxidation by IBN

We have described our finding that cholesteryl nitrite enhanced the autoxidation of methyl linoleate. We have now repeated this experiment using IBN. Since IBN is volatile, methyl linoleate (0.5 g in an evaporating dish), was placed in a sealed glass tank (8 x 21 x 28 cm) also containing a beaker with 2 ml IBN. After 4 to 5 days, some IBN had distilled into the linoleate and IBN in the beaker was replenished. As a control, methyl linoleate was stored in a similar tank without IBN. Table 1 shows that IBN significantly enhanced the formation of IBA-reading material but (unlike cholesteryl nitrite) did not affect (or even reduce) the level of conjugated dienes. This indicates that IBN increased the prcduction of endperoxides, but not that of hydrcperoxides.

In conclusion, the nitrosamine formation and lipid peroxidation experiments suggest that IBN could be carcinogenic in man, either because it produced carcinogenic N-nitroso compounds in vivo, or because it enhanced lipid peroxidation in vitro and hence could be a tumor promoter.

POSSIBLE ASSOCIATION BETWEEN EXPOSURE TO IBN AND KS IN AIDS

At about the time we were studying cholesteryl nitrite, Newell et al. (1985) proposed that IBN could produce N-nitroso compounds in vivo, which in turn could potentiate the induction of AIDS or KS associated with AIDS. After the role of human immunodeficiency virus (HIV) in AIDS was discovered, emphasis shifted to the second of these hypotheses.

About 15 to 25 percent of U.S. homosexual males with AIDS have developed KS; this cancer is often the cause of death (Leu and Odermatt 1985; Safai et al. 1985; Biggar et al. 1985). Heterosexual AIDS patients have a much lower incidence of KS (Haverkos et al. 1985). KS also occurs in immunosuppressed patients who do not have AIDS, but at far lower incidences than in homosexual AIDS patients (Friedman-Kien et al. 1982). Hence, KS in AIDS appears to be associated with the lifestyle of homosexual men in the United States.

Haverkos et al. (1985) reported a significant association of KS in homosexual male AIDS patients with their exposure to IBN measured as total days of use.

There is evidence both for and against nitrites being involved in the genesis of AIDS associated KS. In addition to Involvement in the formation of nitrosamines, IBN is mutagenic in the Ames test (Osterloh and Goldfield 1984), and Dax (this volume) reports an immunosuppressive action of IBN that could contribute to cancer induction. However, three points argue against the involvement of IBN in the etiology of AIDS associated KS. First, the increased incidence of KS in heterosexual AIDS patients in Africa is unlikely to be associated with nitrite abuse (Kestens et al. 1985; Marquart 1986). Second, there is some suggestion that concurrent cytomegalovirus infection is associated with KS in AIDS (Safai et al. 1985). Third, amyl nitrite was used for many years to treat angina pectoris without any evidence of a carcinogenic effect.

Possible Routes of Access of IBN to the Tissue of Origin for KS

KS is a cancer of the endothelium of blood vessels (Leu and Odermatt 1985) or lymph vessels (Beckstead et al. 1985; Jones et al. 1986). The main sites of origin for KS in AIDS are the dermis of the skin or (less often) the submucosa of the oral cavity (Safai et al. 1985; Volberding 1986). Common sites in the skin for AIDS-associated KS are the upper body, arms, and head, including the tip of the nose. The tumor is often multicentric in origin, illustrating the potency of the etiologic agents (Safai et al. 1985).

We favor the hypothesis that, if IBN is involved, its route of access is by direct contact to the skin or oral mucosa with airborne IBN vapor. This could occur directly when IBN is sniffed, which would explain the site at the tip of the nose, or by general exposure to IBN vapor inhaled by the subject or by others in bars, bathhouses or homes (Lauritzen and Wilson 1986). (These places often smell strongly of IBN, as reported at this meeting.) Hence the location of the sites for AIDS-associated KS support our hypothesis about direct contact with IBN vapor. This idea was suggested by our finding that exposure to NO_2 (which, like IBN vapor, is a lipid-soluble gas) results in nitrosating agent formation only in the skin.

Another possibilit y is that IBN vapors are absorbed by the lungs and travel to the skin via the bloodstream. In support of this view, sniffed IBN does reach the peripheral blood vessels, where it

produces vasodilation (Needleman et al. 1985), and the brain, where it produces a “high” sensation. This view could be valid, even though IBN may only persist in the body for about 1 minute after it is sniffed (the time for which the flushing and high feeling persists).

Needs for Research on IBN in Laboratory Animals

Research on the action of IBN in experimental animals would serve to establish more firmly whether IBN is a likely factor in the etiology of KS, and perhaps B-cell lymphoma (Levine et al. 1984; Biggar et at. 1985; Ziegler et al. 1 984), associated with AIDS. Specifically, we propose that research should be undertaken: (1) to determine whether IBN can be an initiator and/or a promoter of carcinogenesis; (2) to determine the route of access of IBN to the dermis; and (3) to establish the mechanisms of any carcinogenic effects of IBN.

With respect to point (1), initiation of carcinogenesis is a single, rapid event, probably involving a mutation of the DNA. Promotion of carcinogenesis is a second stage and usually involves chronic exposure to the active agent. In man, IBN could initiate carcinogenesis via the formation of N-nitroso compounds or by a direct reaction with DNA, and HIV could promote KS development, probably via its immunosuppressive action. Alternatively, HIV could initiate and IBN could promote KS development, perhaps via its effect on lipid peroxidation. A determination of whether IBN initiates or promotes carcinogenesis would help epidemiologists decide whether to estimate IBN exposure before or after infection with HIV.

Hence, we recommend carcinogenesis tests to determine whether IBN is: (1) a complete carcinogen, (2) a promoter of carcinogenesis, especially in the skin, and (3) a cocarcinogen with viruses, including, if possible, HIV in chimpanzees and feline leukemia virus in cats. These tests should be done with IBN administered systemically and by exposure to its vapors.

We further recommend metabolic studies, which should also be performed with IBN given systemically and by exposure to its vapors. These studies will establish the conditions under which various N-nitroso compounds and nonvolatile nitrite esters (which could be long-lasting indirect sources of N-nitroso compounds) are produced from IBN; determine the distribution and metabolism of IBN in the body, including its conversion to inorganic nitrite (which is also a vasodilator); determine whether tissue lipids can be peroxidized by

IBN; and test for DNA damage caused by IBN, including the induction of single-strand breaks and base alkylation (since these types of damage could result in tumor initiation).

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AUTHORS

Sidney S. Mirvish, Ph.D.
Martin D. Ramm, B.S.
David M. Babcook, B.S.

The Eppley Institute for Research
in Cancer and Allied Diseases
University of Nebraska Medical Center
42nd and Dewey Avenue
Omaha, NE 88105

Toxicity of Inhaled Isobutyl Nitrite in BALB/c Mice: Systemic and Immunotoxic Studies

Daniel M. Lewis and Dennis W. Lynch

INTRODUCTION

Initial epidemiologic studies of the outbreak of acquired immunodeficiency syndrome (AIDS) among homosexual men indicated that the use of organic inhalant nitrites (poppers) such as amyl nitrite and isobutyl nitrite (IBN) was a possible risk factor in the development of this disease (Centers for Disease Control (CDC), 1982). Although subsequent studies have shown that the human immunodeficiency virus (HIV) is the etiological agent of AIDS (Popovich et al. 1984), the possible role of inhalant nitrites as a cofactor in some of the illnesses associated with this syndrome has not been excluded.

The use of inhalant nitrites has been reported to be associated with altered ratios of T-helper and T-suppressor cells (Goedert et al. 1982), but other studies failed to confirm this observation (Kornfeld et al. 1982). *In vitro* studies have been reported that show that volatile nitrites can inhibit the responses of mononuclear cells (Hersh et al. 1983; Jacobs et al. 1983), but the *in vivo* effects of these compounds on the immune system have not yet been reported. The pharmacological and toxicological properties of these compounds have been reviewed (Haley 1980; Jackson 1979), and the reported effects include dizziness, headache, tachycardia, syncope, hypotension, increased intraocular pressure, and production of methemoglobinemia (Bruckner and Peterson 1977). In addition, volatile nitrites have been shown to be direct mutagens (Quinto 1980), and are highly flammable. Thus, it is apparent that these chemicals are hazardous, but their immunotoxic properties have not been fully evaluated.
The National Institute for Occupational Safety and Health (NIOSH), as one of the Centers for Disease Control, was requested by the CDC Task Force on AIDS Activity to perform an immunotoxicologic

evaluation of aliphatic nitrites utilizing the NIOSH High Risk Exposure Chambers. IBN was selected as the test chemical primarily because IBN could be obtained and maintained in a more chemically pure state than amyl nitrite. IBN is used as an inhalant, and all volatile nitrites are thought to have similar physiologic effects (Haley 1980). Mice were exposed to various concentrations of IBN and at selected time intervals removed from the chamber and tested for immunocompetency by a variety of techniques. Since there was a general lack of toxicologic data on organic nitrites and on IBN in particular, the subchronic inhalation toxicity of IBN was evaluated concurrently with the immunotoxicity assessment. Exposures were selected to mimic an occupational exposure setting in order to fill this gap in the existing literature, rather than to conduct brief, acute, high-level exposure to imitate exposure by nitrite abusers.

The results of that study are the subject of this report.

EXPERIMENTAL PROTOCOL AND METHODS

The exposure conditions and experimental protocol have been described in detail previously (Lynch et al. 1985; Lewis et al. 1985). Briefly, BALB/c mice were exposed to 300 ppm of IBN vapors in inhalation chambers for 6.5 hours/day, 5 days/week, for up to 18 weeks. The IBN chamber concentrations were monitored hourly using a Miran IA infrared analyzer (Foxboro-Wilkes, Norwalk, CT) and flow rates adjusted to maintain exposure concentrations at the target level. Individual body weights for exposed and control animals were recorded weekly, and all mice were observed twice daily for clinical signs, morbidity, and mortality. At selected time intervals, mice were randomly selected and removed from the exposure chambers and killed with an overdose of pentobarbital sodium. A blood sample was obtained for hematological testing, and the spleen, liver, and thymus removed and weighed. After weighing, the spleens were teased apart, filtered through an 80-mesh wire screen, and the cell suspension collected in Hanks balanced salts solution (HBSS). The cells were washed in HBSS, counted, and divided into three portions. One portion was used to enumerate antibody-producing cells (Dresser 1978) (a measure of B-cell function), one portion was used for the lymphocyte blast transformation assay (LBT) (Luster et al. 1981) (a measure of T-cell function), and the third portion was used to enumerate the T-cell subpopulations by immunofluorescence staining using monoclonal antibodies specific for mouse Thy-1.2, Lyt-1 , and Lyt-2 antigens.

A separate group of animals was used to evaluate cellular immune reactivity *in vivo* by means of a radiometric skin test procedure (Luster et al. 1981). Briefly, 10 IBN-exposed and 10 control animals were immunized wlth Freund's complete adjuvant, which contains mywbacteria, 21 days prior to sacrifice. The animals remained in the exposure chambers during this time. Two days prior to sacrifice they were injected with tritiated thymidine (1.0 uCi/g body weight). One day later the animals were skin tested by injection of 10µl of PPD antigen into the right ear and 10µl of saline into the left ear. Twenty-four hours after skin testing, the mice were killed, the ears excised, and the radioactivity of each ear determined. The ratio of iritated thymldine counts in the right and left ear was calculated to provide an index of antigen-induced inflammation.

RESULT AND DISCUSSION

Immunotoxic effects may be associated with a decline in the general health of an animal. For thii reason, the body weights of the mice were monitored weekly during the exposure period, and at termination of the experiment. No significant differences in weight gain were noted during the exposure period and, as shown in table 1, the terminal body weights were not significantly different. Also shown in table 1 are selected organ weights for mice exposed to 300 ppm of IBN for 13 weeks. The liver weights of exposed males and the thymus weights of exposed females were statistically significantly less than the controls, but these changes could not be related to any gross or histopathological damage in the organs. Histopathologic examinations were made of a number of tissues of these animals, and the only agent-dose-related change noted was focal hyperplasia of the bronchiolar epithelium.

Hematological evaluations of the mice included red cell counts (RBC), white cell counts (WBC), hemoglobin, and *methemoglobin* determinations. In table 2, the results of these analyses are shown. As expected, the exposure to IBN did induce methemoglobin formation. The exposed males also showed lower WBC values than the controls, but these changes were not related to any observed changes in the bone marrow cytology. Depressed WBC counts were not seen in mice exposed to lower concentrations of IBN, nor in the females exposed to 300 ppm; the significance of this observation is unclear.

Several assays were performed to evaluate the immune system of the exposed animals. To evaluate the antibody-producing capabilities of

TABLE1. *Organ and body weights in mice exposed to 300 ppm of IBN for 13 weeks*

Exposure Group	Body Wt. (g)	Liver (g)	Spleen (mg)	Thymus (mg)
Males				
Exposed	31.4	1.94*	197.6	38.9
Control	31.7	2.14	199.5	40.1
Females				
Exposed	25.4	1.53	231.3	33.0*
Control	24.9	1.56	186.3	45.2

*Statistically significant differnece versus controls, p<.05

NOTE: There was 15 mice/sex/groups.

TABLE 2. *Heamtologic data from mice exposed to 300 ppm IBN for 13 weeks*

Exposure Group (millions/ml^3)	RBC (millions/ml^3)	WBC (thousands/ml^3)	Hemoglbin (g/dl)	Methemoglbin (numbe pos/ number tested)
Males				
Exposed	9.5±0.8	2.4±0.8*	15.5±1.3	5/5
Control	9.0±1.1	6.4±1.9	16.0±0.5	0/5
Females				
Exposed	9.0±0.9	5.4±1.7	15.9±0.4	3/5
Control	9.6±1.1	5.4±3.0	16.3±1.1	0/5

*Statisticaly significant from control, P<.05.

the mice, selected animals were immunized with sheep red blood cells, and plaque assays for antibody-producing spleen cells were performed. It should be noted that the mice continued to be exposed to IBN between the time they were immunized and the time the assay was performed (4 days). As shown in table 3, the female mice exposed to 300 ppm IBN for 18 weeks had significantly higher numbers of antibody-producing cells than did the controls. At no other time period or dose level were there any statistically significant

differences Between exposed and control animals. These results do demonstrate that mice can develop an antibody response (humoral response) while being exposed to IBN.

TABLE 3. *Enumeration of antibody-poducing spleen cells in mice exposed to 300 ppm IBN for 18 weeks*

Animals	Number	Plaque Counts (Mean number of Plaque-Forming Cells per 10° Spleen Cells) Exposed	Control	Exposed/Control x 100
Males	5	206±63	199±57	104
Females	5	334±73	129±35	259*
Total	1 0	270±93	164±58	165

*Statisticaly significant from control, P<.01.

To evaluate the cellular immune response, mice were immunized and tested for a delayed hypersensitivity response using a radiometric skin test assay. Thii assay was only performed on animals that had been exposed to 50 ppm IBN for 7 or 13 weeks.

Positive skin test responses were obtained with both the IBN-exposed and control animals (table 4). The exposed animals had slightly higher mean values, but the differences were not statistically significant. The results demonstrate that the animals can develop a cellular immune response while being exposed to IBN.

The cellular immune responses were further evaluated using an in vitro assay. The LBT assay, which measures the proliferative response of splenic lymphocytes to known mitogens, was performed; results are shown in table 5. Each mitogen was tested at several concentrations; the results shown are for the optimal concentration of each mitogen. Only animals exposed to 300 ppm IBN for 18 weeks showed any significant differences in the LBT assay, and, at that time point and for each mitogen, the exposed animals were consistently more responsive than the controls. lt is of interest to note that in the control cultures, i.e., those cultures that received no mitogen and measured the background level of proliferation by the splenic lymphocytes, the IBN-exposed animals had significantly higher levels of thymidine incorporation. lt has been shown that

TABLE 4. *Radiometric determination of the skin test sensitivity of mice exposed to 50 ppm IBN for 7 or 13 weeks*

Ratio of Tritiated Thymidine per mg Tissue of Antigen-Injected Ear/Saline-Injected Ear. Mean ± SE

Treatment	Number	Weeks of Exposure 7	13
Exposed	10	2.98±0.51	2.49±0.69
Controls	10	1.77±0.15	2.19±0.39

TABLE 5. *Results of the LBT assay performed on mice exposed to 300 ppm IBN for 18 weeks*

Mitogen	Number	Exposed	Controls
Phytohemagglutinin	10	67815 ± 9477*	40288 ± 6177
Concanavalin A	10	64096 ± 12948	43628 ± 6811
Pokeweed Mitogen	10	9570 ± 1885*	2887 ± 536
Lipopolysaccharide	10	15724 ± 2312*	5718 ± 884
Control (no mitogen)	10	4596 ± 998*	1422 ± 166

*Statistically significantly difference from control p<.01

NOTE: Results expressed as the mean + SE of cpm of trithted thymidine incorporated for the group. Each animal was tested in replicates of four and the mean value for each animal averaged to give the group moan.

organic nitrites can stimulate guanylate cyclase and increase cyclic guanosine monophosphate (cGMP) levels (Ignarro et al. 1981). An increase in cGMP levels occurs during the initiation of a proliferative response by lymphocytes (Hadden and Coffey 1982). Thus, it is possible that the chronic exposure to IBN may have altered the responsiveness of the lymphocytes to the mitogens or caused the increased background proliferative response seen by affecting the cGMP metabolism. The biological significance of an enhanced LBT response is unclear. Additional studies are needed to determine if this response correlates with resistance to infection, incidence of autoimmune phenomena, or possibly an increased incidence of tumors.

One of the first immunologic abnormalities recognized in AIDS patients was an alteration in ratio of Tcell subpopulations, i.e., an inversion of the ratio of T-helper to T-suppressor cells. For this reason, we determined the percentage of spleen cells expressing either Thy 1.2, Lyt-1, or Lyt-2 surface antigens. The Thy-l antigen is common to all mature T-cells in the mouse. The Lyt-2 antigen is analogous to the CD-8 antigen in man (the suppressor/cytotoxic T-cell subset), and the Lyt-1 antigen is found predominantly on the helper/inducer subset of T-cells in mice but is expressed at low levels on all peripheral T-cells (Ledbetter et al. 1961). Using a fluorescent microscope, we could not accurately enumerate the helper subpopulation. Thus, the ratio of helper to suppressor cells could not be calculated. As shown in table 6, there was no difference in percentages of cells expressing these markers in the IBN-exposed animals. The ratio of Lyt-2 to Thy-l cells was not different, which indirectly indicates that there was no depletion of the helper cell subpopulation.

TABLE 6, *Relative number of spleen cells expressing Thy-l.2 Lyt-1 or Lyt-2 antigens in mice exposed to 300 ppm IBN for 18 weeks*

Treatment Group	(Mean ± SD) Percentae of Cells Expressing Thy 1.2	Lyt-1	Lyt-2	Ratio of Lyt-2/Thy-1.2
Exposed (10)	31.4±3.6[2]	34.2±5.1	19.8±2.8	0.634±0.085
Control (10)	35.5±3.5	38.6±5.1	21.8±3.1	0.616±0.092

CONCLUSION

The results obtained in this study indicate that, at the levels tested, IBN had no significant detrimental effect on the immune system of mice. The toxic effects noted included methemoglobinemia and focal hyperplasia of the bronchiolar epithelium. Thus, while IBN may not be an immunotoxicant, it was not totally innocuous in this study. Numerous other toxic effects of this chemical have been reported by others (Haley 1960; Jackson 1979; Brudner and Peterson 1977), and it should be considered a hazardous substance. Finally, inhalant nitrites may not be responsible for the basic immune defects characteristic of AIDS, but their role as a cofactor in some of the illnesses associated with this syndrome cannot be ruled out.

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AUTHORS

Daniel M. Lewis, Ph.D.
Division of Respiratory Disease studies
National Institute for Occupational Safety and Health
944 Chestnut Ridge Road
Morgantown, WV 26505

Dennis W. Lynch, M.S.
Division of Biomedical and Behavioral Studies
National Institute of Occupational Safety and Health
Robert A. Taft Laboratories
4676 Columbia Parkway
Cincinnati, OH 45226

Altered T-Cell Helper/Suppressor Ratio in Mice Chronically Exposed to Amyl Nitrite

Jesse. Oritz and Vilma L. Rivera

INTRODUCTION

Amyl nitrite (AN) and other volatile nitrites have become popular over-the-counter drugs that are inhaled to alter consciousness, enhance meditation, stimulate dancing, and intensify sexual experience.

The drug was first used in 1867 as a treatment for patients suffering from anginal pain (Sigell et al. 1978). When the Food and Drug Administration designated AN a prescription drug in 1968, butyl and isobutyl nitrite (IBN) were used as substitutes on the street (Cohen 1981). Presently, these aliphatic nitrous acid esters are marketed in small vials (10 to 30 ml) or in small, thin glass capsules (0.18 to 0.30 ml) covered by cloth webbing that may be crushed by the fingers prior to inhalation. These are usually referred to as "poppers." In the United States, approximately $5x10^7$ to $15x10^7$ ml per year of AN are used (Haley 1980; Nickerson et al. 1979).

Dewey et al. (1973) examined the behavioral and toxicological effects of AN in mice and dogs. When intravenous (IV) and intraperitoneal (IP) routes of administration were employed, the medial lethal dose (LDs_{50}) and its 95 percent confidence index for AN in mice was found to be 130 mg/kg by the IP route and 51 mg/kg by the IV route. When 22 vials 300µl/vial) of AN were administered to dogs by inhalation every 30 seconds, the animals exhibited Irritability, tremors, ataxia, and defecation.

The Centers for Disease Control (CDC) (1983) reported that IBN had no immunotoxic effect on mice exposed to vapors at concentrations of 20, 50, and 300 ppm for up to 18 weeks.

Other investigators have shown that inhalation of AN also relaxes most smooth muscle structures, e.g., bronchial, gastrointestinal, rectum proper, and internal anal sphincter muscles (Sigell et al. 1978; Haley 1980; Nickerson et al. 1979). In humans, AN may be dangerous to those who have had cerebral hemorrhage, hypotension, glaucoma, and myocardial disease (except patients with artery spasm) due to its hypotensive and tachycardiac effects (Jorgensen and Lawesson 1982).

Recent reports suggest that AN may be a contributing cofactor to AIDS in the homosexual population (Jorgensen and Lawesson 1982; Goedert et al. 1982; Digiovana and Safai 1981). Further, Goedert et al. (1982) have suggested that use of nitrites may play a role in the imbalance of T-cell ratios among the healthy homosexual population.

While Goedert's hypothesis could not be substantiated by other workers (Durack 1981; Jaffe et al. 1983), in vitro studies using IBN have demonstrated impaired lymphocyte and monocyte function at 1 percent concentration but not at 0.5 percent (Hersh and Reuben 1983; Mayer 1964; Newel et al. 1984). The results demonstrated that these effects were irreversible after 24 hours of exposure. At lower concentrations the drug inhibited lymphocyte blastogenesis, natural killer cell activity, antibody-dependent lymphocyte-mediated cytotoxicity, *in vitro* cell adherence, and transformation of monocytes to macrophages. These agents also inhibited leucine, uridine, and thymine incorporation and inhibition of interferon production by fibroblasts. These inhibitory effects were detectable even at 0.01 percent concentrations. Further, Osterloh and Goldfield (1984) showed that wmmon "street" preparations were effective nitrosating agents at neutral pH. In a recent report presented in Atlanta at the CDC International Conference on AIDS, Haverkos et al. (1985) studied a group of AIDS patients and by multivariance analysis showed that inhalation of nitrites was the most common factor in the genesis of Kaposi's sarcoma, a skin cancer commonly associated with AIDS.

In view of these reports, there is an ongoing reevaluation on the effects of alkyl nitrites and their relationship to opportunistic infections and Kaposi's sarcoma in AIDS patients. As part of this reevaluation, this project studied the *in vivo* effects of AN on T-cell function.

METHOSDS AND MATERIALS

After a 5-day acclimation period, 4-week-old male Charles River Labs CD-1 mice, weighing from 16 to 22 g, were placed in stainless steel cages in a light and dark temperature controlled room. Paired mice were then fed Purina Rodent Chow and distilled water ad *libitum.* The mice were then exposed to distilled AN via inhalation 5 days a week for 21 weeks. Preliminary exposure showed that one 10µl dose of AN was lethal to mice. For this reason, animals were slowly acclimated to the substance by increasing doses as shown in the protocol. The control group was given the same amount of saline solution (0.85 percent NaC). To insure inhalation, the AN or saline was introduced intranasally to a single group of 50 mice using a 10 l micropipette. Mice were exposed as follows:

Exposure Time (Weeks)	Time Since First Exposure (Weeks)	Amount AN Given per Day (µl)	Cumulative Amount Given (µl)
2.6	2.6	2.0	26
2.6	5.2	3.0	65
2.8	8.0	5.0	135
5.0	*13.0	10.0	385
2.6	15.6	0.0 (rest)	--
5.4	**21.0	10.0	655

*Listeria challenge

**T-cell counts

Following exposure, mice were weighed and randomly assigned to group I or group II.

Group I: Listeria monocytogenes (LM) Challenge

A group of 20 mice (10 exposed and 10 control) was randomly selected after a 13-week exposure time and challenged intranasally with 50 µl of an ID_{50} dose of the facultative intracellular pathogenic bacterium LM, containing $2.8x10^6$ organisms per ml. These mice were monitored for mortality death up to 14 days.

Group II: T-Cell Depletion

T-cell depletion was assessed with the remaining group of mice (14 control and 11 exposed) after a 21 -week exposure time. The monoclonal antibodies assay was used to measure different T-cell

subpopulations. Total T cells (Thy-l), helper T cells (Lyt-2 negative), and killer T cells (Lyt-2 positive) were measured using peripheral blood. The bound target antigens were revealed with a fluorescein-labeled goat antirat immunoglobulin G (IgG) (no cross-reactivity with mouse IgG) (Cappel Labs).

Blood was obtained by cardiac puncture on lightly anesthetized mice using a 23-gauge needle attached to a l-ml syringe containing 100 units of heparin. A semi-microtechnique was used to isolate the peripheral blood lymphocytes (Ledbetter and Hersenbrg 1079). Heparnized blood was fractionated by the Ficol-Hypaque gradient centrifugation technique. The washed lymphocytes were Incubated for 45 minutes with the corresponding antibodies. Excess antibodies were washed off and the pelleted cells resuspended with the corresponding flourescien antibodies for 45 minutes. The reaction was stopped by the addition of 2 percent paraformaldehyde and centri-fuged, and the sediment was resuspended in 1 to 2 drops of phosphate-buffered saline (PBS) containing 30 percent v/v glycerol. These cells were observed under the fluorescent microscope.

The mice were then killed and the spleen, kidneys, lungs, and livers collected. The organs were then washed with sterilized PBS, dried, and weighed. Anatomical observations were made for gross pathology. The organs were preserved in Formalin (10 percent) for further studies.

STATISTICAL ANALYSIS

Student's t test was used to test the null hypothesis that exposure of mice to sublethal doses of AN does not affect the following parameters: pathotoxicology (repeated measurement of weight, weight gain, and organ/body weight ratio and cellular Immunity); and T-cell differential count.

In addition, regression analysis was used to measure the relationship between T-cell ratio and the independent variables of body weight and weight gain in group 2.

RESULTS

Host Resistance to LM

Lewis et al. (1983) demonstrated that there were no effects after exposing mice to IBN vapors for 13 weeks. Since our animals were

exposed to IBN by the intranasal route (a slightly different method), we decided to check our experimental animals for signs of immune imbalance after 13 weeks by challenging them to an LD_{50} of LM. Results shown in figure 1 indicate 20 percent of the exposed mice and 40 percent of the controls survived this challenge; however, no significant statistical differences ($p>0.05$) were observed in the survival time of the two groups.

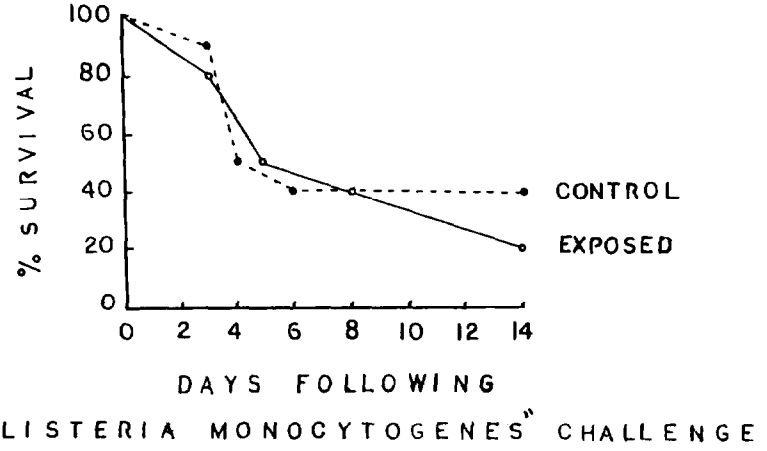

FIGURE 1. *Days following LM challenge*

NOTE: Effect of AN on mice survival following intranasal challenge with LM (pathogenic). Significance was determined by Fisher's exact test: $p>0.05$.

T-Cell Depletion

Results of T-cell differential counts are summarized in table 1. Further, relationships between T-cell helper/suppressor ratio (T-cell H/S ratio), body weight, and weight gain were analyzed (see table 2).

As seen in table 1, the total T cell and suppressor (Lyt-2 positive) cells did not differ significantly between the two groups ($p<0.1578$ and $p>0.4387$, respectively). However, a significant decrease was observed in helper (Lyt-2 negative) cells ($p<0.0037$). As a result, there was a significant shift in the helper to suppressor ratio between the two groups.

Furthermore, a significant degree of correlation ($p<0.01$) was found between T-cell H/S ratio and the independent variables of body weight and weight gain in the exposed group (table 3).

TABLE 1. *Evaluation of T-cell different count folbwing AN exposure (group 2)*

Number	Total T-Cell % from T-Lymphocyte (Mean ± SE)	Lyt-2 Negative *(Mean ± SE)	Lyt-2 positive (Mean ± SE)	Lyt-2 Negative Lyt-2 Positive *(Mean ± SE)
Control (14)	41.22 (±2.89)	37.74 (±3.81)	24.60 (±1.96)	1.53 (±0.24)
Exposed (11)	35.55 (±2.37)	20.88 (±3.44)	27.99 (±4.18)	0.75 (±0.10)
p (value)	=>0.1578	=<0.0037	=>0.4387	=<0.005

*Student's t test was used to mean values. Level of significance p<0.01.

Pathoxicologic Parameter

Mouse body weight and rate of weight gain after termination of each exposure time are represented as means of each group (tables 2 and 4). A decrease In mean body weight was found after an accumulative exposure time of 8 weeks (data not shown) and continued until the end of the experiment (see table 2). After 21 weeks of exposure time, both body weight and weight gain rate were significantly decreased $p<0.02$; $p<0.05$, respectively). When the organ to body weight ratio was evaluated (table 5), no significant differences were found between the two groups. However, examination of lungs,

TABLE 2. *Mouse body weight of group 2 following AN exposure (T-cell depletion)*

	Control (±SE) (n=14)	Exposed (±SE) (n=11)	Level of Significance*
Body Weight Mean (g)	37.62 (1.06)	34.48 (1.12)	$p<0.02$
Weight Gain Mean (g)	18.37 (0.86)	Text (1.11)	$p<0.05$

*Student's t test was used to compare mean values. A value of $p<0.05$ was considered stastically significantly. Total AN=655µl.

spleen, liver, and kidneys of mice exposed to AN showed extensive damage to the lungs (table 6), which was statistically significant ($p<0.01$). Lung damage consisted of (1) hemorrhagic spots; (2) petechiae throughout the lobes; (3) collapsed consolidated sections, which were red and showed the appearance of emphysema; and (4) air pockets with large air bubbles. These gross pathological observations were made in 10 of 11 mice. Spleens in 3 of 11 mice in the exposed group were edematous and showed dark hemorrhagic spots. In the control group, the lungs were normal in 11 mice and edematous in 3. The spleens were normal in 11 mice and edematous without hemorrhagic spots in 3 of the controls; and kidneys and livers appeared normal in both groups.

TABLE 3. *Relationships between T-cell H/S ratio, body weight, and weight gain in mice following AN exposure*

Group Studied (n)	Correlation Coefficient (r)*	
	T-cell H/S Ratio:Body Weight	T-Cell H/S Ratio:Weight Gain
Control (14)	-0.471 ($p>0.05$)	-0.308 ($p>0.05$)
Exposed (11)	0.867 ($p<0.01$)	0.792 ($p<0.01$)

*Regression analysis was used to compare the relationships between the variables mentioned above. A value of $p<0.05$ was considered to be statistically correlated.

DISCUSSION

In summary, the results presented here demonstrate that exposure to sublethal doses of AN can cause a significant decrease in helper cells, thus resulting in an inverted T-cell H/S ratio. Mean body weight decreased after 8 weeks and became statistically significant by the end of 21 weeks of exposure. The cellular immunity parameter (T-cell H/S ratio) showed a statistically significant correlation coeffi cient between T-cell H/S ratio and the independent variables of body weight and weight-gain rate. Thus, as T-cell H/S ratio decreased, weight gain also decreased. The gross pathological observation of selected organs also showed statistically significant damage to the lungs in the exposed group.

The fact that no correlations between T-cell H/S ratio and the independent variables were detected in the control group indicates that the positive correlation detected in the experimental group may be a reflection of AN's toxic effect.

Another parameter tested was the resistance of exposed mica to pathogenic intracellular LM, used to assess the competence of T-lymphocytes and macrophages. Peak resistance was reached on day 6 for the control group (40 percent survival) but was delayed until day 14 (20 percent survival) in the exposed group.

TABLE 4. *Mouse body weight and weight gain following AN exposure and challenge with pathogenic LM*

	Control (±SE) (n=10)	Exposed (±SE) (n=10)	Level of Significance*
Body Weight Mean (g)	36.1 (0.84)	33.4 (0.82)	$p<0.05$
Weight Gain Mean (g)	12.1 (1.07)	13.06 (0.78)	$p<0.25$

*Student's t test was used to compare mean values. A value of $p<0.05$ was considered statistically significant. Total AN=265 µl.

TABLE 5. *Relative* and absolute** organ weights of group 2 following AN exposure*

Group Studied (n)	Organ Weight	Spleen Mean*** (±SE)	Lung Mean*** (±SE)	Liver Mean*** (±SE)
Control (14)	Relative	0.227±0.083	0.584±0.024	6.47±0.247
	Absolute	0.089±0.037	0.229±0.089	2.55±0.105
Exposed (11)	Relative	0.235±0.010	0.623±0.023	6.14±0.118
	Absolute	0.086±0.005	0.227±0.081	2.21±0.061

*Relative=orgsn weight/body weight.

**Absolute=organ weight.

***Studant's t test was used to compare the mean value. A value of $p>0.05$ was not considered statistically significant.

TABLE 6. *Pathological (gross) obsetvations of the lungs following AN exposure (group 2)*

	Lungs	
	Normal	Abnormal
Control (n=14)	11	3
Exposed (n=11)	1	10
Total	12	13

NOTE: Level of significance: $p<0.01$.

These results could be misleading since challenge to LM was done after only 13 weeks of exposure time. Other workers have not shown any effects after exposing mice for 13 weeks. The mice received a total of only 385 I of AN, which is apparently not sufficient to alter survival in vivo, although it might be extremely toxic in vitro. Had this parameter been tested at 21 weeks, there could have been a statistically significant difference.

Inhalation of AN produces effects similar to nitrous acid and N-nitroso compounds. Several studies (Maigetter et al. 1978; Gardner et al. 1977; Fenter et al. 1973; Holt et al. 1979) have reported depression of cellular immunity of various animal species following NO_2 exposure. Holt et al. (1979) exposed BALB/c mice to 10-ppm NO_2 for 30 weeks, and examination of lung pathology showed peripheral air spaces, emphysematous zones, large air spaces in the peripheral lung, and diffuse interstitial pneumonia. Similar gross appearances of the lungs, e.g., hemorrhagic areas, have been reported in humans following exposure to large concentrations of NO_2 (Parks 1974). Other studies have shown that NO_2 exposure increases protein content and glucose metabolism in lung tissue. Cytosolic enzyme activities such as glucose-6-phosphate dehydrogenase, glutathione peroxidase and reductase, and disulfite reductase are also increased in the lung. Experiments with rats exposed to 5, 7, or 10 ppm NO_2 resulted in an increase in glucose consumption via the Hexose

Monophosphate Shunt, increasing production of lactate and pyruvate (Mustafa et al. 1980).

Haley's (1980) review of the physiological effects of AN inhalation mentions that subcutaneous administration of AN in rabbits produced diuresis, albuminuria, and glycosuria. He also reports that after AN inhalation, glucose and lactic acid were detected in the urine. Thus, exposure of mice to AN could have produced the same physiological efects as NO_2 exposure and, as a consequence, the exposed group may have had an increased lipid peroxidation and glucose consumption followed by increased excretion of glucose compared to the control group, leading to lower body weight and lower weight gain. It has also been shown that these aikyl compounds can be nitrosating (Osterloh and Goldfield 1984), thus posing a further hazard in their potential carcinogenic effect. Although this effect has been shown in vitro, there are currently no data on the amounts of nitrosamines formed in vivo in humans. However, this danger should not be ignored.

The work presented here disagrees with the findings reported by the Centers for Disease Control (1983), which attributes differences to the methodologies and substances used. CDC researchers exposed their animals to vapors of IBN for 18 weeks; whereas the present study administered AN intranasally for 21 weeks. It is possible that immunotoxic effects do not reveal themselves until certain concentrations and exposure times have elapsed. Prior to the start of our experiments, we exposed a few animals to 5, 10, and 15 µl of AN. Those animals receiving 10 and 15 µl of AN for the first time died immediately after exposure. Animals receiving 5 µl became very ill and one died within 24 hours; the rest survived. For this reason, we administered AN in small doses and slowly increased the amount given. Eventually the animals were able to tolerate 10 µl of AN without any noticeable ill effects. Apparently animals do develop tolerance to AN; however, felt that a maximum of 10 µl /day was a reasonable sublethal dose.

Several reports in the medical literature suggest that AN might be an inducer of Kaposi's sarwma (Jorgensen and Lawesson 1982, Goedert et al. 1982; Digiovana and Safai 1981; Haverkos et al. 1985) other researchers refute the argument (Durack 1981; Jaffe et al. 1988). More recent in vitro studies using IBN show this agent to be immunotoxic (Hersh and Reuben 1983; Mayer 1984; Newell et al. 1984) at very low concentrations. In addition, CDC's latest epidemiological report demonstrates a possible link between nitrites (poppers) and

Kaposi's sarcoma. A survey of 87 AIDS victims found that significantly more sufferers of Kaposi's sarwma reported heavy use of poppers than did sufferers *of Pneumocystic carinii* pneumonia.

CONCLUSION

The studies presented here show that chronic inhalation of AN can lead to a decrease in helper cells, thus altering the T-cell H/S ratio, which is the same phenomenon that occurs in AIDS victims. This suggests a link between AN inhalation and cellular immunity depression.

In view of the results reported here, there is an urgent need to confirm these findings and to perform microscopic anatomy studies of lung tissue and possibly other organs to elucidate the pathologic effect of AN. In addition, challenge studies with pathogenic and nonpathogenic organisms should be conducted after long-term exposure, along with humoral immunity studies.

Since the termination of this study, various reports in the literature have shown that a more appropriate reagent for measuring helper T-cell populations would be the L3T4 antigen (Swain et al. 1984). For this reason we recommend that these results be confirmed using the more recently discovered antigens. We further recommend that persons prone to using alkyl nitrites should consider the possible adverse health effects that could emanate from inhaling these substances and thus should refrain from using them.

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AUTHORS

Jesse S. Ortiz, Dr.P.H.
Division of Public Health
University of Massachusetts
Amherst, MA 01003

Vilma L. Rivera, M.S.
Associate Hazardous Material
Specialist
State of California
Department of Health Services
Toxic Substances Control Division
107 South Broadway, Room 7011
Los Angeles, CA 90012

Effects of Nitrites on the Immune System of Humans

Elizabeth M. Dax, James E. Nagel, W. Robert Lange, William H. Adler, and Jerome H. Jaffe

INTRODUCTION

Kaposi's sarcoma (KS) is a disease manifestation of unexpectedly high occurrence in homosexual men with acquired immunodeficiency syndrome (AIDS) and has been recognized as such since 1981 (Centers for Disease Control 1981). In other groups of people with AIDS, the occurrence of KS is less frequent (3 to 4 percent vs. approximately 14 percent in the homosexual group) (Centers for Disease Control 1986). Epidemiological evidence indicates that factors associated with the presence of KS in patients with AIDS include sexual activity (Jaffe et al. 1983). drug usage, particularly the use of volatile nitrites (Haverkos et al. 1985), and a history of sexually transmitted diseases and possibly their therapies (Marmor et al. 1982). The association between nitrite abuse and the development of KS has been demonstrated in some studies (Marmor et al. 1982; Haverkos et al. 1985) but has not been supported in the studies of Polk et al. (1987). In some samples the use of volatile nitrites by homosexual men approaches 100 percent (Israelstam et al. 1978; Marmor et al. 1982). Conversely, it has been reported that few heterosexual patients with AIDS use nitrites (Guinan et al. 1984). Thus, the question arises whether the nitrites are surrogate markers of other factors involved in the development of KS, or whether they, themselves, are cofactors in the development of this disease in AIDS patients. It has been suggested that nitrites may be involved in the development of KS by intrinsic immunosuppressive properties.

There are several possible explanations of the nitrites' ability to act as cofactors in the development of KS in AIDS patients. Most important, there is evidence that nitrites may compromise the immune

system. Goedert et al. (1982) found low helper:suppressor T-lymphocyte ratios in eight volunteer, homosexual men who regularly used nitrite compared with seven volunteers who were not regular users. The altered T-lymphocyte ratios were due to decreases in helper cells as well as increases in suppressor cells. Cytomegalovirus (CMV) antibody titers were high in 14 of the 15 volunteers but were not related to either nitrite abuse or T-cell abnormalities. The authors concluded that “nitrites may be immunosuppressive in the setting of repeated viral antigenic stimulation, and may contribute to the high frequency of KS and opportunistic infections in homosexual men” (Goedert et al. 1982, p. 412). In another study, Hersh et al. (1963) examined the effects of nitrites administered in vitro on parameters of lymphocyte function. A dose response inhibition of in vitro blastogenic response to mitogens, natural killer cell activity, antibody-mediated cellular toxicity, and adherence and transformation of monocytes to macrophages was observed. Again, the authors suggested that nitrites have an immunosuppressive effect on mono-nuclear leucocyte function. Animal studies support the proposal that nitrites are immunosuppressive (Lewis and Ortiz, this volume). To date, immunological function following inhalation of nitrites has not Been reported. This paper summarizes preliminary results of such a study.

MATERIALS AND METHODS

Eight HIV-negative male volunteers gave informed consent to participate in this study. They were in residence on the Addiction Research Center ward. During the first week of the study two Baseline immunologicol test batteries were run. Over 4 days of the second week, each volunteer participated in 13 inhalation sessions (0.18,0.3, and 0.46 ml amyl nitrite each three times, and four placebo doses). The placebo, banana oil, was included in each inhalation session with or wlthout amyl nitrite. The drugs were inhaled from a 4-liter flask connected to a collapsible breathing bag which contained a known volume of air. Another inflow valve was opened when the bag was fully collapsed in order to ensure full inhalation of the nitrite dose. Blood was drawn for immunological tests immediately following the last inhalation session, then at 24 hours, 96 hours, and 7 days.

Tests of immune status included white cell counts, absolute lymphocyte numbers, lymphocyte subset representation, in vitro lymphocyte responses to the mitogens Phytohemaglutanin (PHA), Concanavalin A (ConA) and pokeweed mitogen (PWM), natural killer

cell activity, and *in vitro* polyclonal induction of immunoglobulin G (IgG) and immunoglobulin M (IgM) synthesis.

The determination of lymphocyte subsets utilized monoclonal antibodies against membrane markers. The OKT series of antibodies were obtained from Ortho Diagnostics, and the Leu series were obtained from Becton Dickinson. The stained cell preparations were analyzed using an Ortho 50H Cytofluorograph (Nagel et al. 1981 b). The *in vitro* responses to mitogens were determined by culturing 5 x 10^4 lymphocytes in 0.2 ml volumes in indivldual well microculture plates. The cells were grown in RPMI 1640 (Grand Island, NY) supplemented with 10 percent fetal calf serum with 100 ug penicillin and 100 ug streptomycin per ml. Cultures were maintained for 72 hours with 1 Ci 32' H-Trd present for the last 18 hours. Results are presented as the mean cpm for three replicative samples. Natural killer cell activity was determined using Cr^{51} labeled K562 tumor cells as targets. Peripheral blood lymphocytes were diluted to provide effector to target (E:T) ratios of 100:1 to 0.78:1. The resulting cytotoxicity curves were examined for linearity. Results are expressed as specific cytotoxicity for the 12.51 E:T ratio previously described (Nagel et al. 1981 c). *In vitro* immunoglobulin production was analyzed using cultures of peripheral blood lymphocytes in the complete media described above. PWM was used as a polyclonal activator of immunoglobulin production (Nagel et al. 1981 a). The quantitation of IgM and IgG content in culture supermates was accomplished using an ELISA technique with specific enzyme-linked anti-immunoglobulin reagents. Results *are expressed as* the peak amount of IgM and IgG in different cultures stimulated with varying doses of PWM.

RESULTS

The T-lymphocyte line associated with cell-mediated immunity carry surface antigens recognized by the CD_3, CD_4, and CD_8 antibodies for the immature, T4 helper, and T8 suppressor cells, respectively. The B-cell line lymphocytes, which produce immunoglobulins, are identified with the Leu-12 antibody.

The representation of T-lymphocyte subsets as the percentage of total lymphocytes was unchanged after amyl nitrite inhalation. Therefore, there were only minor variations in the lymphocyte helper/suppressor ratio (T4/T8) following inhalation of amyl nitrite. However, the ab solute number of peripheral blood lymphocytes decreased significantly following the inhalation protocol, then returned to baseline levels

after 24 hours, and increased over baseline levels at day 7. The absolute numbers of CD3+ and CD8+ lymphocytes were significantly decreased immediately following inhalation. Natural killer ceils (leu-7+11) doubled in percentage representation between baseline levels and the second day post-inhalation while the level of natural killer cell activity showed an initial significant decrease, then returned to baseline levels by day 4 post-inhalation. The percentage representation of leu-12+B -lymphocyte showed an increase from 12.6 to 17.4 percent between day 4 and day 7, which corresponded to an increase in the *in vitro* PWM-induced immunoglobulin synthesis. The response to the T cell mitogens Con A and PHA was significantly elevated on day 7 following exposure to nitrite. The results showed a nitrite-induced lymphopenia resulting from relatively short exposure. Further, a nonspecific, 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 responses to pathogens.

CONCLUSIONS

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.

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AUTHORS

Elizabeth M. Dax, M.D., Ph.D.
W. Robert Lange, M.D., M.P.H.
Jerome H. Jaffe, M.D.

National institute on Drug Abuse
Addiction Research Center
P.O. Box 5180
Baltimore, MD 21224

James E. Nagel, M.D.
William H. Adler, M.D.

National Institute on Aging
Gerontology Research Center
Francis Scott Key Medical Center
4940 Eastern Avenue
Baltimore, MD 21224

Deliberate Inhalation of Isobutyl Nitrite During Adolescence: A Descriptive Study

Richard H. Schwartz

INTRODUCTION

In the past 10 years, isobutyl nitrite has become popular as a means of getting "high" among heterosexual adolescents, particulariy those who abuse other "gateway" drugs. Several surveys of adolescents and young adults reflect butyl nitrite use in the United States. In 1966 approximately 9 percent of 3,000 American high school seniors surveyed by a standardized indepth questionnaire on drug use patterns stated that they had deliberately inhaled volatile nitrites at least one time in order to self-intoxicate ("get high") (Johnston et al. 1967). In the same study, 1.6 percent of the survey respondents had used nitrite inhalants within the previous 30-day period, and 0.5 percent of the respondents used the drug daily. Annual use of isobutyl nitrite increased from the previous 3-year period. In 1983, 3.6 percent of high school seniors inhaled isobutyl nitrite, while 4.7 percent did so in 1966. The National Institute on Drug Abuse (NIDA) Household Survey of children 12 to 17 years old is in dose accord with the high school senior survey: 9 percent of the 12- to 17-year-old respondents admitted to lifetime use of isobutyl nitrite, and 4 percent were current users (National Institute on Drug Abuse 1986). In New York State, 6 percent of 1.3 million 7th to 12th grade students surveyed inhaled isobutyl nitrite during the 1983 school year, 3 percent were current users, and 1 percent, or 9,000 students, did so at least 10 times in the 30-day period prior to the large statewide survey on drug use frequencies and patterns (New York State Division of Substance Abuse Services 1984).

Isobutyl nitrite, a licit chemical sold allegedly as a room odorizer in some pornography, record, and tape shops, and by mail order from magazine advertisements, is a popular drug of abuse among American teenagers. The drug is chemically similar to amyl nitrite, an inhaled volatile liquid that is used in the emergency treatment of cyanide

poisoning. It has a rather unpleasant chemical odor, which is said to resemble sweaty socks or the smell of a men's locker room.

Isobutyl nitrite is sold legally under various trade names. Among the more common are Rush, Locker Room, Bullet, Quick Silver, Thrust, Lightning Bolt, and Hardware, many of which have sexual connotations. It is estimated that 12 million bottles of the products were sold between 1978 and 1983 (Freezer 1978). The most common sources of these products are underground outlets such as "head shops" and pornography shops.

Labels on some vials of isobutyl nitrite bear clear printed warnings to "avoid inhalation" of the vapors One label even reads "Danger: Excessive Use May Cause Euphoria."

The immediate effect of inhaling isobutyl nitrite is a sensation of warmth and a lightheaded feeling that adolescents call a "head rush." This phenomenon, which is due to profound vasodilation, lasts only a few minutes. Common physiological effects include pounding headaches, dizziness, blurred vision, and pressure in the eyes. Postural hypotension leading to loss of consciousness can easily occur if the drug is inhaled while standing. Prolonged exposure to the vapors can cause irritation to the eyes and the tracheobronchial tree (Covalla et al. 1981). If isobutyl nitrite is accidentally spilled around the nose during inhalation, it can cause dermatitis and pronounced yellow crusting on the upper lip or in the nasal passages (Fisher et al. 1981). In some individuals, the consequences of using this product may be much more severe: some methemoglobin-reductase enzyme-deficient persons develop severe methemoglobinemia or Heinz body hemolytic anemia after sniffing isobutyl nitrite (Horne et al. 1979; Bogart et al. 1988; Dixon et al. 1981).

PATIENT POPULATION AND STUDY DESIGN

We recently had an opportunity to evaluate the extent and consequences of the use of isobutyl nitrite among a select group of youth. The 173 persons in our study were patients in a drug treatment facility which is a long-term therapeutic community for chemically dependent adolescents. These young people were predominantly white and middle class. The vast majority had a diagnosis of primary dependency or abuse of cannabis or alcohol. Most had also abused a wide array of mood-altering drugs including inhalant drugs, LSD, PCP, and cocaine.

The data in our study were gathered from a 21-item questionnaire about isobutyl nitrite use, answered by patients present at the treatment facility on a single day.

After a general announcement about the purposes of the study and the guidelines for voluntary participation, the questionnaire was given to individuals attending a large group therapy session consisting of approximately 200 patients. They were informed that the purpose was for medical research only. Participation was entirely voluntary; there were no rewards or consequences based on a client's decision of whether or not to participate. The confidential questionnaire had been approved by the program director and the professional treatment staff.

RESULTS

Seventy-four (43 percent) of the 173 respondents (85 percent were male, 35 percent were female) to the survey reported that they had abused isobutyl nitrite for the purpose of self-intoxication; however, 52 (30 percent) had used it less than 10 times each. An additional 16 (9 percent) had used it between 10 and 99 times. Six (3 percent) estimated that they had inhaled isobutyl nitrite on more than 100 occasions. Of the 22 individuals who had inhaled the vapors more than 10 times, 19 were male.

The 22 clients (mean age 17 years), who inhaled isobutyl nitrite at least 10 times had almost all been introduced to the drug by a close friend. The substance was almost always used by inhalation directly from the vial.

Isobutyl nitrite had been inhaled in solitude on at least one occasion by 71 percent of the 22 frequent users, a particularly dangerous practice if the user was standing or driving a car at the time because of the cardiovascular effects. Relatively few (17 percent) used the drug before heterosexual intercourse and none admitted to use before anal intercourse. The average cost of a 12 ml bottle of isobutyl nitrite (Rush) was reported at $5 to $6. Most of the young people obtained the drug from a friend or purchased it from "head shops" (33 percent), record stores (17 percent), pornography shops (11 percent), or mail-order catalogues (6 percent).

Nearly half the users described their experiences with isobutyl nitrite as "unpleasant" because of universal symptoms of lightheadedness and dizziness, as well as rapid heartbeat, blurred vision, facial flushing,

and headache. Thirty-four percent reported severe pulsatile headache, 17 percent reported burning in the nose, and 11 percent reported nausea immediately after inhalation of the drug. Probably because of the unpleasant odor and physiological effects of the volatile vasodilator drug, the euphoric effect ("high") was described as only "fair to good" by 56 percent of the respondents.

CONCLUSION

We believe that the results reported may reflect the extent and side effects of the use of isobutyl nitrite among adolescents in drug treatment programs. Being honest and open about their drug problems was an integral part of the treatment program that these individuals were undergoing. Conclusions from three published studies support the validity of self-reporting of drug use by adolescents (Smart and Jarvis 1981; Needle et al. 1983; Benson and Holmberg 1985).

The widespread and apparently increasing use of isobutyl nitrite as a euphoric agent by teenagers warrants consideration of banning the sale of isobutyl nitrite.

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AUTHOR

Richard H. Schwartz, M.D.
Clinical Professor
Child Health and Development
George Washington University
School of Medicine
Washington, DC 20037

Nitrite Inhalants: Contemporary Patterns of Abuse

W. Robert Lange, Elizabeth M. Dax Charles A. Heartzen, Frederick R. Snyder, and Jerome H.Jaffe

INTRODUCTION

Amyl nitrite has been used medically since 1867 in the management of coronary insufficiency (Brunton 1867), and even though the agent is used infrequently in clinical therapeutics at this time, it does have a contemporary role in diagnostic cardiology and diagnostic radiology and in the management of cyanide poisoning. The history of butyl and isobutyl nitrites (collectively referred to hereafter as butyl nitrite) is more recent. Whereas amyl nitrite is a prescription drug, butyl nitrite is marketed as a room odorizer. Even though it has no clinical or practical utility, it is widely available and has been endowed with a certain mystique.

Beginning in the late 1960s and into the early 1970s, reports began to surface that nitrite inhalants were being abused by both sexes in an attempt to augment the physical pleasure of sexual intercourse (Everett 1972). Soon nitrite inhalants became known as “poppers” (an onomatopoetic street name derived from the sound of breaking an amyl nitrite pearl), and abuse patterns took two distinct courses. As a street drug, poppers were frequently used on social occasions, often in discotheques, to promote a sense of abandon while dancing, to expand creativity, to stimulate music appreciation, and to enhance meditation (Sigell et al. 1978). Others used them on a more general basis for a euphorigenic rush, an altered state of consciousness, and even as a stimulant (Nickerson et al. 1979). There is no scientific evidence, however, that any of these effects are associated with the use of nitrite inhalants.

A more widespread pattern of use was associated with overt sexual activities. Purported attributes included prolongation of penile erection and relaxation of rectal smooth muscle and anal sphincter tone, thus facilitating intromission (Labataille 1975). Other alleged drug effects included heightened libido, i.e., an aphrodisiac, prevention of premature ejaculation, increased volume of ejaculate, and promotion of a generally intensified or prolonged sexual experience (Israelstam et al. 1978).

Although awareness of adverse health effects of nitrite abuse was raised in the 1970s (Louria 1976), real concerns surfaced with the advent of the acquired immunodeficiency syndrome (AIDS) epidemic, and the growing body of data suggesting that the volatile nitrites may influence immunomoduiation or otherwise be a cofactor in the pathogenesis of human immunodeficiency virus (HIV) infection and certain AIDS-related syndromes. For example, of the first five homosexual men with AIDS reported to the Centers for Disease Control, all had used nitrite inhalants, but only one had abused intravenous drugs and only two were reported to be promiscuous (Centers for Disease Control 1981).

Contemporary patterns of abuse of nitrite inhalants are not clearly delineated. On one hand, a readily available drug with the reputation of being an aphrodisiac would appear to have appreciable abuse liability. On the other, the close association to and growing concern over AIDS might be expected to alter patterns of abuse. This study was a descriptive survey of two populations with a high probability of using nitrite inhalants.

METHODS

Two distinct high-risk populations were surveyed: predominantly heterosexual substance abusers in six regions of the United States and homosexual men in Baltimore.

Substance Abusers

Substance abusers were surveyed in Baltimore, MD, during two separate time frames: 1981 through 1984 (n=365) and February through October 1986 (n=246). in addition, substance abusers in five other regions of the United States were queried about nitrite abuse patterns as part of a national HIV seroprevalence survey conducted between September 1986 and February 1987. The following regions were surveyed: New York City (n=267); Tampa, FL (n=59);

San Antonio, TX (n=102); Denver, CO (n=99); and Los Angeles, CA (n-409). The Baltimore participants during both time periods were comprised of both volunteers for National Institute on Drug Abuse-Addiction Research Center (NIDA-ARC) research studies as well as clients in treatment for substance abuse disorders. The former tended to be recreational drug users not physically dependent on any substance at the time of their participation; the latter had heavier drug use histories. The subjects in the five locations other than Baltimore were all individuals recently enrolled in a drug treatment program.

Male Homosexuals

Between April and August 1966, a sample of 80 male homosexuals was studied. Of these, 20 were interviewed while participating in the activities of a homosexual support group, and 60 were interviewed in gay bars in Baltimore, MD, and Washington, DC. They were queried specifically in regard to their current and past use of both amyl and butyl nitrites, including methods and reasons for using these drugs as well as associated drug effects and side effects.

For the purpose of this investigation, current use was defined as use at any time during the 6 months prior to the survey. To assess possible differences between nitrite inhalant users and nonusers, the data were analyzed by either of two methods. Variables measured on an interval scale were subjected to independent samples r-tests, whereas categoricai variables were analyzed using the chi-square statistic.

RESULTS

Substance Abusers

Table 1 describes the Baltimore data for the two time frames. Among recreational users (NIDA-ARC clients), the prevalence rate of nitrite use during the preceding 6 months was 11 percent for both periods. The participants with the heavier drug use patterns (clients in treatment) also had a higher frequency of nitrite use, with the current prevalence rate being 22 percent. Nitrite inhalant use was more common in whites than in members of minority groups (x^2=6.80, $p<0.05$). The sexual orientation of the earlier samples is not known, but 7 percent of both 1986 samples admitted to a history of

TABLE 1. *Prevalence of nitrite use in Baltimore substance abusers*

Sample	Dates Studied	No.	Mean Age (Years)	White (Percent)	Male (Percent)	Prevalence of Use (Percent)
Early 1980s						
ARC Volunteers	1981-84	203	32±6	56	90	11
Clients in Treatment	1982-84	162	35±9	54	66	17
1986						
ARC Volunteers	1986	63	30±7	40	90	11
Clients in Treatment	1986	183	32±6	44	78	22

homosexuality, and there was no statistically significant association between homosexual behavior and nitrite use in these groups.

ARC subjects were asked when they first began using nitrites in relation to other substances of potential abuse, and these results are depicted in table 2. The inhalable nitrites tend to be a drug class whose use is initiated much later in life, with the mean age of first use at 25.6 years. This compares to an age of 14.6 years for glue, the other principal inhalant of abuse.

TABLE 2. *Age of first use for substances of abuse for Baltimore ARC volunteers*

Substance	Mean Age ± SD
Alcohol	13.9 ± 3.6
Glue	14.6 ± 3.2
Benzedrine/Speed	17.2 ± 3.1
Marijuana	17.6 ± 5.5
Heroin	18.5 ± 3.5
Cocaine	20.4 ± 4.4
Nitrites	25.6 ± 7.8

Table 3 shows the geographic differences in nitrite abuse patterns. Among abusers in treatment, use of nitrite inhalants ranged from a high prevalence of 40 percent in Tampa to a low of 7 percent in the New York City area (Brooklyn and Harlem). In all locations, experience with amyl nitrite was reported more frequently than experience with butyl nitrite.

TABLE 3. *Proportion of* exclusively heterosexual substance abusers *in treatment reporting nitrite inhalants, by residence*

City or Region	Number Queried			EH Reporting Nitrite Use (Percent)
	Total	Exclusively Heterosexual (EH)	EH Reporting Nitrite Use	
Tampa	59	53	21	40
Denver	99	90	34	38
San Antonio	102	93	28	30
Baltimore	182	172	39	23
Los Angeles	409	397	55	14
New York city	267	241	16	7
Total	1,118	1,046	193	18

Among substance abusers, nitrite use was reported more frequently among whites than blacks or Hispanics, and more frequently among men than women. Thirty percent (142/475) of ,whites reported nitrite use compared with 13 percent (31/246) of blacks and 10 percent (35/350) of Hispanics. The highest rates of nitrite use for all six regions were reported by whites. Twenty-one percent (159/743) of men reported nitrite use compared to 16 percent (57/366) of women. Within each region, men reported nitrite use more frequently than women except in San Antonio. There, 14 of 30 women (47 percent) and 12 of 72 men (29 percent) reported nitrite use.

Since these data were collected as part of an HIV antibody survey, the data were analyzed to ascertain whether there were differences in nitrite use on the basis of HIV seropositivity. Among substance abusers in Baltimore, there was a significant association between nitrite inhalant use and HIV seronegativity (x^2=121.6, $p<0.001$), and this was felt to be secondary to the ethnicity of the sample: HIV

seropositivity was independently associated with blacks, whereas nitrite use was independently associated with whites. In New York, there was a tendency for an association between nitrite use and seropositivity, and in Denver, there was a distinct association. However, among the five HIV positives in Denver, three admitted to being homosexual, and this likely confounds the data in an area with few HIV-seropositives.

Male Homosexuals

The mean age of the homosexual sample was 30.0 years (range 18 to 56 years). The component enrolled in the support group tended to be older (mean age 39.5 years, range 23 to 56 years) than those recruited in gay bars. Fity-five (89 percent) admitted to ever having used nitrite inhalants, and there were no demographic differences between users and nonusers. The mean age of first use among the homosexuals interviewed was 22.2 years (range 18 to 42 years), and the mean age of peak use was 25.4 years (range 17 to 45 years).

Of those who had used poppers, 43 (78 percent) reported use of only amyl nitrite, and the remaining 12 (22 percent) had used both amyl and butyl. Among those using both, there was a preference for amyl nitrite, which was regarded as being more potent and less associated with side effects. No homosexual subject in our sample admitted to having used only butyl nitrite.

Patterns of nitrite use were assessed for the homosexual men surveyed in Baltimore. Only 13 users (23 percent) reported having used nitrite inhalants at times other than during sexual activity, and this was primarily while dancing. Among the various sexual behaviors, use was most often associated with masturbation (64 percent). Twenty-six (47 percent) had used nitrite inhalants concomitantly with one or more other drugs, with concomitant marijuana being the most frequent (96 percent).

Despite the propensity for using nitrites during overt sexual activity, the predominant reason given by the homosexual group for using the substance was to get high, rather than for any anticipated physiologic effect. Twenty-five (45 percent) reported some side effect associated with nitrite use, the most common being headache. Three participants related that nitrite use Caused them to lose their erections, and none credited the drug with facilitating erection, heightening libido, or enhancing sexual enjoyment.

Homosexuai subjects reported that the duration of the subjective effects of nitrite inhalation was brief. The modal duration was 1 minute, with a range of 4 seconds to 15 minutes. The reported modal frequency of we was once weekly, and the modal intensity of peak use was four times per week. The style or technique of use was correlated with the intensity of use, with heavy or current users tending to inhale more deeply. They also reported a longer retention of inhalation than did light users. The time interval since most recent use ranged from 1 to 156 months, with a mean of 25.2 months, and the mean period since peak use was 4.1 years (range 1 to 11 years). From the data obtained during this survey, it appears that the use of nitrite inhalants within the gay community has decreased appreciably in recent years. Health-related concerns were the most frequently cited reasons for the discontinuance of the substance.

DISCUSSION

Ongoing surveillance in Baltimore afforded the opportunity to monitor changing patterns of nitrite inhalant use among substance abusers. Over the period since 1981 (a time which predates the AIDS epidemic) through 1996, there appears to have been no change in the patterns of nitrite use by substance abusers. Consistently, 11 percent of recreational abusers participating in NIDA-ARC research protocols have reported recent use of nitrite inhalants, as have approximately 20 percent of heavier drug users enrolling in treatment. Although it is difficult to rule out homosexual behavior among male substance abusers in the study, the use of nitrites by female substance abusers suggests that male homosexuals are not the only users of nitrites. Even though some evidence is emerging suggesting that parenteral drug abusers are attempting to procure new needles when they are available (Des Jarlais et al. 1985), there is still a basic recalcitrance to modify their drug use in the face of the risk of overdose death, or their needle-sharing behavior in the face of the AIDS epidemic. Consequently, there is probably little reason to anticipate that this high-risk group will modify its use of nitrites out of concern for the effects of these substances on the immune system.

There appear to be ethnic as well as geographic variations in nitrite abuse, and in some of the locations studied, these factors may confound each other. For example, Denver and San Antonio had the highest frequencies of use of poppers, and the proportions of these

samples which were white were 60 percent and 62 percent, respectively. New York City had the lowest frequency Of use, and that sample was only 18 percent white.

Within populations that have, a propensity to misuse a wide spectrum of drugs, nitrites appear to be substances whose abuse begins relatively late in life. This may reflect either a delayed availability or a delayed popularity of nitrites. Butyl nitrites were not generally available before 1970; therefore, they were not available during teenage years for men and wormen currently in their thirties and forties. Whereas the prevalence of nitrite inhalant use among substance abusers has remained constant in addition to being initiated late, other inhalant use such as glue sniffing has classically been a phenomenon Of young abusers; and, baaed on ongoing surveillance by the ARC, its abuse in the Baltimore area appears to be waning appreciably.

The natural history of addiction included what has been referred to as the “maturing-out phase” (Winick 1972). For a variety Of reasons, many long-term, hardcore addicts either become abstinent or manifest marked changes in their drug use patterns beginning in their late twenties and continuing through their thirties. Many will abuse alcohol more heavily during this period. The late use Of nitrites suggests that these substances may, in sime drug users, be associated with this process.

Within the homosexual population surveyed in the Baltimore-Washington, DC, area, the current level of nitrite use (21 percent) is the same as that observed in heavy drug users in the same locate (22 percent). The observation that nitrite use in the gay community is waning is encouraging; however, it is of concern that many homosexual men who continue to use nitrites do so for the euphorigenic properties, and will often use poppers with other sub stances of abuse, notably marijuana. A has been observed that homosexual men who use recreational drugs are significantly more likely to engage in high-risk sexual practices capable Of transmitting HIV infection than are those who do not (Polk, unpublished data). That both substance abusers and homosexual men use nitrite inhalants primarily to get high, and that both appear to have much more experience with amyl nitrite rather than the butyl derivative, were unexpected findings.

We conclude that heterosexual drug abusers in the Baltimore area do not appear to have modified their nitrite use appreciably in recent

years. however, data from this sample, which admittedly may not be representative of the overall homosexual community, would suggest that homosexual males probably have decreased their use of nitrite inhalants. This appears to be a result of a greater awareness of AIDS risk in this population, and a consequent trend to modify their high-risk behavior.

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AUTHORS

W. Robert Lange, M.D., M.P.H.
Elizabeth M. Dax, M.D., Ph.D.
Charles A. Haertzen, Ph.D.
Frederick R. Snyder, Ph.D.
Jerome H. Jaffe, M.D.

Addiction Research Center
National Institute on Drug Abuse
Francis Scott Key Medical Center
Baltimore, MD 21224

Epidemiologic Studies-Kaposi's Sarcoma Vs. Opportunistic Infections Among Homosexual Men With AIDS

Harry W. Haverkos

INTRODUCTION

Kaposi's sarcoma (KS) is an important manifestation of AIDS. It is the most common cancer reported among AIDS patients and its occurrence among gay men in New York and California was one of the earliest harbingers of the AIDS pandemic (Centers for Disease Control 1991).

KS associated with AIDS is unique both clinically and epidemiologically. Unlike "classical" (or non-AIDS-related) KS among elderly men, or "endemic" KS in some parts of Africa, KS in patients at risk for AIDS is associated with human immunodeficiency virus (HIV) infection and cellular immune impairment (Biggar et al. 1984; Stahl et al. 1982). Furthermore, KS occurs much more commonly among gay men than among non-gay AIDS patients and is the only AIDS-related illness in which the proportion of cases has declined (Haverkos et al. 1985; Haverkos 1987b).

Nitrite inhalants were investigated as a possible cause of AIDS early in the epidemic, partly because of the preponderance of homosexual men who used nitrites among the early patients with AIDS. During pilot testing of questionnaires in 1981, Centers for Disease Control (CDC) investigators found that nitrites were used by nearly all homosexual men with AIDS. In addition, a 1981 survey of 420 men attending sexually transmitted diseases clinics showed that homosexual men reported use of nitrites far more frequently than did heterosexual men, and that the amount of use directly correlated with the number of different sexual partners (Centers for Disease Control 1982). In 1982, Marmor and colleagues repotted significant associations between use of amyl nitrite and sexual activity, and the

development of AIDS (all 20 patients in the study had KS) among homosexual men (Marmor et al. 1982).

On the other hand, when Marmor et al. reanalyzed their data incorporating other factors and using multivariate analysis, they found that still other variables, including frequency of receptive anal intercourse and antibody titers to cytomegalovirus appeared to differentiate patients with AIDS from controls (Marmor 1984; Marmor et al. 1994). Although AIDS patients used greater quantities of nitrites than did controls in the initial CDC study, Jaffe showed that nitrite inhalant use did not appear to be important in distinguishing patients with AIDS from controls. Measures of sexual activity were the best markers for AIDS in that study (Jaffe et al. 1993). In addition, studies conducted by that National Institute for Occupational Safety and Health (NIOSH) for the CDC showed no immunotoxic reactions in mice exposed to isobutyl nitrite vapors (Lewis et al. 1985).

This paper reviews six epidemiologic studies conducted among gay men with AIDS to examine the role of nitrite inhalants, not as a cause of AIDS, but as a cofactor in the development of KS as a major manifestation of AIDS. Aspects of each study reviewed include: Basic study design, wording of the questionnaire regarding nitrite inhalant use (kindly provided by each of the investigators), and published results comparing nitrite inhalant use of KS patients compared wlth those who developed opportunistic infections and not KS. All six studies included questions about sociodermographic variables, medical history, use of drugs, and sexual history. Patients were invited to provide Mood and/or other specimens for laboratory analysis. More details about the six studies are available in the references.

REANALAYSIS OF CDC STUDIES

Basic Design: Eighty-seven gay men with AIDS (47 with KS, 20 with *Pneumocysts carinii* pneumonia (PCP), and 20 with both diseases) had participated in 3 different studies in 1981 and 1982 conducted by CDC in large metropolitan areas. Patients were interviewed by CDC personnel. HIV testing was not performed.

Questionnaire: There were 10 questions about nitrite inhalant use on the questionnaires. Patients were asked if they had ever used inhalant sexual stimulants or “poppers” prior to the onset of illness. If patients responded “yes” to the first question, they were asked to

give the calendar year they first used them, the last year they used them, how many months during that time period they used them, how many days per average month of use, and the number of "sniffs" per average day (or night) of use. Questions about the location of use (bathhouses, discos, Other) and types of nitrites (ampules, labeled bottles, unlabeled bottles) were asked. Respondents were asked to identify specific brand names of labeled bottles used and to identify Where they purchased unlabeled bottles of nitrites.

Results: Eighty-four of the 87 (97 percent) AIDS cases in the studies reported nitrite inhalant use. The median frequency of use was 394 days. Sixty percent of KS patients and those with both diseases used nitrites more than 394 days compared with 10 percent of PCP patients. Compared with PCP patients, KS patients and those with both diseases reported more sexual partners, more "recreational" drug use, higher incomes, and higher rates of non-B hepatitis. Multivariate analysis showed that the variable most strongly associated with KS was the use of large quantities of nitrite inhalants (Haverkos et al. 1985b).

MOUNT SINAI SCHOOL OF MEDECINE, NEW YORK

Basic Design: A longitudinal study of 42 homosexual men with persistent generalized lymphadenopathy was started at Mt. Sinai School of Medicine in 1981. Over the 4 1/2 years of the study, 12 patients (29 percent) have developed AIDS, 8 with KS. Questionnaires were self-administered.

Questionnaire: The questionnaire contained eight questions about nitrite inhalant use and was based on the one developed by CDC. Mt. Sinai investigators deleted questions on the brand names of labeled bottles and place of purchase of unlabeled bottles and changed the question about location of use in bathhouses to ask about use during sex or at discos.

Results: All 42 participants had used nitrites. The amount of nitrite use was statistically associated with the development of KS (Mathur-Wagh et al. 1984; Mathur-Wagh et al. 1985).

UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO

Basic Design: An AIDS case-control study among gay men in San Francisco started in 1993 and evolved into a longitudinal study. By April 1986, 158 men in the initial cohort had developed AIDS, 108 had

KS. Trained interviewers administered the questionnaires. This study is unpublished, but was presented at the First International AIDS Conference in Atlanta (Osmond et al. 1985) and at a National Institute of Allergy and Infectious Diseases-sponsored meeting in April 1986 (Haverkos 1987a).

Questionnaire: The questionnaire contained seven questions about nitrite inhalant use: first and most recent nitrite use, number of days or nights of use during an average month, and number of "hits" on a typical day or night of use. It also asked about the amount of use in the last 30 days and last year.

Results: Patients with KS were 2.2 times more likely to report greater than four "hits" per night than patients with opportunistic infections reported. Patients with KS, compared to patients with opportunistic infections, were more likely to report a large number of sexual partners, practice analingus, use large quantities of recreational drugs by routes other than intravenous, and receive metronidazole therapy for intestinal parasites. Patients with opportunistic infections were more likely to report syphilis and to use drugs intravenously than were patients with KS. Multivariate analysis showed that the variable most strongly associated with KS was the use of more than four "hits" of nitrite inhalants per night of use.

NATIONAL CANCER INSTITUTE

Basic Design: Two hundred forty-five men (85 from Manhattan and 160 from Washington, DC) were prospectively followed starting in 1982. Self-administered questionnaires were given to participants at entry and at followup visits. Nineteen have developed AIDS, 8 of whom had KS (Goedert et al. 1986).

Questionnaire: The questionnaire contained 15 questions about nitrite inhalants. Patients were asked if they ever used nitrite inhalants ("poppers"), what year they first used them, and to estimate the number of days of use during the past 30 days. The participants were asked to complete a grid containing four questions each for their use in 1982, 1981, and 1980. They were asked to estimate the number of days of use in each year, the number of sniffs on a typical day of use in that year, the types of nitrites (unlabeled brown bottle, "snapper," brand name) used at least twice a month during that year, and whether nitrites were used during sex acts, at other times, or both for each year. The questionnaire contained a listing of examples of brand names of labeled bottles.

Results: “Nitrite inhalant use, measured as frequency during the previous year or intensity (number of sniffs) during the previous 6 months, was not significantly associated with AIDS, Kaposi’s sarcoma, or *Pneomocystis* pneumonia (Goedert et al. 1986, p. 332).” Several variabies were associated with the development of KS, including receptive fellatio, frequent enemas/rectal douches, high levels of antibody to hepatitis B antigen, and use of methaquaalone (Goedert et al. 1986).

MULTICENTER AIDS COHORT

Basic Design: The Multicenter AIDS Cohort Study (MACS) is a prospective study of homosexual men in Baltimore/Washington, Chicago, Los Angeles, and Pittsburgh supported by the National Institutes of Health (NIH). Four thousand nine hundred fifty-men were enrolled in 1984 and are evaluated every 6 months. Questionnaires are administered by trained interviewers. One thousand eight hundred thirty-five were HIV seropositive on entry, 59 have developed AIDS (24 with KS) during a median followup of 15 months (Polk et al. 1987).

Questionnaire: The questionnaire contains eight items concerning nitrites and concentrates on use in the previous week, 8 months, and 2 years. Emphasis is also placed on the use of nitrite inhalants during sex in the previous 6 months and 2 years.

Results: No association between nitrite inhalant use and KS was reported. Significant associations for KS were found for decreased hemoglobin level, decreased T-helper lymphocyte count, and increased immunoglobulin A levels (Polk et al. 1987).

SAN FRANCISCO HEPATITIS B COHORT STUDY—CDC

Basic design: In 1984, followup of 6,700 homosexual men enrolled In a 1978 hepatitis B study was initiated. Ninety-six of the men were found to have AIDS (54 with KS) and were available for interview. Questionnaires were administered by trained personnel (Darrow et al. 1986, Darrow et al. 1987).

Questionnaire: The questionnaire contained three questions about nitrite inhalant use as follows: "Before your onset date for AIDS, about how many days in an average month would you use nitrite inhalants: 1. unlabeled bottles, 2. labeled bottles, and 3. ampules?”

Results: No differences in *nitrite* use were found between disease groups. The development of AIDS in men infected with HIV may be related to duration of infection, older age, and repeated exposures to HIV.

DISCUSSION

The results of epidemiologic studies concerning nitrite inhalant use and the development of KS among homosexual men with AIDS are inconclusive. Of the six studies reviewed in this paper, three find a strong association between larger quantities of nitrite inhalant use and KS, and three do not confirm the association.

The questionnaires used in the six studies vary significantly in the amount of information sought concerning nitrite inhalant use. Studies begun in 1981 and 1982 asked more detailed questions about nitrites than later studies asked, possibly because nitrites were considered a possible cause of AIDS early in the epidemic. After HIV was discovered as the cause of AIDS, epidemiologic studies tended to focus more on sexual activities than on nonintravenous drug use. In addition, nitrite inhalant use among gay men may be declining since the AIDS epidemic (Lange et al., in press). If so, questions asked in 1985 and 1986 about nitrite use in the previous 6 months or 2 years cannot reflect accurately the amount of nitrite use at earlier times, and, possibly more important, nitrite use at the time of HIV infection.

The differences in outcomes of these studies may be due to differences in study methods. The sample s&es of the studies are small. The largest study evaluated 150 AIDS patients, 100 with KS (Osmond et al. 1985). The smallest study evaluated 12 patients, 8 with KS (Mathur-Wagh et al. 1984). The various patient recruitment processes may have introduced unsuspected selection biases. Several of the studies used self-interviews, others used trained personnel to administer the questionnaires. Although there is no evidence to suggest that self-interviews are less reliable than face to face interviews, information bias must be considered in any study involving personal items, such as sexual and drug history.

There have been several hypotheses concerning the possible mechanism by which nitrite inhalants may promote KS in users infected with HIV. First, nitrite inhalants may act directly on the immune system (Goedert et al. 1982). Second, nitrosamines, among the possible metabolites of nitrites, are carcinogenic (Jorgensen and

Lawesson 1982). Interactions of nitrites and mouse skin lipids have been shown to produce potentially carcinogenic substances (Mirvish et al. 1986). Third, the vasodilatory action of nitrite inhalants may, in some way, promote KS, a malignancy of the endothelial cells lining blood vessels. Finally, use of nitrites may only be a marker for another cofactor, e.g., an as-yet "unidentified" microorganism. National Cancer Institute investigators have proposed a hepatitii A-like organism as the KS cofactor (Weiss and Biggar 1986).

Several epidemiologic observations suggest that an infectious cofactor is less likely than nitrites. The "unidentified" virus must be highly associated with gay sexual activity. This would imply a sexually transmitted and/or bloodborne agent. Clearly the KS cofactor is not boodborne. Blood donors who develop KS have transmitted HIV infection to recipients, but those recipients do not develop KS (Curran et al. 1984). There is no documented evidence that the cofactor is sexually transmitted; there is no consistent pattern of KS transmission among clusters of homosexual men linked by sexual contact and developing AIDS (Auerbach et al. 1984). KS has not been reported in a female partner of a bisexual man with KS. The strong association of KS with increased income in one study is consistent with the argument that a drug is more likely to be the cofactor than a sexually transmitted agent (Haverkos et al. 1985b).

RECOMMENDATIONS

More epidemiologic and laboratory studies are needed to ascertain the role, if any, of nitrite inhalants as a cofactor in AIDS-related KS. Investigators need to develop questions that quantitate lifetime nitrite exposure and measure dose more accurately. (The concept of "pack-years" to quantitate cigarette use may be a useful model.) Questioning heterosexual AIDS patients who have KS about nitrite use may be helpful. Study of animal models infected with retroviruses and challenged with large quantities of nitrites before, during, and/or after retrovirus infection would be useful. Finally, the U.S. Public Health Service could organize a multiagency task force to resolve this issue as was done for aspirin use and Reye's syndrome.

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AUTHOR

Harry W. Haverkos M.D.
Chief, Clinical Medicine Branch
National Institute on Drug Abuse
Parklawn Building, Room 10A-08
5600 Fishers Lane
Rockville, MD 20857

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