POSSIBLE LONG-TERM HEALTH EFFECTS OF SHORT-TERM EXPOSURE TO CHEMICAL AGENTS

Volume 2 Cholinesterase Reactivators, Psychochemicals, and Irritants and Vesicants

Prepared by the
Panel on Cholinesterase Reactivator Chemicals
Panel on Psychochemicals
Panel on Irritants and Vesicants
Committee on Toxicology
Board on Toxicology and Environmental Health Hazards
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PANEL ON CHOLINESTERASE REACTIVATOR CHEMICALS

Donald Ecobichon, McGill University, Quebec, Canada, Chairman
Mohamed Abou-Donia, Duke University Medical School, Durham, North Carolina
Wolff D. Dettbarn, Vanderbilt University School of Medicine, Nashville, Tennessee
Walderico M. Generoso, Oak Ridge National Laboratory, Oak Ridge, Tennessee
Wayland Hayes, Vanderbilt University School of Medicine, Nashville, Tennessee
Richard Johns, Johns Hopkins University School of Medicine, Baltimore, Maryland
Lewis H. Kuller, University of Pittsburgh, Pittsburgh, Pennsylvania
Frank S. Standaert, Georgetown University Medical School, Washington, D.C.
Irwin Wilson, University of Colorado, Boulder, Colorado

Technical Consultants

Ronald J. Kassel, U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland
Joseph S. Wiles, Baltimore, Maryland
J. Henry Wills, Uniformed Services University of the Health Sciences, Bethesda, Maryland

PANEL ON PSYCHOCHEMICALS

Robert Snyder, Rutgers University, New Brunswick, New Jersey, Chairman
Monique C. Braude, National Institute on Drug Abuse, Rockville, Maryland
Sidney Cohen, University of California, Los Angeles, California
Walter J. Decker, University of Texas Medical Branch, Galveston, Texas
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Marvin S. Legator, University of Texas Medical Branch, Galveston, Texas
William R. Martin, University of Kentucky, Lexington, Kentucky
A. Thomas McLellan, Veterans Administration Medical Center, Philadelphia, Pennsylvania
Charles P. O'Brien, University of Pennsylvania, Philadelphia, Pennsylvania
Wolfgang H. Vogel, Thomas Jefferson University, Philadelphia, Pennsylvania

Technical Consultants

Ronald J. Kassel, U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland
James S. Ketchum, University of California at Los Angeles, School of Medicine, Los Angeles, California
S.N. Pradhan, Howard University, Washington, D.C.
Fred Sidell, Biomedical Laboratories, Aberdeen Proving Ground, Maryland
Joseph S. Wiles, Baltimore, Maryland

PANEL ON IRRITANTS AND VESICANTS

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William J. Blot, National Institutes of Health, Bethesda, Maryland
George Hoffmann, Holy Cross College, Worcester, Massachusetts
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Kim Thorburn, University of California at Los Angeles, California
John H. Weisburger, American Health Foundation, Valhalla, New York
William Willoughby, Johns Hopkins University Medical School, Baltimore, Maryland
John Zapp, Kennett Square, Pennsylvania

Technical Consultants

Charles Hassett, Towson, Maryland
Ronald J. Kassel, U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland

National Research Council Staff

Francis N. Marzulli, Project Officer, Panel on Cholinesterase Reactivator Chemicals, Panel on Psychochemicals, and Panel on Irritants and Vesicants

Devra Lee Davis, Executive Director, Board on Toxicology and Environmental Health Hazards (BOTEHH)
Seymour Jablon, Director, Medical Follow-up Agency
Robert J. Keehn, Statistician, Medical Follow-up Agency
Edna Paulson, Manager, Toxicology Information Center (BOTEHH)
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Ellen Silbergeld, Environmental Defense Fund, Washington, D.C.

National Research Council Staff

Devra Lee Davis, Executive Director

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The following persons provided technical assistance:
Gary Keilson, University of Virginia Medical School, Charlottesville, Virginia
Lester Miller, U.S. Army Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland
Gordon Newell, EPRI, Palo Alto, California
James Norman, Medical Follow-up Agency, National Research Council
Robert Tardiff, Environ Corporation, Washington, D.C.
James Vick, Food and Drug Administration, Washington, D.C.

PREFACE

The Department of the Army asked the Committee on Toxicology, in the Board on Toxicology and Environmental Health Hazards of the Commission on Life Sciences, National Research Council (NRC) to conduct a study of the possible chronic adverse health effects on servicemen of experimental exposure to various chemicals at the U.S. Army Laboratories (formerly the Army Chemical Center), Edgewood, Md. The Edgewood tests were conducted over a 20-yr period ending in 1975, to learn how five major classes of chemicals tested for various military applications may affect humans. Some 6,720 soldiers took part in the programs, and 254 chemicals were administered by various routes.

In 1982, the Committee reported (Volume 1) on possible long-term effects of two pharmacologic classes of chemicals (anticholinesterases and anticholinergics) tested at Edgewood. This volume reports on long-term effects of three additional classes of chemicals tested at Edgewood: cholinesterase reactivators, psychochemicals, and irritants and vesicants. Studies of these substances, including LSD (discussed in a separately prepared report), constituted the main thrust of the Edgewood experiments.

After completion of Volume 1, three new panels were established to identify and assess evidence on the possible long-term health effects or delayed sequelae of the three chemical classes tested. This was done over a period of a year, during which each panel met three times. Pertinent material was examined to evaluate the possibility that experimental exposure of soldiers may have resulted in untoward health effects. The three panels were separately concerned with four cholinesterase reactivator chemicals (oximes); two types of psychochemicals (phencyclidine and dimethylheptylpyran and congeners), administered in pure form, as opposed to street drugs; and mustard gas and several lacrimatory and respiratory irritants (such as CN, CS, CR, and CA).

The charges to each panel were as follows:

- To determine whether there was sufficient evidence to assess the likelihood that the test chemicals had had long-term health effects or delayed sequelae.

- To determine, on the basis of this evidence, whether the chemicals, as administered, are likely to have produced such adverse effects in the test subjects.

As part of this undertaking, the NRC staff conducted interviews with administrators, investigators, nurses, and technicians and reviewed documents to identify practices and procedures followed in testing chemicals at Edgewood. The interviews included persons who had a wide spectrum of viewpoints, but who were essentially in agreement regarding the conduct of human testing. Committees were formed at Edgewood to review classified chemicals and develop reports for declassification and use by NRC panels. Extensive extracts were prepared of preclinical animal and human protocols and of technical reports at Edgewood libraries and other facilities. A repository was established at Edgewood (August 1980) for storing selected reports and needed information obtained from other sources. Research and experimental case files on volunteers were extracted and summarized. Digests of the literature were prepared.

This report presents the conclusions of the three panels based on available toxicologic and epidemiologic data, including specific information on the frequency, routes, and amounts of the substances administered and the known immediate and long-term effects of the substances under the prevailing experimental conditions.

A third report is intended to provide a final evaluation of the chemicals tested. Volume 3 will report on the current health status of the soldier participants on the basis of a recent questionnaire and interpret its impact on the conclusions reported in Volumes 1 and 2. The followup questionnaire to volunteer subjects may add descriptive data, but, because of the limited sample size, it is unlikely to provide evidence of a cause-and-effect relation between exposure to these chemicals and development of delayed disease.

EXECUTIVE SUMMARY

In 1980, the Board on Toxicology and Environmental Health Hazards of the National Research Council's Commission on Life Sciences began a program to evaluate the long-term health effects of chemical agents administered to military volunteers at the Army Chemical Center, Edgewood, Md., during the 1950s, 1960s, and 1970s. This work was conducted at the request of the Department of the Army. The tests were conducted to find out how various potential chemical warfare agents affect human performance. It was felt that animal tests were inadequate for this type of evaluation and that only humans could provide definitive information.

The first report (Volume 1) reviewed data on 15 anticholinesterase and 24 anticholinergic agents, to which almost half of some 6,700 subjects were exposed at Edgewood.

The current report, prepared by three panels of the Board's Committee on Toxicology, evaluates possible delayed health effects of three additional classes of compounds that were tested on most of the remaining volunteer subjects: cholinesterase reactivators, psychochemicals, and irritants and vesicants (blistering chemicals). The cholinesterase reactivators, of which there are four, are used as antidotes for anticholinesterase poisoning. The psychochemicals include phencyclidine, an anesthetic with substantial disorienting effects that is also available (with impurities) as the street drug PCP, and 10 related dibenzopyrans that are central nervous system depressants capable of producing orthostatic hypotension. The irritants include the well-known lacrimatory chemical CN, the riot-control agent CS, and other ocular and respiratory irritants. Mustard gas was the vesicant whose effects were studied.

The Committee established three panels to identify and assess evidence on the possible long-term health effects or delayed sequelae of the chemicals tested. As in the work that led to Volume 1, the chairman of each panel was selected from the Committee on Toxicology.

The specific charges to each panel were as follows:

- To determine whether there was sufficient evidence to assess the likelihood that the test chemicals had had long-term health effects or delayed sequelae.
- To determine on the basis of this evidence whether the chemicals, as administered, are likely to have produced such adverse effects in the test subjects.

The conclusions in this volume are based on available epidemiologic, toxicologic, and mortality data that were reported in Volume 1. They also rely on a review of test-subject exposure data obtained from Edgewood, all of which were available to panel members. Long-term clinical followup was not conducted. The subjects tested were healthier than the control subjects with whom they were compared, and both groups were healthier than the general population, reflecting the better health status of those in military service. The analyses presented here necessarily reflect the limitations of the available data. These conclusions might change in the light of information gained through a study of morbidity, which will be based on a questionnaire and an analysis of admissions to Army and Veterans' Administration hospitals (to be reported in Volume 3).

CHOLINESTERASE REACTIVATORS

On the basis of an examination of toxicologic literature, case reports from Edgewood volunteers, and a review of mortality data conducted by the National Research Council Medical Follow-up Agency, the Committee found no evidence of chronic disease in animals or humans associated with single or repeated doses of the cholinesterase reactivators (oximes). The lack of followup data on volunteers prevents certainty in predicting occurence or absence of delayed effects. The compounds are eliminated very rapidly from the body, but they produce a variety of acute effects that are short-lived and reversible, such as gastrointestinal distress after oral administration, pain at an injection site, dizziness, headache, and ocular discomfort. The Committee found no conclusive studies of carcinogenicity, mutagenicity, teratogenicity, or reproductive anomalies associated with the four oximes and therefore did not reach a conclusion in this regard.

PSYCHOCHEMICALS

The Committee found the evidence on the long-term health effects of the tested psychochemicals to be sparse. The target organs that may be involved in prolonged or delayed effects of phencyclidine are the brain and cardiovascular system. Target mental or cardiovascular effects did not take place within a week of exposure to the drug. No case reports have identified long-term effects or mental or cardiovascular effects soon after first exposure.

The margin of safety of a tested chemical is sometimes estimated by considering the ratio of the lethal dose to the pharmacologically effective dose (the dose at which some detectable biologic effect occurs). On the basis of animal data on the psychochemicals tested, the margin of safety for short-term effects is large for acute intravenous, intragastric, intraperitoneal, and subcutaneous administration and somewhat smaller for inhalation of the aerosolized form.

On the basis of the scientific literature alone, it is not possible to predict whether any long-term effects would be asociated with the small exposures used. However, evaluation of this toxicity literature and the Edgewood studies led this Committee to conclude that, at the doses and frequencies of phencyclidine used at Edgewood in a small number of test subjects, it is unlikely that detectable long-term or delayed effects have occurred or will occur.

Acute administration of the dibenzopyrans (dimethylheptylpyran and congeners) produced various degrees of physical incapacitation in Edgewood subjects, mainly because of moderate to marked and prolonged orthostatic hypotension. The duration and intensity of effects varied among most doses and subjects. Despite the variations, there is a large pharmacologic margin of safety in the use of these compounds in animals. The dibenzopyrans produced more potent long-lasting orthostatic hypotension and weaker (but otherwise similar) psychologic effects than Δ-9-tetrahydrocannabinol during the Edgewood experiments. There is no information on chronic effects of dibenzopyrans.

IRRITANTS AND VESICANTS

The Committee analyzed published studies describing the in vivo and in vitro properties of the agents used and reviewed short-term data collected by the U.S. Army on volunteers. The ability to provide definitive answers to the questions raised by the charge to the Committee was limited by the absence of long-term followup studies of the soldiers and by the sparseness of chronic effects studies of these compounds in animals or in humans after industrial exposure.

In general, the Committee found insufficient evidence to evaluate these chemicals, except mustard gas. Mustard gas is an experimental mutagen and human carcinogen at high doses.

Data on the irritants are insufficient to evaluate their mutagenicity, carcinogenicity, or other long-term effects. Tests of all theses chemicals involved few exposures and low doses.

MUSTARD GAS (H)

Mustard gas is highly reactive and has vesicant and systemic toxic effects. It is an alkylating agent that is mutagenic in various laboratory test systems, including mammalian germ cells, but data are inadequate to predict the extent of its genetic risk in humans. Mustard gas is also carcinogenic in experimental animals and humans. Other possible long-term effects of mustard gas are related to its local toxicity; specifically, it can cause blindness, possible skin tumors from some cases of permanent scarring of the skin, and chronic bronchitis. Reported instances of long-term injury, such as carcinogenesis in workers in a Japanese mustard production plant, were associated with exposure at high, long-term dosages. Information is insufficient to project risks associated with smaller exposures to mustard gas; however, serious long-term adverse effects in the small number of soldiers who received one or a few low-dose exposures at Edgewood seem unlikely (except for possible skin tumors and some cases of permanent scarring). Some of those exposed at Edgewood suffered skin injuries that took several weeks to resolve. However, in view of the small number of persons tested (about 150 healthy men) and the very low dosages involved, it is unlikely that a statistically significant increase in the risk of cancer or other chronic disease can be detected in those exposed to mustard gas at Edgewood. When exposed, the Edgewood subjects were wearing gas masks and impregnated clothing--an ensemble being tested for efficacy against toxic contamination.

o-CHLOROBENZYLIDENE MALONONITRILE (CS)

Results of experimental studies in microorganisms and short-term experiments in laboratory animals suggest that long-term medical abnormalities in soldiers exposed to CS are unlikely. Acute tissue changes produced in animals and humans seem reversible and not likely to become chronic in the absence of recurrent exposures. Followup information on the long-term state of health of exposed soldiers is not available, but no reports indicate that Edgewood subjects have experienced any long-term sequelae.

CHLOROACETOPHENONE (CN)

CN, a moderately toxic irritant, has immediate effects on the eyes, skin, and respiratory tract. CN is a strong skin-sensitizing agent, but is rarely lethal. The Committee found no evidence of lasting ocular or respiratory effects in 99 volunteers exposed experimentally at Edgewood between 1958 and 1972 when subjects were evaluated 2 wk after cutaneous administration or inhalation of aerosol. Allergic contact dermatitis or hypersensitivity in these volunteers on re-exposure to CN is possible. There has been no systematic study of the possible mutagenic and neoplasm-promoting effects of CN with current scientific methods.

DIBENZ[b,f][1,4]OXAZEPINE (CR)

CR, a mild lacrimatory irritant, manifests less acute toxicity than CN and CS. At low doses, it causes transient effects. There are a few studies on long-term health effects, including potential mutagenicity and teratogenicity. The available data are insufficient to predict long-term health effects. The small number of exposures and the small number of subjects exposed to CR at low doses at Edgewood make the occurrence of demonstrable effects in these subjects unlikely.

CHLOROPICRIN (PS)

PS is acutely toxic and has a variety of sensory effects in animals. It has not been evaluated thoroughly for mutagenicity or carcinogenicity. Like those exposed to mustard gas, the subjects exposed to PS were wearing gas masks, and small numbers of soldiers were exposed to small doses. PS is unlikely to have produced detectable long-term health effects in volunteers exposed at Edgewood.

BROMBENZYL CYANIDE (CA), DIPHENYLAMINOCHLORARSINE (DM), and 1-METHOXY-1,3,5-CYCLOHEPTATRIENE (CHT)

CA, DM, and CHT are unlikely to have produced measurable long-term health effects in volunteers exposed at Edgewood. But there are no specific toxicologic data on the mutagenicity and carcinogenicity of these compounds. CHT is less toxic than CN or DM when administered acutely.

NONANOYL MORPHOLIDE

The Committee does not expect long-term health effects in volunteers tested with nonanoyl morpholide at the dosages used at Edgewood. As with CA, DM, and CHT, specific toxicologic data regarding its potential in this regard are not available.

123 IRRITANT CHEMICALS

A total of 123 irritant chemicals were tested on only two subjects each. There are no data on their mutagenicity, carcinogenicity, or other long-term health effects. However, because the exposures were small, detectable adverse effects seem unlikely.

OVERVIEW

The Army's studies on human subjects were designed entirely to evaluate short-term physiologic and pharmacologic effects. A review of all available data reveals that these data are inadequate to provide definitive answers regarding the likelihood that the test chemicals produced or did not produce long-term health effects or delayed sequelae. Information on long-term health effects of the test chemicals on animals or humans is lacking, as is followup information on the current health status of the subjects. It therefore cannot be determined whether some subjects may have sustained long-term or delayed effects. Analysis of a questionnaire and of admissions to Army and Veterans' Administration hospitals (Volume 3) may provide further information on the current health status of these subjects.

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INTRODUCTION

In the spring of 1980, the Department of the Army asked the Committee on Toxicology of the National Research Council's Board on Toxicology and Environmental Health Hazards to study the possible chronic or delayed adverse health effects incurred by servicemen who had been exposed experimentally to various chemicals at Aberdeen Proving Ground, Edgewood, Maryland, during the years 1958-1975. The Edgewood tests were conducted to learn how potential chemical warfare agents might affect humans over a short period and how such affected humans might respond to therapy against such agents. The Army believed that relevant information could not be obtained from animal experimentation alone and that it was therefore necessary to confirm animal findings by using human volunteers.

Some 6,720 soldiers took part in this program. To understand the extent to which they might have experienced unanticipated long-term or delayed adverse effects, an extensive search of reports, records, and other data was undertaken. This search and study and evaluation of all available information on the involved chemicals themselves were accomplished by expert panels under the direction of the Committee on Toxicology.

To facilitate evaluation of the hazards associated with a long list of chemicals, the chemicals were grouped according to pharmacologic class. Of the five major groups of related chemicals, the largest and most important were the anticholinesterase and anticholinergic chemicals; these were reviewed first. Other chemical groups included cholinesterase reactivators, psychochemicals, and irritants and vesicants, and the effort culminated in a report issued in June 1982, Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents: Volume 1--Anticholinesterases and Anticholinergics.[1] That report dealt with 24 anticholinergic and 15 anticholinesterase chemicals that were administered to approximately 3,200 subjects.

The present report evaluates toxicologic and epidemiologic data relevant to the testing of approximately 750 subjects exposed to cholinesterase reactivators, about 260 exposed to psychochemicals, and 1,500 exposed to irritants or vesicants. A remaining group of subjects used largely in tests involving placebo or innocuous chemicals or conditions is available for comparison and will be discussed in Volume 3.

This report is the work of three panels of scientists--the Panel on Cholinesterase Reactivator Chemicals, the Panel on Psychochemicals, and the Panel on Irritants and Vesicants. The chairman of each panel was selected from the Committee on Toxicology, and the members were selected on the basis of their knowledge of the compounds in question or because they represented required disciplines.

Methods of selecting, testing, and handling subjects used for experimental work at Edgewood changed with time. Indeed, consent forms and methods of obtaining consent changed for all U.S. investigators during the period in which these tests were performed. A short history of the Edgewood testing program taken from Volume 1 is reproduced in Appendix A with a standard operating procedure for screening and selecting volunteers, which was issued by the Clinical Research Department at Edgewood on August 12, 1968.

LSD was among the psychochemicals tested at Edgewood, but its effects were not within the purview of the National Research Council's evaluations. Effects of LSD on 741 soldiers tested at Edgewood are described in a report by the U.S. Army Medical Department and the U.S. Army Health Services Command, issued in February 1980.[2]

Volume 3 will contain an evaluation of the current health status of Edgewood subjects based on their responses to a questionnaire and discussion and conclusions of the entire evaluation effort.

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2

CHOLINESTERASE REACTIVATORS

BACKGROUND

Rapid advances in chemistry during the nineteenth and twentieth centuries, coupled with the success of mustard gas as a toxic weapon in World War I, attracted attention to the warfare potential of chemical agents. This led to support for research on lethal nerve agents during and immediately after World War II. The research was followed by the development of treatment methods, and prominent among these was the use of cholinesterase reactivators to reverse the lethal effects of anticholinesterase nerve gases.

The termination of World War II brought to light the highly potent chemical-warfare agents of the organophosphorus ester class that had been synthesized by both sides during the conflict. The properties, pharmacology, and toxicology of these anticholinesterase agents were discussed in Volume 1 of the National Research Council report.[17] Such agents as diisopropyl fluorophosphate (DFP), sarin (GB), soman (GD), and tabun (GA) were a fascination to pharmacologists who conducted extensive studies in the early postwar years. It soon became evident that no available antidotes could block the pharmacologic activity of these chemicals, alleviate the signs and symptoms of toxicity, or restore normal bodily functions after exposure. Atropine readily antagonized the muscarinic actions, including those in the central nervous system (CNS), but elicited no reversal of the nicotinic effects. Better forms of therapy were sought, particularly to alleviate the nicotinic effects of anticholinesterase agents.

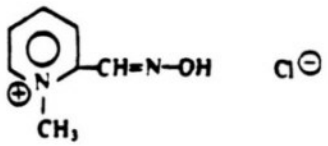
The first suggestion of a practical form of antidotal therapy came in 1949 from Hestrin,[9] who found that acetylcholinesterase (AChE) catalyzed the formation of acetohydroxamic acid when incubated with sodium acetate and hydroxylamine. Critical in vitro studies in the next decade led to the development of a practical approach to therapy. The crucial concept in these studies was the recognition that the compound formed when AChE reacted with a phosphorus ester was a covalent phosphoryl-enzyme intermediate similar to that formed in the hydrolysis of acetylcholine.[23] Wilson and colleagues, beginning in 1951, demonstrated that AChE inhibited by alkyl phosphate esters (tetraethyl pyrophosphate, TEPP) could be reactivated by water, but that free enzyme formed much more rapidly in the presence of hydroxylamine.[20,21] Similar results

were obtained by Hobbiger.[10] In 1953, Wilson and Meislich[26] synthesized nicotinehydroxamic acid and the corresponding methiodide; these agents were far more potent reactivators of alkyl phosphate-inhibited AChE than was hydroxylamine. In 1955, Wilson described picolinehydroxamic acid as the best reactivator among a series of quaternary hydroxamic acids, noting that oximes also were reactivators. In in vitro studies, Davies and Green[4] and Childs et al.[3] reported that 2-formyl-N-methylpyridinium iodide oxime (2-PAM iodide form) was 170 times more active than picoline-hydroxamic acid in reactivating TEPP-inhibited AChE in vitro, 30 times more active in reactivating DFP-inhibited AChE, and 400 times more active in reactivating GB-inhibited AChE. Similar findings were reported by Wilson and Ginsburg.[24,25]

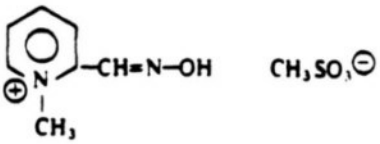
The first investigators to show the marked antidotal properties of pralidoxime compounds (Figure 2-1) were Kewitz and Wilson.[13] Askew[1] noted marked species differences in the rate and effectiveness of oximes and hydroxamic acids in reactivating alkyl phosphate-inhibited AChE. Other in vivo studies demonstrated that some oximes were effective in antagonizing the blockade of neuromuscular transmission after treatment of animals with TEPP, DFP, or GB.[12] The methyl methanesulfonate derivative of 2-PAM, P2S (Figure 2-1), was shown to be very effective by virtue of greater water solubility.[5] Additional studies demonstrated that the most effective treatment of organophosphorus ester-poisoned animals was to use oximes concomitantly with atropine. The general conclusions were that atropine minimizes or abolishes the toxic actions of AChE-inhibiting compounds on muscarinic and central cholinergic sites and the pyridinium and other quaternary hydroxamic acids and oximes reverse the toxic effects of the organophosphorus esters by reactivating the inhibited AChE.[14,19]

Success with the pyridinium oximes led to an intensive search for more effective oximes and the discovery of an especially potent derivative, 1,3-bis(4-formylpyridinium)propane dibromide bisoxime (TMB-4) (Figure 2-1), which reactivated serum-inhibited AChE in vitro more rapidly than 2-PAM or P2S.[11,18] TMB-4 was an effective chemical adjunct to atropine in sarin-poisoned mice, rats, rabbits, cats, and dogs.[2] Further modifications of the bispyridinium structure--insertion of different groups between the pyridinium rings--led to the synthesis of bis(4-formyl-N-methylpyridinium oxime) ether bichloride, known originally as LuH6 and later as toxogonin (Figure 2-1).[7] Its advantage was that it was less toxic than TMB-4 and also capable of reactivating AChE inhibited by a variety of organophosphorus esters.[6,15]

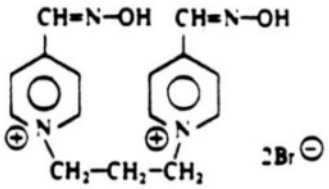
With the demonstration of species variability in response to the various oximes,[1] it was noted that results obtained with a particular organophosphate were not obtained with other agents, even if



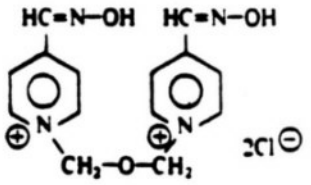
2-PAM. Pyridine-2-aldoxime methiodide methyl chloride



P2S. Pyridine-2-aldoxime methyl methanesulfonate



TMB-4. N,N-trimethylene bis(pyridine-4-aldoxime bromide)



Toxogonin. Bis(4-formyl N-methylpyridinium oxime) ether dichloride

FIGURE 2-1 Structural formulas of cholinesterase reactivators tested at Edgewood

they formed the same phosphorylated enzyme. This meant that screening of the reactivators for suitable antidotal properties necessitated in vivo testing with as many organophosphorus esters as possible. It also meant that data from animal studies could not be extrapolated to humans without considerable caution, necessitating testing in humans. The effectiveness of oximes in treating poisoning by organophosphorus ester chemical-warfare agents stimulated the U.S. Army to initiate studies with four compounds--2-PAM, P2S, TMB-4, and toxogonin--in military volunteers from 1958 to 1975, to assess their effectiveness against low doses of suitable militarily important organosphates. This chapter examines some pertinent features of the pharmacology and toxicology of the pralidoxime reactivators (Table 2-1). It presents conclusions of the Panel on Cholinesterase Reactivators as to the potential of these agents to impair chronically the health and well-being of the volunteers who participated in the studies conducted by the Department of the Army. It focuses on the four agents tested at Edgewood. A detailed report of the pharmacology and toxicology of the four oxime reactivators is in Appendix B.

TABLE 2-1 Subjects Tested with Cholinesterase Reactivators at Edgewood

Tox. No.	EA No.	Compound	CAS NO.	No. Subjects (Approx.)
D-1	2170	2-PAM: pralidoxime chloride (or iodide) protopam chloride (or iodide); 2-formyl-N-methylpyridinium chloride (or iodide) oxime; pyridine-2-aldoxime methochloride (or methiodide)	51-15-0	607
D-2		P2S: methyl methanesulfonate derivative of 2-PAM; pralidoxime methanesulfonate; Protopam methanesulfonate	154-92-2	95
D-3	3475	Toxogonin: LuH6; bis(4-formylN-methylpyridinium oxime) ether dichloride	114-90-9	41
D-4	1814	TMB-4; 1,1'-trimethylenebis(4-formyl-pyridinium) bromide (or chloride) dioxime; trimedoxime; 1,3-bis (4-formylpyridinium)propane dibromide (or dichloride) bisoxime	56-97-3 (bromide) 3613-81-9 (chloride)	32

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REVIEW OF AVAILABLE INFORMATION ON 2-PAM, P2S, TMB-4, AND TOXOGONIN

CHARACTERISTICS

Pralidoxime chloride (EA 2170), $[C_7H_9N_2O]Cl$ (Figure 2-1), is a white, nonhygroscopic, crystalline powder, with a molecular weight of 172.63, a melting point of 226-227°C, and a solubility in water of over 1 g/ml. It is a cholinesterase reactivator and was tested alone and with anticholinesterase chemicals on human volunteers at Edgewood. It is manufactured by Ayerst Laboratories, approved by the FDA, and widely used in the United States as an antidote to alleviate the acute responses to poisoning by organophosphorus insecticides and other anticholinesterase chemicals.[4,11,73]

Pralidoxime methanesulfonate (P2S), $[C_7H_9N_2O]CH_3O_3S$, consists of hygroscopic crystals with a molecular weight of 232.28 and a melting point of 155°C.[11,117] This is reportedly the preferred oxime in the United Kingdom.[73]

Toxogonin (EA 3475), $[C_{14}H_{16}Cl_2N_4O_3]$, is a grayish-white powder that exists as the chloride with a molecular weight of 359.22, a melting point of 229°C, and free solubility in water. A dibromide compound with a melting point of 202°C is also used. This oxime is much used in many European countries.[73]

TMB-4 (EA 1814), $[C_{15}H_{18}N_4O_2]Br_2$, exists as odorless, light tan crystals with a molecular weight of 446.21 and a melting point of 238-241°C and is also available as the chloride.[11,73] Russian and Yugoslavian investigators have reported the use of TMB-4 as an antidote in anticholinesterase poisoning.[17,73]

All four cholinesterase reactivator compounds are pralidoximes. The first two are monoquaternary oximes; the last two are bisquaternary oximes.

BIOCHEMISTRY

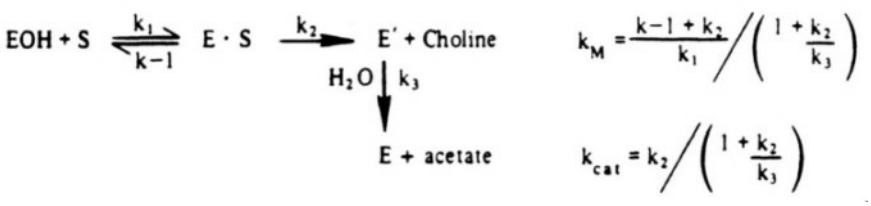
The body contains two main classes of cholinesterase: acetylcholinesterase (EC 3.1.1.7) and butyrylcholinesterase (EC 3.1.1.8).[27] The former, sometimes referred to as true cholinesterase, is mainly a tissue enzyme and is found mainly in such tissues as the synapses of the cholinergic system; it is also found in other tissues, such as erythrocytes, where its function is uncertain. The latter, referred to as pseudocholinesterase, is a soluble enzyme that is synthesized in the liver and circulates in the plasma.

Acetylcholine is the optimal substrate for acetylcholinesterase, whereas butyrylcholine is the optimal substrate for butyrylcholinesterase.[80,104] Acetylcholinesterase does not hydrolyze butyrylcholine very efficiently.

Acetylcholinesterase can be extracted from tissues to various extents by saline buffers, but often a detergent is necessary to solubilize the larger part of the enzyme. Even a single tissue usually yields a number of enzyme forms with different sedimentation coefficients.[72] Some of the forms are highly asymmetric; some tend to aggregate, unless the salt concentration is high. Regardless of the source or the molecular form, the kinetic properties of acetylcholinesterase are similar. Although it contains a number of catalytic subunits, there are no homotropic allosteric effects, nor do there appear to be any physiologic regulators of its activity.

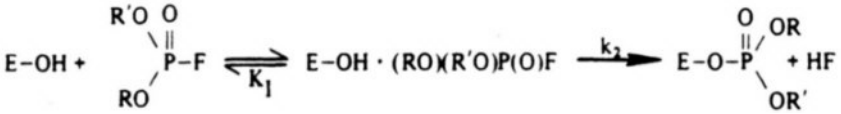
Acetylcholinesterase is subject to substrate inhibition at high concentrations, but Michaelis kinetics are observed at lower concentrations, because the substrate constant and the Michaelis constant differ by a factor of 100. Turnover numbers run about 2-9 x 10^5 min^{-1}, and K_m (Michaelis constant) values are about 0.2 mM.[76,116] whatever the source, the enzyme is subject to inhibition by the same reversible and irreversible inhibitors. Most of the kinetic work has been done with the saline-extracted 11S enzyme from electric eel and the detergent-extracted 6S enzyme from erythrocytes. The former is a tetramer derived from the native enzyme by the action of proteases; the latter is a dimer.[89]

The following diagram illustrates the reaction of acetylcholinesterase (E) with acetylcholine (S) to produce an acetylenzyme intermediate (E') with later hydrolysis of the intermediate and regeneration of the enzyme.[113, 114] K_M is calculated as shown in the diagram. The catalytic constant, k_{cat}, refers to the overall decomposition of the enzyme intermediate (E.S, E').

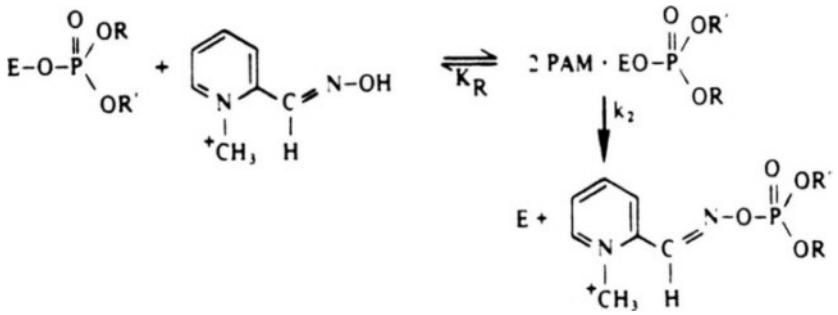


In acetylation, the enzyme acts as a nucleophile, and choline as a leaving group; in deacetylation, water is the nucleophile, and the enzyme is the leaving group. The enzymic nucleophile is the hydroxyl group of a specific serine residue. The mechanisms of both acetylation and deacetylation probably involve the formation of tetrahedral intermediates. The active site contains an imidazole group that functions first as a general base and than as a general acid in each step and whose ionization determines in part the pH variation of enzyme activity.

Details of enzyme-substrate and enzyme-inhibitor reactions were described in Volume 1 (p. 8). In summary, organophosphate esters with good leaving groups phosphorylate the enzyme by a mechanism similar to acetylation of the enzyme.



These substances are sometimes called irreversible inhibitors, because the hydrolysis of the phosphorylated enzyme by water is slow. Various nucleophiles containing a substituted ammonium group will dephosphorylate the phosphorylated enzyme much more rapidly than water. This was recognized early in the quest for a practical reactivating agent when choline was found to be a reactivator. Because a hydroxyl group in an alcohol is a weak nucleophile at neutral pH, the capacity of choline to function as a reactivator must be a consequence of its molecular complementarity with the enzyme and the increased acidity of the alcohol. The idea arose of finding a rigid structure that would include a quaternary ammonium group and an acidic nucleophile that would be complementary with the phosphorylated enzyme in such a way that the nucleophilic oxygen would be positioned close to the electrophilic phosphorus atom. This led to the pralidoxime compounds (PAMs). The syn isomer of 2-PAM (2-PAM-syn) turned out to be especially active. The reaction of 2-PAM with the phosphorylated enzyme is shown in the following diagram.



The pseudo-first-order rate constant for reactivation of 11S enzyme is:

$$k\,(\text{pseudo-first-order}) = \frac{k_2}{1 + \frac{K_R}{2\text{-PAM}}}$$

When the oxime concentration is smaller than K_R ([2-PAM] $\ll K_R$), the reactivation follows second order kinetics, and the rate constant can be described as shown below.

$$k\,(\text{second-order}) = \frac{k_2}{K_R}\,(2\text{-PAM})$$

The value for KR as determined for eel cholinesterase is about 10^{-4} M, and that for k_2, about 4 min^{-1}, so the second-order rate constant, k_2/K_R, is 4 x 10^4 M^{-1} min^{-1}.

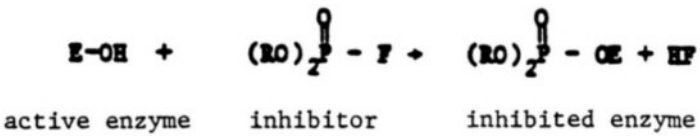
Thus, the biochemical characteristics affecting the reactivation of cholinesterase are complex and only partially understood. Knowledge of the kinetics of the various rate-determining processes is essential to the understanding of the inhibitor-reactivation process.

MECHANISMS OF ACTION

The therapeutic action of oximes resides largely in their capacity to reactivate cholinesterases without contributing markedly toxic actions of their own at recommended or usual doses. Other actions may contribute to their effectiveness: they may react directly with the anticholinesterase agent,[41,45] block its reaction with cholinesterase,[90] modulate the release of acetylcholine, [30,74] block acetylcholine's agonistic activity

(cholinolytic effect),[15,35,51] or increase excretion of the anticholinesterase agent.[48,86] Some of the other possible mechanisms are discussed later, but the interaction between oxime and free or unbound organophosphorus ester is considered below.

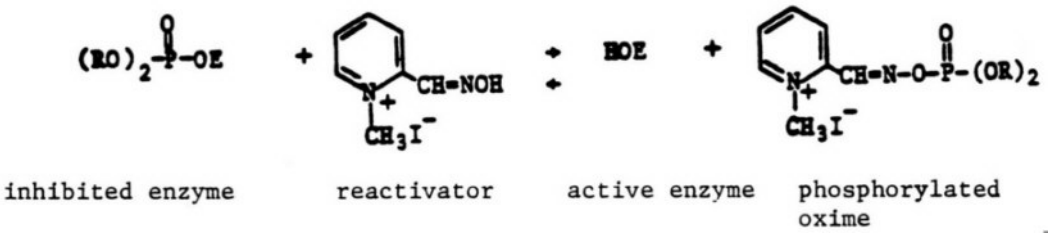
The basic reaction believed to occur in the inhibition of acetylcholinesterase by some alkylphosphates is shown in Reaction 1.



(1)

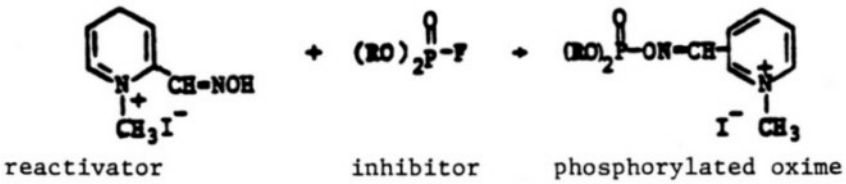
The inhibited enzyme is stable in aqueous environments, and lethality results from a blocking of transmission at cortical cholinergic synapses, such as those involved in breathing.

The reactivation of the inhibited enzyme can be achieved with selected oximes (such as 2-PAM), as shown in Reaction 2.



(2)

The reactivation is an equilibrium reaction. Under some conditions--e.g., when the oxime reacts directly with the organophosphate ester (Reaction 3) or when the equilibrium constant is less than 1 (Reaction 2)--the phosphorylated oxime can inhibit the enzyme by driving the reaction to the left.



(3)

Thus, the phosphorylated oxime can itself be a potent cholinesterase inhibitor. Wilson and Ginsburg advanced this concept when they noticed incomplete reactivation of acetylcholinesterase by 2-PAM and suggested that the phosphorylated oxime reinhibited the enzyme (according to Reaction 2).

Studies of the in vitro reaction between the alkaline oximes and sarin (isopropyl methylphosphonofluoridate, GB) revealed that 2-PAM reacted rapidly with the organophosphorus ester in solution at physiologic pH and temperature (Figure 2-2).[43]

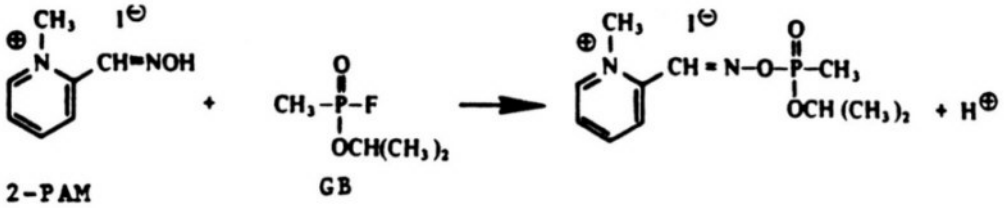


FIGURE 2-2 Suggested interaction between 2-PAM and GB (isopropyl methylphosphonofluoridate). Reprinted with permission from Hackley et al.[45]

Many of the oximes reacted in two distinct acid-producing steps: an initial phosphonylation of the oxime and then a breakdown into secondary products.[44] Although the phosphonylated intermediate of 2-PAM could not be prepared, the phosphonylated derivative of 4-PAM was synthesize d and found to be a potent inhibitor of eel acetylcholinesterase and to be toxic to mice, having an intravenous LD_{50} of 0.2 mg/kg of body weight.[44] Green and Saville found that MINA (monoisonitrosoacetone or 1,2-propanedione 1-oxime) underwent a stoichiometric reaction with sarin.[41] Additional studies confirmed that 2-PAM, 4-PAM, and probably TMB-4 were converted to potent inhibitors of AChE when they reacted with sarin in vitro.[45] That TMB-4 was converted to a potent anticholinesterase agent when it reacted with sarin in vitro was confirmed in studies that suggested that this bis-quaternary oxime could exist either as a monophosphonylated or as a diphosphonylated compound (Figure 2-3).[64] Zech et al. demonstrated that 2-PAM, 4-PAM, and TMB-4 were more potent inhibitors of cholinesterases after incubation with dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) or derivatives.[119] Later studies, in which sarin was incubated with P2S and toxogonin, demonstrated that unstable phosphonylated oximes were formed, but that these intermediates were potent inhibitors of cholinesterase.[26]

Although it appears that these phosphonylated oximes rapidly degrade in aqueous solutions, it has been suggested that they can have a harmful effect on the oxime therapy of poisoning by some organophosphates if they are formed in vivo, react with excess (“free”) circulating ester in cases of severe poisoning, and create a new inhibitor as potent as or more potent than the original anticholinesterase. The inhibitory potency of phosphonylated oximes has been estimated from their effects on the course of the reactivation of phosphonylated AChE when they were formed during the reaction.[2,91] Thus, it appears that the potential for the phosphorylated oximes to inhibit cholinesterases in vivo is substantial, but depends entirely on the stability of these intermediates in the body.

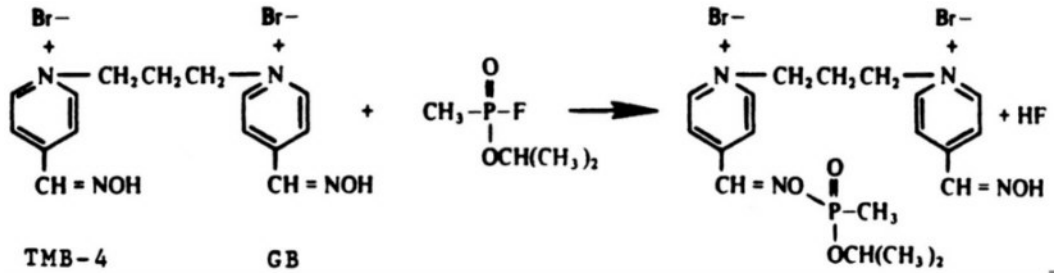


FIGURE 2-3 Interaction between TMB-4 and GB to form the monophosphonylated oxime. Reaction with a further molecule of GB would form the diphosphonylated compound.

A practical limitation on the usefulness of reactivators comes about because cholinesterase inhibited by organophosphates undergoes a secondary reaction termed “aging.” This phenomenon is discussed in Volume 1 of this report series. In brief an alkyl group is cleaved from the phosphorylated enzyme, leaving an “aged” enzyme that is resistant to reactivation by the oxime. The rate at which a phosphorylated enzyme “ages” is determined by the alkyl groups on the organophosphate, as described in Volume 1.

PHARMACOKINETICS

The pharmacokinetic features of these drugs, based on the evidence produced and the conclusions drawn by various investigators, are described in Appendix B. This section touches on the highlights and restates the material from another viewpoint.

Two features of the cholinesterase reactivating agents are critical for their pharmacokinetics:

Oxime reactivators (R-CH=NOH) are weak acids that partly ionize at biologic pH. This property allows them to function as nucleophiles and displace organophosphate moieties from inhibited acetylcholinesterase. It also makes them vulnerable to decomposition by other mechanisms in the body.

The oximes contain a quaternary ammonium group that contributes to their acidity and their strong binding to the inhibited enzyme. This appears to be a key structural element in known reactivators, but it tends to make them poorly soluble in lipids. Practically, this means that the drugs are slowly absorbed from the gastrointestinal tract, have difficulty entering the brain, do not easily enter hepatic cells to be biotransformed, and are not reabsorbed from the renal tubular urine.

2-PAM has been studied extensively by pharmacokinetic means, and data are available on its absorption, tissue distribution, blood concentration, and elimination by humans and animals. Although it has been given as various salts, the particular form administered is of no consequence once the material is absorbed and dissociated into the cation by body fluids.[61,100,106]

2-PAM is poorly and variably absorbed from the human gastrointestinal tract; only 10-50% of an oral dose is recovered in urine unchanged or as metabolites.[58,61,99,100,106] The absorption occurs, at least in dogs, in a segment of the ileal-jejunal area[68] either by passage through the small membrane pores in this section or by some more active absorption process that carries a variety of quaternary ammonium compounds into the blood. The overall absorption rate is lower than the elimination rate; that is, absorption is the rate-limiting step. Hence, blood concentrations are always low after oral administration, and substantial blood concentrations are difficult to produce or maintain when the drug is given by this route.[28,99] Marked gastrointestinal distress is produced by orally given oximes, particularly if therapy is continued.[99] 2-PAM may effectively be given either intravenously or intramuscularly, although the latter route is more erratic and slower in producing high blood concentrations and may involve some local pain.[96]

Like most quaternary ammonium compounds, 2-PAM is distributed mainly to extracellular water, and some tissue sequestration occurs.[100] 2-PAM is only weakly bound to plasma proteins.[34,100]

2-PAM crosses the blood-brain barrier with difficulty. 2-PAM in rat brain, 10 min after injection, is only about 5-12% of that in plasma; higher percentages are in the more heavily vascularized areas, such as cerebral and cerebellar cortex and inferior colliculi.[34] This low brain-to-blood ratio persists, but over the next 6 h the brain and blood come closer to equilibrium as the blood

concentration decreases sharply.[34] The low brain concentration is puzzling, inasmuch as the oximes depress anticholinesterase-induced seizures and void the part of the weakness of respiratory muscle contractions that is thought to be due in part to the action of organophosphate in the brain by suppressing anticholinesterase inactivation of central respiratory drive.[34] Hypoxia might cause disruption of the blood-brain barrier, thereby allowing the oxime to enter the brain. Some anticholinesterases may also facilitate the entry of oximes into the central nervous system.[34] Regardless of mechanism involved, oximes can cause remarkable improvement in CNS signs and symptoms in some patients. There is some evidence that peripheral actions, such as might occur at neuromuscular junctions, could contribute to muscle weakness.[60,70,88]

2-PAM is eliminated rapidly by man and animals. In humans, biologic half-life is about 1-2 h.[100] This short half-life is due in part to metabolism, but more to the fact that renal clearance approaches 700 ml/min,[100] i.e., almost that of p-aminohippuric acid (PAH). Investigators have therefore suggested that 2-PAM might be secreted into the urine.[14,50,100,108]

Interpretation of data on renal elimination is complicated by the presence of a quaternary group on one end of the oxime molecule and a weak acid at the other (Figure 2-1). They imply different mechanisms of renal handling. Swartz and Sidell [108] showed that in human beings both chemical groups are important. The more important of the two actions is the active secretion of the compound by the organic-base-secreting system of the kidney. This process can be slowed by one-third by coadministering high doses of thiamine.[57] Das Gupta et al.[25] claimed that the inhibiting effect of thiamine occurs in female, but not in male, rats. The net clearance of 2-PAM approaches that of PAH,[100] but is not as great; hence there may be some simultaneous active reabsorption of the material.[50] Like most cations, 2-PAM in the urine does not readily diffuse back from renal tubule to blood. The process here is complicated, in that the oxime has a pKa of 7.8-8.[108] It is more ionized at lower pH, and the renal loss is inversely proportional to pH.[14] Although the pH/pKa relation and active absorption contribute to the resulting or biologic half-life, active secretion into the urine is the predominant feature, and the total half-life is correspondingly short. Because of this, it is difficult to accumulate and maintain a substantial concentration of material.

The rapid renal elimination does not leave much time for metabolism of 2-PAM; about 90% of injected material is recovered unchanged in urine.[100] Nonetheless, 2-PAM is metabolized by liver, primarily by removal of the oxime moiety, and a number of metabolites have been identified,[32,78,111,112] some of them in rat brain, plasma, and urine.[34]

The blood concentrations, time-courses, and half-lives (1.2-1.4 h) of 2-PAM and toxogonin are similar in humans,[100] but toxogonin has a smaller volume of distribution than 2-PAM (174 vs. 795 ml/kg) and a smaller renal elimination (clearance, 133 vs. 717 ml/min). The smaller volume of distribution and slower clearance imply different distribution and handling of the two oximes. They also imply that, if toxogonin and 2-PAM were given in equimolar concentrations, the toxogonin plasma concentration would be greater than that of 2-PAM.

TMB-4 is unstable in fecal matter in vitro; only about 3% of an oral dose is found in urine and 2% in feces.[61]

ADVERSE EFFECTS

The adverse effects of various oximes (including diacetylmonoxime, which was not used at Edgewood) in humans are listed in Table 2-2, Table 2-3, Table 2-4, Table 2-5, Table 2-6 through Table 2-7. These tables list only significant findings.

Although Hopff and Waser[54] have listed mechanisms of action whereby reactivators of inhibited cholinesterase could be harmful, the real cause of most of the observed adverse effects is obscure. One exception is the slight inhibition of cholinesterase caused by a single dose of TMB-4 (dibromide form).[61]

Regardless of the underlying mechanism, the observed adverse effects are usually mild and brief. Many last only a few minutes; almost all disappear within a few hours after a single dose. A number of investigations, especially those concerning metabolism or pharmacokinetics, revealed no adverse effects, even though they were looked for.[31,101,107] Gastrointestinal disturbance after oral administration of oximes, especially P2S and TMB-4 chloride, may be severe enough to require discontinuation.[20] But doses of P2S as high as 8 g caused no symptoms (including gastritis) when given as tablets coated with dimethylaminoethyl methacrylate, each containing 400 mg of the drug and 71 mg of excipient.[100]

Local pain and increased creatine phosphokinase in the blood follow intramuscular injection. Using 2-PAM chloride and saline, Sidell et al.[95] showed that the degree of tissue injury was directly related to the osmolarity of the injected solutions when the volume was kept constant and directly related to the volume when the osmolarity was constant. However, under identical conditions, 2-PAM chloride was somewhat more injurious than saline.

Hepatic injury has been recorded in connection with 2-PAM (chloride), TMB-4 (chloride) and toxogonin. In the case of 2-PAM chloride, hepatic injury was manifest by transient increases in SGPT

TABLE 2-2 Adverse Effects Observed in Humans After Administration of 2-PAM Iodide

Effects	Dose and Route	References
Taste (bitter, metallic, salty, or mushy)	[b]	
Iodism	≥5 g, po	61
Local irritation	≥1 g, iv, NEG	56
Headache	≥1 g, iv	56
Dizziness and nausea, possibly progressing to vomiting	≥1 g, iv	56
Blurred vision, impaired accommodation	≥1 g, iv	56
Muscular weakness and/or malaise, fatigue, etc.	[b]	
Gastrointestinal distress	[b]	
Paresthesia and/or anesthesia	[b]	
Rash	[b]	
Bleeding	[b]	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	≤10 g, po, NEG	61
	≤1 g, iv, NEG	56
Initial hypertension followed by hypotension	[b]	
ECG changes	[b]	
Icterus and/or clinical test implicating liver	[b]	
Other biochemical changes	≥1 g, iv, NEG	56
Loss of consciousness	[b]	
Convulsions	[b]	

[a] po = oral; iv = intravenous; NEG = administration failed to produce effect indicated.
[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-3 Adverse Effects Observed in Humans After Administration of 2-PAM Chloride

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	[b]	
Iodism	[b]	
Local irritation	0.6 g, im	101
	≥0.175 g, im	101
Headache	≥2 g, iv	20
Dizziness and nausea, possibly progressing to vomiting	[b]	
Blurred vision, impaired accommodation	≥2 g, iv	20
	0.25 g, iv	108
Muscular weakness and/or malaise, fatigue, etc.	[b]	
Gastrointestinal distress	≥8 g, po	99
	≥2 g, po qid, 2 d	99
	2.1 g, iv	20
	2 g, po, bid, 180 d NEG	20
Paresthesia and/or anesthesia	[b]	
Rash	[b]	
Bleeding	[b]	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	≤3.7 g, po, NEG	61
≥4 g, po, NEG		20
2 g, iv		20
2 g, iv		20
1 g, iv, NEG		20
≤2 g, po, qid, 3 d, NEG		20
2 g, po, bid, 180 d, NEG		20
0.35 g, iv, NEG		108
Initial hypertension followed by hypotension	[b]	
ECG changes	3 g, iv	20
	2 g, po, bid, 180 d	
Icterus and/or clinical test implicating liver	2 g, po, bid, 180 d	20
Other biochemical changes	≤3 g, iv, NEG	20
	≤2 g, po, qid, 3 d, NEG	20
Loss of consciousness	[b]	
Convulsions	[b]	

[a] in = intramuscular; iv = intravenous; po = oral; qid = 4 times a day; bid = 2 times a day; NEG = administration failed to produce effect indicated.
[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-4 Adverse Effects Observed in Humans After Administration of P2S

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	[b]	
Iodism	[b]	
Local irritation	≥0.7 g, im	6
	0.5 g, im	52
Headache	[b]	
Dizziness and nausea, possibly progressing to vomiting	[b]	
Blurred vision, impaired accommodation	0.5 g, im, NEG	52
	1 g, po, qid + 0.5 g, im	52
Muscular weakness and/or malaise, fatigue, etc.	[b]	
Gastrointestinal distress	≥2.5 g, po, bid, 180 d	20
Paresthesia and/or anesthesia	[b]	
Rash	[b]	
Bleeding	[b]	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	≤5 g, po, NEG	61
	3 g, po, NEG	20
	3g, iv	20
Initial hypertension followed by hypotension	[b]	
ECG changes	3 g, po, NEG	20
	3 g, iv	20
	2.5 g, po, bid, 77 d	20
Icterus and/or clinical test implicating liver	[b]	
Other biochemical changes	[b]	
Loss of consciousness	[b]	
Convulsions	[b]	

[a] im = intramuscular; po = oral; iv = intravenous; qid = 4 times a day; bid = 2 times a day; NEG = administration failed to produce effect indicated.
[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-5 Adverse Effects Observed in Humans After Administration of TMB-4Dichloride

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	[b]	
Iodism	[b]	
Local irritation	[b]	
Headache	[b]	
Dizziness and nausea, possibly progressing to vomiting	≥1 g, po, 70 d	20
Blurred vision, impaired accommodation	[b]	
Muscular weakness and/or malaise, fatigue, etc.	≥1 g, po, 70 d	20
Gastrointestinal distress	≥1 g, po, 70 d	20
Paresthesia and/or anesthesia	≥1 g, po, 70 d	20
Rash	≥1 g, po, 70 d	20
Bleeding	≥1 g, po, 70 d	20
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	[b]	
Initial hypertension followed by hypotension	4 g, po 2 g, iv	20
ECG changes	4 g, po, NEG 2 g, iv	20
Icterus and/or clinical tes implicating liver	≥1 g, po, 70 d	20
Other biochemical changes	[b]	
Loss of consciousness	[b]	
Convulsions	[b]	

[a] po = oral; iv = intravenous; NEG = administration failed to produce effect indicated.
[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-6 Adverse Effects Observed in Humans After Administration of Toxogonin Chloride

Effects	Dose and Route[a]	References
Taste (bitter, metallic salty, or mushy)	0.175 g, im	97
	≥3 g, po	98
	≥1.84 g, po	102
Iodism	[b]	
Local irritation	≥0.175 g, im	97
	0.25 g, im	33
Headache	≥2.76 g, po	102
Dizziness and nausea, possibly progressing to vomiting	7 g, po	98
Blurred vision, impaired accommodation	[b]	
Muscular weakness and/or malaise, fatigue, etc.	≥2.76 g, po	102
Gastrointestinal distress	[b]	
Paresthesia and/or anesthesia	0.175 g, im	97
	≥3 g, po	98
	0.25 g, im	33
	≥2.76 g, po	102
Rash	[b]	
Bleeding	[b]	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	0.175 g, im	97
	≤4.6 g, po, NEG	102
Initial hypertension followed by hypotension	[b]	
ECG changes	[b]	
Icterus and/or clinical test implicating liver	0.25 g, im, 2 d, NEG	16
	≤4.6 g, po, NEG	102
Other biochemical changes	[b]	
Loss of consciousness	[b]	
Convulsions	[b]	

[a] im = intramuscular; po = oral; NEG = administration failed to produce effect indicated.
[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-7 Adverse Effects Observed in Humans After Administration of Diacetylmonoxime

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	≥1 g, iv	56
Iodism	[b]	
Local irritation	≥1 g, iv	56
	≥0.5 g, iv	42
Headache	[b]	
Dizziness and nausea, possibly progressing to vomiting	≥1 g, iv	56
	≥0.5 g, iv	42
Blurred vision, impaired accommodation	≥1 g, iv	56
Muscular weakness and/or malaise, fatigue, etc.	[b]	
Gastrointestinal distress	[b]	
Paresthesia and/or anesthesia	≥1 g, iv	56
	≥0.5 g, iv	42
Rash	[b]	
Bleeding	[b]	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	[b]	
Initial hypertension followed by hypotension	[b]	
ECG changes	[b]	
Icterus and/or clinical test implicating liver	[b]	
Other biochemical changes	[b]	
Loss of consciousness	≥1 g, iv	56
Convulsions	≥1 g, iv	56

[a] iv = intravenous.
[b] Effect looked for, but not observed at any dose or by any route.

and SGOT in 4 of 29 subjects, positive thymol flocculation test in 4 subjects, and positive urobilinogen test in 11 subjects. This occurred when substantial oral doses (2 g twice a day) were administered for 6 mo. In the case of TMB-4 chloride, hepatic enzyme changes were accompanied by icterus, petechial bleeding, and prothrombin times of 40% and 50% of normal, respectively, in two of 11 subjects. All adverse effects were marked by the time TMB-4 chloride had been administered for 6 wk orally at increasing rates of 0.8-2.3 g. All symptoms except icterus subsided completely in 1 or 2 wk after administration was stopped; clearing of icterus required 3-6 wk. The investigators considered the icterus similar to that produced by chlorpromazine.[20]

GASTROINTESTINAL EFFECTS

Direct effects of oximes on the gastrointestinal tract are related entirely to the route of administration--they are seen only after oral administration. Ingestion of tablets of 2-PAM or related salts is often accompanied by transient diarrhea and cramps. The full explanation is not known. In the case of P2S, it has been suggested that a dose of 20 mg/kg inhibits ATPase and therefore produces excess intestinal fluid and diarrhea. The effect depends on concentration and is reversible. 2-PAM has no effect on cyclic AMP (cAMP), adenylate cyclase, or phosphodiesterase.[66]

Repeated oral administration of 2-PAM or TMB-4 in dogs may lead to areas of acute necrosis and to deposition of fibrous tissue in the stomach.[1] Whether those effects are due to a specific action of the oxime or to chronic irritation related to repeated administration is not known. Nor are the long-term effects of such chronic administration and consequent fibrosis known. However, single doses, even orally, have not been reported to produce tissue changes. No change was found in the gastrointestinal tracts of rats given P2S (200 mg/kg) orally each day for 50 d.[23]

Large doses of 2-PAM or toxogonin (at 7100 mg/kg) cause death that may be associated with acute enteritis,[37] but small doses do not seem to produce tissue changes.[23] With small doses, enteritis is not sufficiently severe to cause death.

CARDIOVASCULAR EFFECTS

The adverse effects of cholinesterase reactivating chemicals on the human cardiovascular system are shown in Table 2-2, Table 2-3, Table 2-4, Table 2-5, Table 2-6 through Table 2-7. These effects may be classified as hypertension characterized by moderate increased systolic and diastolic pressure with hypertension followed by hypotension, and ECG changes.[20,61,97,102,107] The

occurrence of adverse cardiovascular effects depends on dosage and route of administration. In general, these effects were seen when large doses of the cholinesterase reactivators were given intravenously or intramuscularly or after repetitive oral doses. Although therapeutic doses of toxogonin produced no effects on heart rate or blood pressure in humans, 2-PAM caused a significant increase in cardiac output in dogs.[21] 2-PAM increases blood pressure in experimental animals and humans.[18,20,38,60,67,103,118] It causes an increase in mycocardial contractility in experimental animals[85] and cardiac arrythmias when used in humans to treat organophosphorus insecticide poisoning.[18,24]

The mechanisms by which 2-PAM exerts its cardiac effects have been studied in experimental animals. At least three classes of action have been attributed to the effects of altered calcium metabolism on autonomic ganglia. A sympathomimetic action of 2-PAM was postulated to explain the increase in blood pressure and the augmented myocardial contractility by one or more of the following mechanisms: 2-PAM may not block the release of the endogenous compounds, but may prevent the uptake of catecholamine;[10] it may stimulate the release of norepinephrine;[18] it increases myocardial contractility by directly stimulating beta receptors;[38] and it increases blood pressure by directly stimulating alpha receptors.[85]

2-PAM increased the contractile force of stimulated rabbit atria that did not result from an increase in the frequency response.[21] Similar results were obtained in rabbits pretreated with reserpine, a catecholamine-depleting agent. These findings are in agreement with previous reports that the cardiovascular effects of 2-PAM on anesthetized dogs were not due to a release of tissue catecholamine.[38,103] other studies, however, have shown that 2-PAM affects blood pressure in vivo by changing the release or metabolism of endogenous norepinephrine.[10,118]

Most attention has been focused on adrenergic receptors. Recent study of the mechanism of the effect of 2-PAM on cardiac action in rabbit atria indicated that 2-PAM exerts a direct action on vascular smooth muscle that is not mediated through stimulation of the alpha receptors.[21] These results have led to the conclusion that the effect of 2-PAM on the cardiovascular system cannot be explained entirely by its sympathomimetic effect. This conclusion was supported by the finding that 2-PAM caused an increase in the contractile force of isolated aortic strips from rabbits and the finding that phentolamine, an alpha-adrenergic blocking drug, did not affect this response of the aorta. These results contradict the previous finding that phenoxybenzamine, an adrenergic blocking drug, decreased the pressor effect of 2-PAM.[118] The latter finding may be explained by the inhibiting effect of phenoxybenzamine on the druginduced calcium movement in vascular smooth muscle.[94]

Conflicting results have been reported in regard to the involvement of beta receptors in the mechanism of the inotropic effect of 2-PAM. It was concluded that the direct stimulation of the myocardium by 2-PAM that could be blocked by dichloroisoproterenol (DCI) was responsible for the increase in blood pressure.[38] A recent report, however, has indicated that the increase in blood pressure caused in the dog by 2-PAM was not blocked by propranolol.[103] Similar results were obtained with rabbit atria in vitro[21] in a study that also showed that 2-PAM augments muscle contractility and alters calcium movement.

The studies reported above implicated calcium movement in the mechanism.[21] It was postulated that 2-PAM may increase the rate of calcium movement either by interfering with calcium binding to its binding site or by directly stimulating the release of bound calcium.[21] It was suggested that 2-PAM may not have increased cell permeability or affected the rate of re-entry of calcium into the cell, or 2-PAM may have increased calcium distribution in the vicinity of myofilaments.

Studies of the efflux of ^{45}Ca by stimulated rabbit atria have characterized three calcium pools. Phase I may represent extracellular washout of the ^{45}Ca that binds to the surface of muscle membrane and is characterized by a high rate constant. Phase II may represent loosely bound calcium present in cell membrane and calcium released at the sarcoplasmic reticulum. Calcium in this pool is directly related to contractility.[65,84,93] Phase III may represent the tightly bound calcium that exchanges very slowly and does not play a role in maintaining calcium concentrations. Recent study has shown that the storage or release of calcium at the sarcoplasmic reticulum and other loosely bound calcium sites (cell membrane) that are involved in muscle contractility can be directly affected by 2-PAM.[21] These results indicate that 2-PAM increases the rate of release of Phase II calcium.

It has been postulated that 2-PAM exerts its cardiac action in rabbit atria through its alteration of calcium metabolism. The relaxation phase of skeletal muscle contraction seems to be directly affected by the sarcoplasmic reticulum because of its ability to sequester calcium actively.[29,46] A similar role has been suggested for the sarcoplasmic reticulum in cardiac muscle.[46,83] The onset of muscle contraction takes place when calcium reaches a critcal concentration. This contraction is later reduced by the increased calcium-sequestering activity of the sarcoplasmic reticulum. Thus, 2-PAM can affect this process by decreasing the rate of calcium uptake by the sarcoplasmic reticulum, which results in increasing the time required to reduce the calcium concentration enough to allow relaxation to take place. This was demonstrated by the increase in the relaxation phase. It was suggested that this

delay in binding of calcium to the sarcoplasmic reticulum may increase the active state that increased the contractile force.

2-PAM either activates or blocks autonomic ganglia, depending on dose and route and speed of administration. The changes in ganglionic function induced by 2-PAM will be reflected in sympathetic and parasympathetic activity, which may cause changes in cardiovascular functions. These are discussed further in the next section.

NEUROPHARMACOLOGY

The four oximes have, in addition to reactivation of phosphorylated AChE, a variety of actions that can be demonstrated in the absence of previous exposure to organosphosphorus compounds. Experiments that involved only short-term exposure to high concentrations of 2-PAM showed the following short-lived and reversible actions of oximes in nervous tissues: inhibition of AChE, agonist-antagonist effect on cholinergic receptors, ganglionic blockade, and presynaptic actions, such as modification of the release of acetylcholine (ACh) from the nerve terminal.

Effects of Oximes on AChE Activity

ACh contraction of frog rectus abdominis muscle was potentiated by 2-PAM, and progressive AChE inhibition was seen with increasing concentrations of 2-PAM. 2-PAM (at 1.5 x 10^{-3} M) produced a 66% inhibition.[36] This is far higher than the concentration that would be produced in vivo by administration of ordinary doses. Similar effects were seen on isolated rabbit intestine and atrium[15] and on chicken muscle.[22] In phrenic nerve diaphragm preparations of rat, 2-PAM increased the neuromuscular block induced by depolarizing blocking agents and antagonized the nondepolarizing block induced by curare.[36]

Receptor Interactions

Receptor interactions were seen at nicotinic and muscarinic receptors. No correlations were found, however, between atropine-like or curare-like actions of these compounds and their protective effects against organophosphate poisoning.

Nicotinic Receptor Interactions. After producing an initial brief augmentation of the twitch response and fasciculations,[63] 2-PAM and TMB-4 at 1-20 mg/kg abolished the indirectly stimulated twitch response of skeletal muscle, but did not alter the response to direct stimulation. Neostigmine and edrophonium antagonized, whereas

d-tubocurarine increased these effects, which are due to competition for nicotinic acetylcholine receptor sites.[9] Both agents antagonized the response to decamethonium and reduced the effects of carbamoylcholine.[36]

Muscarinic Receptor Interactions. Excitatory muscarinic effects, such as temporary stimulation of salivation and stimulation of intestinal peristalsis, were seen with 2-PAM. Atropine-like actions were seen at high concentrations (15-20 mg/kg or more), and, when injected rapidly, 2-PAM caused temporary diplopia (nicotinic block) and loss of accommodation in the eye.[56] Both TMB-4 and 2-PAM blocked bradycardia induced by vagal stimulation. At low concentrations, neither compound affected normal intestinal peristalsis, but they did block peristalsis caused by increased vagal stimulation. TMB-4, 2-PAM, and toxogonin antagonized the effect of acetylcholine, acetyl- -methylcholine, and other agonists on isolated guinea pig ileum.[62]

Ganglionic Blocking Action

Doses of 2-PAM larger than 40 mg/kg, as well as TMB-4 and toxogonin, produced a temporary block of the cardiac response to vagal stimulation and of the nictitating membrane response to preganglionic, but not postganglionic, stimulation. There was transient hypotension due to block of ganglionic transmission.[9,63,71]

Presynaptic Effects of Oximes

Concentration-dependent presynaptic effects of 2-PAM on the release of acetylcholine from terminals of nerves innervating the rat diaphragm muscle were seen; at concentrations of 10^{-4}-10^{-3} M 2-PAM stimulated the release of acetylcholine; higher concentrations led to a total block of the evoked release of acetylcholine.[30,40]

MUTAGENIC, REPRODUCTIVE, AND CARCINOGENIC EFFECTS

No information on mutagenic, carcinogenic, reproductive, or teratogenic effects of any of the compounds in question is available.

None of the compounds or their in vivo intermediates is likely to bind covalently with DNA and other macromolecules. Other ways of binding with DNA, such as intercalation, cannot be ruled out. There is some membrane transport, albeit limited. It is not possible, therefore, to conclude that they are not mutagens, teratogens, or carcinogens.

THERAPEUTIC USE

Little information is available on the treatment of humans exposed to military agents. However, pralidoxime is an FDA-approved marketed drug in the U.S. and there is substantial experience with therrapeutic use on civilians exposed to agricultural organophosphorus products.

That poisoning by diethoxy compounds can be treated effectively by oximes is nearly universally agreed.[55,59,82,87,92,109] There is general agreement that the therapeutic effects of oximes are less spectacular in poisoning by dimethoxy compounds as a group,[87] and several reports[7,8,53,77,105] indicate lack of efficacy. However, oxime treatment of patients poisoned by dimethoxy compounds has been beneficial in many cases.[3,12,13,79,87] Thus, 2-PAM in particular and oximes in general are recommended for therapeutic use in severe poisoning by either diethoxy or dimethoxy phosphorus compounds.[47]

Two factors contribute to the safety of oximes in the treatment of acute poisoning by organophosphorus compounds: recommended doses are small, compared with doses likely to cause even mild toxic effects in normal subjects; and adverse effects of oximes are reduced in the presence of poisoning by an organophosphorus compound.

More reports deal with 2-PAM than with other oximes in the treatment of poisoned patients. High dosages have been used in patients who survived without sequelae. Milthers et al.[77] reported the administration of 21 g. Gitelson et al.[39] administered total doses as high as 24 g in 6 d. Namba[81] reported the use of 40.5 g in 7 d, with 26 g in the first 54 h. Hiraki et al.[49] administered 65 g in 16 d. Warriner et al.[110] gave 92 g in 23 d; this case is especially instructive, because the patient suffered a relapse on the tenth day of illness after his dosage was reduced, but responded favorably to a temporary return to full dosage (12 g/d).

In one case of successful treatment with toxogonin, 16 doses of 250 mg each (total, 4 g) were administered during the first day of illness and somewhat smaller doses later.[5]

DELAYED AND LONG-TERM EFFECTS

Appendix B reviews some important animal studies of cholinesterase reactivator chemicals. The extensive literature reviewed offers little definitive information with which to project possible long-term effects or delayed sequelae in human subjects tested at Edgewood. These compounds have a short biologic half-life of 1 to 3 h. However, no chronic studies were found. Consequently, the carcinogenic potential of cholinesterase reactivators remains

unknown.[61,96,98,99] Acute effects have been reported that may be rev ersible, but these require additional study. Reports in the Polish li terature (see Appendix B) that single exposure to obidoxime (toxogonin) may affect the integrity of renal tubules need exploration and confirmation. These findings certainly raise questions about the long-term use of these compounds in humans. Another finding that needs further investigation, substantiation, and extension is the apparent production of muscle necrosis from intramuscular injection of 2-PAM[1,95] and P2S.[1] It is not known to what extent and under what circumstances this effect occurs in humans. Oral administration of cholinesterase reactivators for up to 17 wk produced erosion of the mucosa along ridges of the rugae in the fundus of the stomach and fibrosis in vicinities of the cardiac and pyloric sphincters.[1] The finding that an azotemic subject had markedly decreased clearance from blood[56] is of interest, although probably not pertinent to the Edgewood studies on human subjects, because the volunteers were in good physical condition when tested. These findings require further analysis to determine dose-frequency and dose-response relationships and their potential significance with regard to human experiments conducted at Edgewood.

EFFECTS ON VOLUNTEERS

The medical records of the volunteers who received cholinesterase-reactivating chemicals consisted of the test protocol, physicians' orders, nursing notes (including clinical observations), a checklist of symptoms, and laboratory and performance test results. The reports of physicians' examinations and physical findings were generally not included. Volunteers were identified by number. The Committee on Toxicology's assessment was based on records and summaries provided by the Department of the Army and NRC staff. The procedures were described fully in Volume 1. In most cases, the analysis was based on summaries of drug administrations prepared by a consultant to the Panel.

The number of subjects tested with each compound, the number of records examined, routes of administration, and doses are shown in Table 2-8, Table 2-9, Table 2-10 through Table 2-11. In some instances, the compounds were given before or after anticholinesterase compounds or in conjunction with other drugs; thus, some of the results shown here were also presented in Volume 1.

The data on TMB-4 (Table 2-11) are difficult to interpret, because, in the instances on which clinical data are available, other potent drugs were given as well.

The manifestations experienced by the subjects in these tests (Table 2-8, Table 2-9, Table 2-10 through Table 2-11) were the moderate clinical effects that

TABLE 2-8 Manifestations after Administration of 1-7 Doses (on Different Days) of 2-PAM (Chloride Form) to 79 Subjectsa

Manifestations[b]	Intravenous,5 mg/kg, to total of 500 mg, N = 29	Oral, 5-7 g total, N = 13	Intramuscular, 2.5 mg/kg, to total of 600 mg, N = 37c
None	0	6	0
Dry mouth[b]	2	--	--
Dizziness	23	1	--
Diplopia	8	--	--
Eye discomfort	12	--	--
Blurred vision	9	1	5
Mood elevation	1	--	--
Voiding difficulty	2	--	--
Nausea	6	--	1
Vomiting	1	--	--
Uric acid increase	1	--	--
Faintness	1	--	--
Claustrophobia	1	--	--
Abdominal cramps	1	--	--
Diarrhea	--	3	--
Muscle pain	--	--	37
Tachycardia[b]	--	--	1
Grand mal seizured[d]	--	--	1

[a] Task plan: absorption efficacy of 2-PAM (chloride form) as function of pH (intramuscular studies). Studies conducted: plasma and urine content, renal clearance, CNS effects, blood pressure, and in some cases, pupil size and number facility. Test conditions: heat and exercise in some tests. Clinical evaluation tests: uric acid, creatinine, BUN, SGOT, and CPK in serum. Records on 79 subjects selected, on basis of high dose or high frequency of administration, from records on 607 subjects tested.
[b] Dry mouth and increased heart rate were associated with atropine administrated to some subjects.
[c] Several subjects received atropine in addition to 2-PAM.
[d] Subject 6849 (see text).

TABLE 2-9 Manifestations after Administration of Toxogonin to 41 Subjectsa

Manifestations	Intravenous,[b] 0.5-1.0 µg/kg, 60-128 mg infused, N = 11	Oral, 1-9 g total, N = 20	Intramuscular, 2.5-10 mg/kg, N = 10
None	0	3	0
Hot, cold, numb, tingling sensation	10	9	10
Eye discomfort	1	2	2
Dizziness	1	2	1
Dry mouth	--	--	2
Local pain	--	--	10
Peculiar taste	--	10	1
Drowsiness	1	5	--
Blurred vision	1	1	--
Headache	--	3	--
Nausea	--	3	--
Vomiting	--	1	--

[a] Records on all 41 subjects tested were summarized.
[b] Five subjects, single injection; six subjects, infusion as total dose in combination with aminohippuric acid (PAH).

TABLE 2-10 Manifestations after Administration of P2S to 75 Subjectsa

Manifestations	Intravenous, 5 mg/kg, N = 3	Oral 2-9 g total, N = 49	Oral, Before Nerve Agent, 1-3 g total, N = 12	Intramuscular After Nerve Agent, 1-2 g total, N =11
None	3	38	11	10
Headache (slight)	--	2	--	--
Voiding difficulty	--	4	--	--
Diarrhea	--	4	--	--
Stomach ache	--	2	--	--
Nausea	--	1	--	--
Ventricular extrasystoles	--	--	--	1
Anxiety, agitation, vomiting	--	--	1[b]	--

[a] Clinical tests included CBC, blood oximes, creatine, and urinalysis. Results were in normal range after transitory manifestations. Nerve agent resulted in decrease in RBC cholinesterase content. Records on 75 of 95 subjects tested were summarized; each subject was tested once with P2S.
[b] Subject 2307 (see text).

TABLE 2-11 Manifestations after Administration of TMB-4 to 24 Subjectsa

Protocol	No. Subjects
TMB-4 alone	6[b]
With intravenous soman	1
With intravenous soman and P2S	1[c,d]
With percutaneous VX and atropine	2
With percutaneous VX, atropine, and 2-PAM (chloride)	1
TAB[e]	3[d]
TAB and heat stress	2[d]
TAB and physostigmine	4
Manifestations[f]	**No. Subjects**
None	--
Dry mouth	9
Blurred vision	6
Lethargy, sluggishness	6
Dizziness	4
Dreaming (hallucinations)	3
Nausea	2
Restlessness	2
Muscle twitching	2
Heat, flush	1
Headache	1
Abdominal discomfort	1
Anxiety, agitation, vomiting	1[c]

[a] Records on 24 of 34 subjects tested were summarized; clinical data available on 11, only laboratory data on eight, and no data (except RBC cholinesterase content) on five.
[b] Clinical data available on one.
[c] Subject 2307; see text.
[d] Clinical data available.
[e] TAB = TMB-4, atropine, and benzactyzine.
[f] Among 11 with clinical data.

have been reported in the literature, and they are discussed in Appendix B. In all but two instances (described below), moderate effects disappeared within 24 h.

One volunteer (2307) received 2 g of P2S and 1 g of TMB-4 orally 68 min before being given 1.5 µg of soman by intravenous infusion. He had minor symptoms, but in 12 h became anxious, restless, and agitated. He was transferred to Walter Reed Hospital. His Multiphasic Multiple Personality Inventory (MMPI) and psychiatric interview before exposure were described as "doubtful in regard to suitability, but not grossly pathological." Postexposure physical and neurologic examinations showed no organic signs of neurologic disease. The discharge diagnosis from Walter Reed Hospital in 1961 specified acute, moderate anxiety reaction manifested by restlessness, anxiety, agitation, and hysterical reaction. He has experienced further problems requiring inpatient and outpatient psychiatric care. The Veterans' Administration diagnoses between 1966 and 1980 noted infantile personality with strong paranoid trends, organic brain syndrome, and severe anxiety neurosis with depression. This subject was also noted in Volume 1 (p. 30).

A second subject (6849) experienced a grand mal seizure 3 h after receiving 300 mg of 2-PAM (chloride form) intramuscularly. He regained consciousness within 5 min; he had bitten his tongue. He was initially lethargic, but felt well 10 h later. He was transferred to Walter Reed Hospital, but followup records are not available. He had received 300 mg of 2-PAM intramuscularly 5 and 8 d before the episode. The only symptom on those occasions was local pain at the injection site.

Although 2-PAM is an FDA-approved drug, data submitted to the FDA to obtain approval are proprietary and were not released when requests for them were made to the FDA and to the manufacturer. A report of the FDA Adverse Drug Experiences Monitoring Program (October 30, 1983) contained one adverse reaction (hypotension following 1 g by intravenous injection).

In summary, with the possible exception of those two cases, the records contained no evidence of delayed or persistent effects after administration of the cholinesterase reactivators. Such data cannot, however, address the issue of long-term effects or delayed sequelae.

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CONCLUSIONS

On the basis of an examination of toxicologic literature, case reports from Edgewood volunteers, and a review of mortality data (reported in Volume 1), the Committee found no evidence of chronic disease in animals or humans associated with single or repeated doses of the cholinesterase reactivators (oximes). The paucity of chronic-exposure data from animals and the lack of followup data on volunteers prevent certainty in predicting occurrence or absence of delayed effects. The compounds are eliminated very rapidly from the body, but they produce a variety of acute effects that are short-lived and reversible, such as gastrointestinal distress following oral administration, pain at an injection site, dizziness, headache, and ocular discomfort. The Committee found no data on the basis of which to determine or rule out carcinogenicity, mutagenicity, teratogenicity, or reproductive effects of the four oximes and therefore did not reach a conclusion in this area.

3

PSYCHOCHEMICALS

BACKGROUND

Military interest in psychochemicals stems from the late 1940s. L. Wilson Green of the Chemical Corps Technical Command at Edgewood proposed that modern military use of psychochemicals might permit the conquering of an enemy without the need for weapons of mass destruction. Such use, he suggested, might reduce the wholesale killing, human misery, and property destruction normally experienced in warfare. He proposed a search for a stable chemical with the capacity to produce mental abnormalities of military importance; 61 chemicals were suggested as a starting point for this search.[1]

Over the next few decades, scientists at Edgewood engaged in toxicologic and clinical evaluations of the biologic effects of a wide variety of chemicals that could alter the state of mind or mood, largely by affecting the brain. Among those tested on human volunteers were LSD, a hallucinogen; BZ (3-quinuclidinyl benzilate) and related anticholinergic compounds; phencyclidine, an anesthetic with marked disorienting after-effects; and dibenzopyrans, CNS depressants with powerful capacity to produce orthostatic hypotension.

LSD and the anticholinergics were the subjects of earlier extensive evaluations.[2,3] This chapter is concerned only with phencyclidine and dimethylheptylpyran (dibenzopyran) and its isomers.

Table 3-1 lists the compounds tested, approximate numbers of subjects, routes of administration, and dosages.

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VOLUNTEER SCREENING, SELECTION, AND CLASSIFICATION

In the clinical charts at Edgewood, severe adverse psychologic reactions to SNA or to the cannabinoids appeared less common than one might expect from experience in civilian laboratories. If these findings were valid, they might be accounted for by subject selection procedures and special characteristics of the experimental milieu.

Studies of workers often find that workers are healthier than the general population. This “healthy-worker effect” is generally ascribed to the fact that the working population is in better overall health than the general population, which includes the very sick, the elderly, and the hypersusceptible.

The selection of volunteers for testing at Edgewood most likely introduced a “healthy-test-subject effect” into the study. In fact, those selected for exposure to the test chemicals were healthier than those used as controls or used in nondrug tests of equipment. The control groups consisted of those who were rejected for drug testing and thus possibly less healthy. Because the exposed subjects were healthier at the start than the nonexposed subjects, comparisons between these two groups may well yield results that understate the relative risk to the exposed subjects. For the study of neurologic processes and psychologic functioning, subtle effects i subjects would not be readily evident in a comparison of them with the less healthy, nonexposed subjects.

In addition, there was a great deal of preselection, in that all the subjects were soldiers--healthy enough and functioning well enough to meet the criteria for entry into the Army. A detailed set of guidelines, most completely spelled out in a document dated August 12, 1968, described a standard operating procedure in the clinical research department (Appendix A, part 2) for forming the exposure group. Multiple criteria were used in the psychologic screening of volunteers. A “yes” answer on any of various items in the medical history without explanations based on further examination by a medical officer would routinely be cause for rejection. When General Test (GT) scores were in the very low range (below 90 or 80), the volunteer was rejected.

Minnesota Multiphasic Personality Inventory (MMPI) profiles were used as approximate guidelines or rules of thumb, rather than to

TABLE 3-1 Psychochemicals Tested on Human Volunteers at Edgewood

Military Designation	Tox. No.	Chemical Type	Common Name	Abbreviation	CAS No.	No. Subjects Tested	Dosage, µg/kg	Route[a]
EA 1476	H-1	Dibenzopyran	Dimethylheptyl pyran	DMHP	32904-22-6	38	0.4-4,000	PO
EA 2233	H-2	Dibenzopyran	Dimethylheptyl pyran	DMHP	39624-99-2	95	0.5-5.4	IM
	0.5-8.3	IV						
	2.5-51.4	PO						
EA 2233-1	H-3	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-75-5	23	1-10.5	IM
EA 2233-2	H-4	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-76-6	15	0.5-7.5	IV
EA 2233-3	H-5	Dibenzopyran	Dimethylheptyl pyran	DMHP	15077-23-3	20	1.0-10.5	IM
EA 2233-4	H-6	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-77-7	16	0.7-7.8	IV
EA 2233-5	H-7	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-78-8	6	0.7-3.5	IM
EA 2233-6	H-8	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-79-9	6	0.7-6.4	IM
EA 2233-7	H-9	Dibenzopyran	Dimethylheptyl pyran	DMHP	15206-43-6	4	5.3-6.4	IM
EA 2233-8	H-10	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-80-2	6	0.7-3.5	IM
EA 2233-24	H-11	Dibenzopyran	Dimethylheptyl pyran	DMHP	39624-99-2	30	1.2-2.5	IV
EA 2148-A	C-1	Heterocyclic amine	Phencyclidine	SNA	956-90-1	29	2.5-43	PO[b]

[a] PO = oral; IM = intramuscular; IV = intravenous

[b] Some subjects exposed to aerosol at 250-888 mg/m^3; Cts 249-2,955 mg·min/m^3.

provide firm cutoff points. In general, any prospective subject who had five or more clinical scales on the MMPI above 65 units was not included in the experiments. A volunteer with high L and K scales was considered sutiable for the experiments only after careful review of family history and of other indication of possible psychologic problems. Any subject with high PD, PA, and SC scales (psychoticism) was rejected, as was a subject with high PD, MF, and MA patterns (sociopathic deviation), particularly if there was a history of "acting out." Careful review of the overall clinical picture, history, etc., took place if the HS, D, HY, PT, and SI patterns (neuroticism) were high. Apparently, exceptions were made--for example, if an ambitious college graduate had high PD and MA scales, but no history of acting out. In doubtful cases, corroborating evidence from the family history was reviewed. Particular attention was paid to the family and developmental histories of prospective volunteers, especially a history of trouble in school, contact with a psychiatrist for anything other than routine screening, fighting in association with drinking, and overt expressions of hostility.

Screening of the histories and MMPI profiles took place before arrival at Edgewood. After arrival, volunteers were interviewed by officers in the Psychopharmacology Branch. On the basis of further testing (Sentence Completion and Picture Frustration tests), physical examination, and interview, subjects were classified on a four-point scale. Those rated A were considered suitable for psychochemical testing; those rated B were suitable for low-dose psychochemicals only; those rated C were not suitable for psychochemicals; those rated D were suitable for equipment testing only. The main criteria for an A or B rating were absence of evidence of psychologic problems, absence of a tendency to somaticize or act out intrapsychic tension, good ego strength, flexibility, maturity, good sense of identity, normal MMPI, and family history. Subjects who seemed to be particularly at ease when handling anxiety and hostile or aggressive impulses were rated A+--suitable for psychochemical tests considered to be of greater than usual stress. Those rated B were similar to the A group, but had had occasional experiences that suggested less control or minor personality disturbances. Any subject who showed a tendency toward psychosomatic reaction or aggressive acting out, who appeared to be dull or nonverbal, or who had obvious neurotic traits, immaturity, or rigidity was not included in any psychochemical experiments.

The criteria described in the standard operating procedure appear to be those used either consciously or deliberately in many civilian laboratories that conduct research with psychoactive drugs with normal volunteers. It appears that the subjects actually given psychochemicals in these experiments were selected from an optimal pool of mentally and physically healthy persons.

Apparently, the precise configuration and staffing of the research unit changed over the years during which these experiments occurred. Facilities for the subjects included a controlled environment with padded rooms (to protect subjects from harm from hypotension) and an adjacent communal area. During the first hours of the experiment, each subject was in his own padded area with a padded stool. Each subject had a nurse or aid in the room during his progress. Later, the subjects were allowed access to dayroom facilities, where they played cards. When further along in recovery, they could play table tennis. Throughout the entire recovery process, they could be observed through a window.

The notes, comments, and data in the charts reflected what must have been a supportive and well-staffed research unit. The only negative comments from subjects in the charts had to do with the quality or preparation of the food. Many positive comments reflecting careful attention, support, and, in general, informed participation in the experiments occurred throughout the charts over the years. Retropective chart reviews always involve guessing and speculation. A best guess is that a supportive atmosphere and careully screened and well-informed (with respect to experimental procedures, goals, pitfalls, etc.) subjects were important in determining how well these volunteer subjects tolerated the experiments.

It is difficult to generalize about the experimental design used in studies of multiple drugs and spanning 10 yr or more. Some generalizations are relevant in assessing the quality of data, pattern of effects, and possible consequences. In the usual sequence of experiments, the effects of low doses were investigated first in a few volunteers, particularly when the route of administration was being changed or when a compound that had been studied previously only in animals (such as the cannabinoids) was being studied. Then, depending on the pattern and duration of effects, a small to moderate-sized group of subjects were tested with a few doses in what appeared to be a safe but pharmacologically active range. Later studies followed up on interesting or possibly worrisome side effects, such as borderline changes in hepatic or renal function. Particularly in later studies based on earlier observations, interventions were made with assumed antidotes or antagonists; for example, drugs that increased blood pressure were given in conjunction with or after dimethylheptylpyran to investigate the intriguing and important postural hypotension.

The protocols appeared to be flexible and generally conservative, with variations often following up preliminary observations. A critical and skeptical reviewer, in retrospect, might say that there was too great emphasis on browsing and that the changes in protocol, with small groups tested under any single protocol, precluded definitive conclusions. These experiments were analogous to the Phase 1 clinical trials in human beings now conducted with therapeutic drugs,

in which examination of small groups, attention to borderline biochemical abnormalities, revision of protocols, and attention to hunches about mechanisms of drug action, dose, etc., are appropriate research strategies.

Placebo controls were not used and were probably not appropriate, given the goals of the research. One must remember that when these studies began, in the early 1960s, psychopharmacology, particularly optimal research strategy and design as we know them today, was truly in its infancy. Not until the mid-1960s was there a general consensus in a minimally acceptable design for studying psychochemicals, and even now there may be disagreement. The experimental design used in the experiments at Edgewood compares favorably with the pharmacologic research at other research centers.

In the Edgewood studies, SNA was administered intravenously, orally, and by inhalation.

Intravenous doses of 0.1 mg/kg given to 10 volunteers produced onset of physiologic and psychologic effects within 3-5 min of injection that peaked about 10 min to 1 h after injection. Most of the symptoms were nonmeasurable 5-6 h later. As in all studies with SNA, individual variation was great. For example, of the 10 volunteers given intravenous SNA, four became withdrawn and drowsy and answered when spoken to, but otherwise were silent. The effects on proprioception were manifested by limb numbness, vertigo, ataxia, and a feeling of detachment. Various degrees of amnesia regarding the events after the first hour after injection were common. Nausea was common, but considered to be less than expected by the investigators, possibly because the subjects were nonambulatory. Increases in systolic and diastolic blood pressure of up to 20 or 30 mm Hg occurred soon after injection and lasted for 2-3 h. It was judged by subjects and observers alike that SNA would impair performance in a military field situation.

After oral doses of up to 30 mg (0.48 mg/kg), similar signs and symptoms appeared in a single test subject. At 30 mg, approximately the first plane of stage three anesthesia was produced. The one subject used was unresponsive to sensory stimuli, although corneal reflexes were intact. Blood pressure was slightly increased. An hour after the drug administration, he was responsive to stimuli, but still groggy; 8 h later, he had recovered almost totally, but was amnesic regarding the events of the hours after the drug was administered. Two volunteers given a combination of SNA (20 mg) and alcohol had intense “manic” reactions, with much agitation and restlessness. Perceptual and cognitive effects lasted 4 d in one and 2 d in the other. At lower oral doses (5 and 10 mg), volunteers described their subjective feelings as similar to those of alcohol intoxication. Skin temperature and heart rate increased slightly. Vertigo, ataxia,

weakness, and nausea were mild and not sufficient to interfere with treadmill exercises that were part of the experiment. Subjects appeared slow to think and slow to make decisions and reported a subjective slowing down of time. The subjective effects peaked at about 2-4 h after oral administration and, after the 10-mg dose, were still present to a slight degree at 13-14 h. After the 5-mg dose, effects were generally milder, and subjective symptoms were gone in about 8 h. Perceptual motor functioning, as judged by Purdue pegboard and Minnesota rate manipulation tests, was definitely impaired after the 10-mg oral dose, markedly impaired after 15 mg, and nearly absent after 5 mg.

REVIEW OF AVAILABLE INFORMATION ON PHENCYCLIDINE

CHEMISTRY

Phencyclidine (Sernyl, SNA)--1-(1-phenylcyclohexyl)piperidine, $C_{17}H_{25}N$ (molecular weight, 243.38)--is an arylcyclohexylamine. It is used as the hydrochloride, $C_{17}H_{25}N$ HCl (molecular weight, 279.84), which is crystalline with a melting point of 214-218°C. Its hydrobromide salt is also crystalline with a melting point of 214-218°C. The hydrochloride is soluble in water, methanol, ethanol, aniline, and methylene chloride, and the base is almost insoluble in water and soluble in toluene, methanol, ethyl acetate, kerosene, and methylene chloride. The base (SNB) is crystallin melting point of 46-46.5°C.

The chemical structures of SNA, ketamine, and SNB are shown in Figure 3-1.

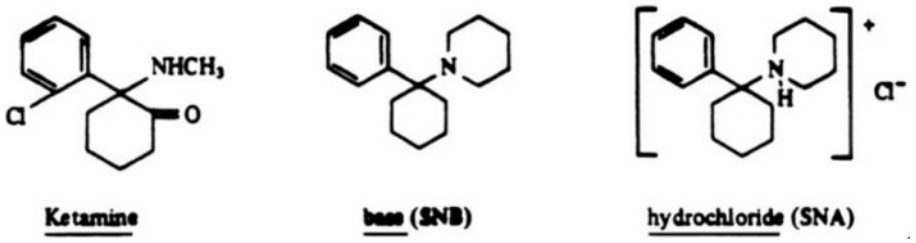

FIGURE 3-1 Structure of ketamine, SNA, and SNB.

SNA was originally synthesized and developed as an anesthetic agent for human use by Parke, Davis and Co. under the name Sernyl.[13,14 and 15] Its human use was soon abandoned, because it sometimes produced postoperative thought disturbances and agitation. It is currently used, under the name Sernylan, as an immobilizing agent in veterinary medicine.

In pharmaceutically pure form, SNA is a white powder that dissolves readily in water. When distributed illicitly, phencyclidine,

often called PCP, is highly variable in appearance (powder or tablets in many colors or as liquid), contains many impurities, is often adulterated, and quite often is misrepresented as another drug, such as marijuana constituents, mescaline, psilocybin, lysergic acid diethylamide (LSD), or even amphetamine or cocaine.

The compound tested at Edgewood was pure phencyclidine provided by a pharmaceutical manufacturer and should not be confused with the substance sold on the street as PCP.

The structure of SNA was modified by replacement of its phenyl, cyclohexyl, and piperidine rings and by introduction of substituents onto those rings. Replacement of the phenyl ring with a thienyl ring increased central activity, but bulkier aromatic rings were inactive.[39] Replacement of the piperidine ring by $NHCH_3$ and some substitutions in the other rings led to the development of ketamine, an effective dissociative anesthetic agent.

ABSORPTION, FATE, AND ELIMINATION

Two recent reviews[3,22] contain considerable information on the absorption, distribution, metabolism, and excretion of phencyclidine.

Absorption

Early studies in experimental animals and human subjects[46] established that SNA is well absorbed when administered by inhalation, percutaneously, intraocularly, orally, intramuscularly, intravenously, and intraperitoneally. Dogs exposed to aerosols generated from a 15% aqueous solution of SNA developed prostration, hypersalivation, and exophthalmos; approximately 50% of the dogs had tremors and convulsions. SNA and SNB were dissolved in a number of vehicles, and these preparations were applied to the skin of rabbits. Ataxia confirmed the percutaneous penetration of the drug. This clinical sign also occurred after an alcoholic solution of the base or the salt was instilled in the eyes of rabbits. A human volunteer given SNA orally at 0.48 mg/kg reached plane 1 of stage 3 anesthesia 1 h after administration. Intravenous administration of SNA at 0.01 mg/kg in 10 male volunteers resulted in onset of mental and physical effects within 3-4 min. Nineteen men were given aerosolized SNA in methylene chloride solution. At an exposure as low as 250 $mg \cdot min/m^3$, one subject developed visual disturbances and light-headedness in 7 min and perioral and distal paresthesias within 10 min. Inadvertent exposures by inhalation have been reported. Aniline et al.[4] found SNB in the blood of a 65-yr-old woman who occupied a second-floor apartment directly above a clandestine laboratory making SNB by an open-vat process. Pitts et al.[52] reported

intoxication in two chemists, employed in a law enforcement laboratory, who handled confiscated samples of SNA.

Absorption through the placenta has been shown in mice and rabbits[50] and is likely to occur in humans.[30] In the mouse, SNA concentration was 10 times higher in fetal tissue than in maternal blood. Furthermore, SNB appeared rapidly in the milk, reaching concentrations 10 times those in maternal plasma. Fetal SNA concentrations in the rabbit peaked 12 h after parenteral administration to the dam. A neonatal infant whose mother had used SNA during pregnancy manifested abnormal behavior consistent with effects often seen with this drug. However, blood SNB concentrations were not measured in either child or mother. Aniline and Pitts studied three women who used SNA during pregnancy;[3] blood and urine SNB concentrations were determined. Concentrations in cord blood were 2-3 times higher than those in the mother in all cases.

Distribution

In Sprague-Dawley rats given SNA at 50 mg/kg intraperitoneally, concentrations in adipose tissue 1 h after injection were 13 times higher than those in brain and more than 20 times higher than those in blood.[34] Thus, SNB is highly lipophilic in its distribution; indeed, SNB remained in fat at approximately 10 µg/g 48 h after injection--that is equivalent to the highest blood concentration attained (3 h after injection). Brain and blood concentrations were virtually parallel. In one case of an SNB-related death in a human subject,6 the brain:blood ratio of SNB concentration was 6:1, and the liver:blood ratio was 2:1. In a second case in which it was established that the deceased had smoked SNB, concentration ratios were as follows: liver:blood, 46:1; lung:blood, 2:1; and kidney: blood, 1:1. In a third case, in which intravenous use of SNB was documented, the liver:blood and bile:blood ratios were 4:1 and 1:1, respectively. A knee synovial fluid:plasma ratio of 9:1 was found in a living subject who had inhaled (“snorted”) SNB.

Receptor sites specific for binding SNB have been found in rat brain and other organs.[66] The greatest specific binding was found in the cerbral cortex and the corpus striatum. Binding was less in the thalamus and hippocampus and least in the medulla oblongata, pons, olfactory bulb, hypothalamus, and cerebellum; none was found in the spinal cord. Specific binding was also found in heart, liver, lung, and kidney.

Distribution studies of [^{3}H]SNB in selected brain regions[68] revealed concentrations from 10.4 nmol/g in cerebellum to 16.4 nmol/g in anterior cingulate cortex. Another study[37] showed that radioactivity from administration of [^{3}H]SNA was distributed almost

evenly over the major anatomic areas of the brain; only the hypothalamus had a high concentration. Most of the radioactivity was associated with the soluble cell fraction, and very little was detected in nuclear and mitochondrial fractions. Chronic administration of SNA altered the distribution of [^{3}H]SNB and its metabolites in the central nervous system; radioactivity in the cortex was 7-31% less than that in the whole brain. Other areas, particularly the hypothalamus, had a higher concentration relative to that in whole brain. A recent report[44] indicated that 30 min after intravenous or oral administration of [^{3}H]SNA to mice the highest concentration was in the stomach. The next highest concentrations were in fat (by the intravenous route) and in liver and intestine (oral route) and the lowest in brain and plasma (either route).

Metabolism

A proposed scheme of the metabolism of SNB in man was reported by Jasinski et al.[35]

After mice became tolerant to SNB, hepatic microsomal cytochrome P-450, cytochrome b5, nicotinamide adenine dinucleotide phosphatase, and NADPH-cytochrome c reductase activities were increased and thus presumably involved in SNB metabolism.[49] These findings confirmed previous observations that chronic SNB administration to the mouse increased liver microsomal hydroxylation of aniline, pentobarbital, and hexobarbital and the N-demethylation of aminopyrine and ethylmorphine.[32] 4-Phenyl-4-piperidinocyclohexanol (PPC), one of the major metabolites of SNB, can exist in cis and trans isomers. Both isomers were found to be biologically active in the mouse, producing ataxia and seizure activity (the latter at high doses). The trans isomer was only slightly more active than the cis isomer.[11] The presence of an additional metabolite in the urine of human subjects using SNB has recently been confirmed;[16] the metabolite had been found in rat and rabbit liver and in dog urine. This metabolite was identified as 5-(1-phenylcyclohexylamino)valeric acid. In man, PPC is highly conjugated as the glucuronide and is excreted mainly in that form.[35] Details of the biotransformation of SNB have been extensively reviewed.[40]

Elimination (Pharmacokinetics)

It was recognized many years ago that SNB had a prolonged action.[46] A human volunteer given SNB orally at 0.48 mg/kg was not responsive to stimuli until 4 h after treatment, at which time he was stuporous, and his arms and legs remained in any position in which they were placed. Full recovery required about 7 h. In Sprague-Dawley rats, half-lives of SNB were approximately 1 h in blood, 2 h

in brain, and 3-4 h in adipose tissue.[34] Plasma half-lives of 2.4 h (monkey) and 2.9 h (dog) have been reported.[69] The half-life in humans varies considerably from subject to subject. Acidification of urine results in a markedly decreased half-life.[24] Some indication of the slow elimination of SNB is found in an investigation of coroners' cases of persons who died of accidental causes;6 the highest concentrations of SNB in brain and other organs were noted 7-10 d after single high doses of the drug. In brains of SNB-tolerant mice, the half-life was much shorter than that in control animals.[49] SNB metabolites remained in the liver of mice up to 14 d and in the lung up to 21 d.[44] Measurable concentrations of SNB persisted in a human subject for at least 6 mo after the last known exposure.[52] All available information indicates that the pharmacokinetics of SNB are highly dose-related.

The recently reported findings of Cook et al.[19] on the biodisposition of phencyclidine after oral or intravenous administration of trace amounts of radiolabeled material are presented in Table 3-2.

Quantitatively similar dispositional kinetics in the dog were recently published;[71] again, there was a wide variability in half-life, as well as a very small renal clearance.

These results indicate that, in addition to effectiveness by inhalation and parenteral administration, SNA is well absorbed in man when administered orally. The drug is 60-70% bound to plasma proteins, the volume of distribution is high (approximately 500 L in an 80-kg man), clearance is largely a result of metabolic processes, the half-life is quite variable from one person to another, and the drug and its metabolites are excreted principally in the urine, regardless of whether it is given orally or intravenously.

ANIMAL TOXICOLOGY

Several animal species (mouse, goat, cat, dog, guinea pig, rabbit, and rat) were given graded doses of SNA intravenously to determine its LD_{50} and its median effective dose (ED_{50}) for several gross manifestations--ataxia, salivation, prostration, and convulsions. Table 3-3 lists the LD_{50}s and ED_{50}s for these effects. It shows that in various species the LD_{50}s ranged from 11.7 to 17.9 mg/kg, and the pharmacologic dose ranged from 50 to 80 µg/kg; thus, the margin of safety of this compound was high. Ataxia, salivation, and prostration occurred within 1-5 min, and convulsions developed within 1-40 min. Death usually occurred in less than 15 min in the guinea pig, rabbit, and cat; in 1-3 h in the mouse; and in 5 min to 24 h in the dog. At 3-5 mg/kg intravenously, SNA caused a decrease in blood pressure, a decrease in heart rate, and minor cardiac irregularities in cats. It also significantly depressed respiration, but

TABLE 3-2 Biodisposition of Phencyclidine in Mana

Disposition of trace amount of phencyclidine:	
Oral bioavailability	72 ± 8%
Plasma concentrations (1 h after 1-mg intravenous dose)	10 pmol/ml
Plasma binding	68-69%
Volume of distribution	6.2 ± 0.3 L/kg
Total clearance	380 ± 80 ml/min
Renal clearance	33 ± 8 ml/min
Apparent terminal half-life	17.6 h (7-50 h)
Renal excretion (10-d collection)	73 ± 4%
Fecal excretion (10-d collection)	5 ± 1%
Metabolites of phencyclidine in urine (97% of total ^{3}H label):	
Phencyclidine (SNB)	16%
1-Phenylcyclohexylamine (PCA)	trace
1-(1-Phenylcyclohexyl)-4-hydroxypiperidine (PCHP)	7%
4-Phenyl-4-piperidinocyclohexanol (PPC)	18%
4-(4'-Hydroxypiperidino)-4-phenylcyclohexanol (dihydroxy metabolite)	3%
5-(1-Phenylcyclohexylamino)valeric acid	15%
Unidentified polar metabolites	38%

[a] Data from Cook et al.[19]

TABLE 3-3 Lethal and Effective Intravenous Doses of SNA in Various Speciesa

	ED_{50}, mg/kg[b]						
Species	Salivation	Ataxia	Prostration[c]	Convulsions[c]	LD_1, mg/kgb	LD_{50}, mg/kg	Highest No-Effect Dose, mg/kg[b]
Mouse	NR	NR	0.3	NR	Ca. 11.3	16.0[d] (14.4-18.8)	
Goat	0.5	0.025	0.1-0.5 (6 h)	0.1-0.5	Ca. 8.9	12.0 (10.9-13.2)	<0.025
Cat	<0.5	0.025-0.05	5.0-7.9	Irregular at 7.9 and above (6 h)	Ca. 12 (3 d)	Ca. 15	0.025
Dog	0.5	0.05-0.1	0.1-0.5	0.75 (1.2 h)	Ca. 14.6 (2-6 h)	17.9 (16.7-19.1)	0.05
Guinea pig	NR	<0.25	0.25-0.5 (15-30 min)	5.0 (20-60 min)	Ca. 7.1	12.0 (10.0-14.4)	<0.25
Rabbit	NR	0.20-0.79	0.5-4.0 (5-15 min)	Irregular at 7.9 and above (3 h)	Ca. 9.4	11.7 (10.9-12.6)	0.05
Rat	NR	0.4	NR	NR	NR	NR	0.25

[a] Data from McNamara et al.[46]
[b] NR = not recorded
[c] Times in parentheses are durations of action or times of effect.
[d] Intraperitoneal LD_{50} in mice was 66±5 mg/kg; most of the mice died within 10 min of injection.

had no neuromuscular blocking effect; that indicates that its respiratory depressant effect is not central.[46,62]

Death from SNA intoxication appears to involve both cardiovascular and respiratory systems.[46,62] SNA increases the effects of pentobarbital on both respiration and heart rate. The depressant effects of SNA on the myocardium and on the Purkinje system are antagonized effectively by epinephrine, but not by Metrazol (pentylenetetrazol) and caffeine. Artificial respiration restored cardiac stability and regularity. It was suggested that SNA interferes with oxygen utilization in animals. The circulatory and respiratory responses to SNA were considered to be mediated through the medulla oblongata.[46,62]

The primary action of SNA is on the brain.[3] In low doses, it produces tranquilization in the pigeon, cat, dog, guinea pig, and monkey. In high doses, it causes repetitive convulsions. The inhalation toxicity of SNA and its base (SNB) given as aerosols was studied in mice. The LCt_{50} for SNA was 22,000 mg·min/m^3. Mice and possibly dogs were more susceptible than other species to the lethal effects of aerosols of SNA. The LCt_{50}s for rats and guinea pigs were in excess of 115,000 mg·min/m^3.[46] Recent data of Davis et al. show that a presursor in synthesis of SNA, piperidinocyclohexanecarbonitrile (PCC), increases the toxicity of SNA.[21]

Early studies (1957) by Parke, Davis in monkeys showed no evidence of tolerance or persistent toxic effects in monkeys after repeated parenteral administration at 1.25 or 5 mg/kg once a day, 5 d/wk, for 43 doses.

No persistent toxic signs were seen in dogs after intramuscular injections at 1 mg/kg. Hematologic and biochemical values in the animals remained normal.

More recent studies,[8,9,12] at dosages that produce behavioral changes, have shown that chronic intramuscular or intraperitoneal administration of SNA results in development of tolerance to several test situations in several animal species. Tolerance to roughly 2-4 times as much SNA as initially given was evident in the behavioral effects of SNA in these species. Tolerance to SNA was also observed in intramuscular SNA self-administration studies in the monkey. In the development of tolerance, such pharmacologic factors as dose per injection, injection frequency, and duration of chronic exposure were considered to play a more important role than behavioral factors (e.g., reinforcement loss).[9]

Rhesus monkeys on chronic SNA intramuscular administration have also been shown to develop dependence. In experiments in which rhesus monkeys were given unlimited access to SNA, animals self

administered amounts sufficient to maintain continuous gross intoxication for periods of 50 d or longer. Unlike other hallucinogens, SNA is avidly self-administered by monkeys. Abrupt discontinuation after long-term intoxication produced withdrawal symptoms that could be reversed by further SNA administration. The spectrum of symptoms varied somewhat among the animals. Four hours after drug administration was stopped, SNA intoxication decreased and animals started eating and became less ataxic. At 8-12 h, they became markedly hyperresponsive with a distinctive oculomotor hyperactivity that was different from the nystagmus observed during intoxication. They usually refused preferred food. During the peak hour (at 12-16 h) of withdrawal, several of the following abstinence symptoms were manifested: piloerection, tremor, diarrhea, continuous vocalization, hyperresponsivity, oculomotor hyperactivity, priapism, bruxism, and ear and facial twitches. Abdominal contraction, emesis, and convulsions occurred in some animals. Food intake decreased by 50% during withdrawal. The symptoms gradually diminished over the next 24 h. Plasma concentrations of SNA during self-administration ranged from 105 to 208 ng/ml.

The large margin of safety for this compound when given intravenously, intragastrically, intraperitoneally, and subcutaneously is decreased when the compound is given in the aerosolized form. Tolerance and dependence have been observed after chronic administration.

NEUROPHARMACOLOGY

The neurochemical effects of phencyclidine in animals have been well reviewed.[3,22,45,51,53] A summary of these reviews and of some recent papers is presented below.

At behaviorally effective intraperitoneal doses (10 mg/kg), SNA has been reported to increase serotonin (5-HT) concentrations in rat brain.[60] 5-Hydroxyindoleacetic acid concentrations are first decreased and then increased. It has been claimed that SNA causes a decrease followed by a compensatory increase in 5-HT turnover. However, these studies have been criticized on experimental grounds,[36] and later studies showed that SNA produces a decrease in 5-HT.[58] No effects on aromatic amino acid decarboxylase and monoamine oxidase, two enzymes involved in 5-HT formation and destruction, were observed SNA has been shown to reduce the concentration of the 5-HT precursor tryptophan. The uptake of 5-HT into rat brain preparations in vitro and into the brain stem in vivo is somewhat inhibited. There also seems to be a strain difference in response to SNA.[61] In general, acute administration of SNA does not seem consistently to cause marked changes in brain serotonin content or turnover.

Similarly, the effects of SNA on the catecholamine (norepinephrine, epinephrine, dopamine) systems have been widely studied, but the results are far from clear. SNA has been reported to increase rat brain tyrosine[59] and to decrease rat brain norepinephrine (NE) and to increase dopamine (DA) slightly.[60] Again, conflicting results are available.[31] Apparently, changes in these biogenic amines depend strongly on the brain region studied and the dose used.

Changes in turnover rates of various catecholamines have been claimed, but methodologic difficulties prevent firm conclusions,[36] and later studies found no effects on NE turnover. The uptake of NE and DA into rat cortical preparations is markedly inhibited in vitro[57] and in vivo[29] by SNA; interestingly, SNA is 100 times more potent than ketamine in this respect. In vivo, SNA is a competitive inhibitor of striatal DA uptake and hypothalamic NE uptake in rats.[29] Tyrosine hydroxylase is inhibited and the catecholamine metabolite homovanillic acid is decreased after acute SNA administration; the latter effect is lost during chronic treatment. Adenylate cyclase and phosphodiesterase activities are decreased.[55] However, most effects appear to involve DA. SNA increases the accumulation of 3-methoxytyramine, and that indicates that the synaptic concentrations of DA are increased. SNA also has been shown to affect the release and uptake of DA from brain slices in vitro.[5] SNA decreases DA receptors in the brain. Behavioral studies have indirectly implicated the involvement of the DA system.[27] In general, effects on NE and epinephrine neurons appear smaller than those on DA neurons. It might be concluded that one of the main actions of SNA is on the DA system.[47] This might lead one to be concerned about the possibility that a parkinsonian syndrome will develop in later life; however, that seems unlikely.

The effects of SNA on the acetylcholine (ACh) system have been less well studied.[36] It has been claimed to raise rat brain ACh content slightly and transiently when given intravenously;[17] however, other studies reported no effects on rat brain ACh content concentrations when it was given intraperitoneally.[23] SNA does not release brain ACh. It has been shown to increase the activity of acetylcholinesterase in whole brain and to decrease choline acetyltransferase (CAT) activity in the cerebellum[56] while stimulating CAT in the hippocampus.[53] The effects on CAT disappear during chronic treatment. SNA has been shown to interact with muscarinic, nicotinic, and opiate receptors.[64] It inhibits the potassium-stimulated release of ACh from slices of the striatum, but not from the hippocampus. Some evidence suggests that this inhibition of ACh release is mediated via DA. At the neuromuscular junction, SNA blocks neuromuscular transmission, prolongs the action potential, and potentiates muscle twitch.

SNA does not affect the concentrations of histamine and glutamic acid, but it decreases the activity of glutamic acid decarboxylase and reduces the concentrations of gamma-aminobutyric acid (GABA) in rat brain.[25] GABA release is inhibited by acute administration, but not by chronic treatment.[25] SNA increases serum creatinine phosphokinase content in stressed rats. Recently, SNA was found to decrease methionine-enkephalin content in the medulla oblongata and midbrain in the mouse, whereas other areas remained unaffected.[48]

SNA seems to block K^+ conductance specifically.[1] It also affects ion movements and has been shown to be a potent blocker of both the K^+ channel (EC_{50}, 2.6 μM) and the Na^+ channel (EC_{50}, 9.2 μM).[1,63]

In summary, SNA administered acutely has potent pharmacologic and toxic properties and thus must have pronounced effects on biochemical events in the brain. These actions are thought to occur predominantly at the synaptic level. SNA seems to affect a number of neurotransmitters, biochemicals, and enzymes in the brain. Most evidence points to an action on the dopamine and acetylcholine systems. These changes acount for observed abnormalities in behavior and awareness. In addition, SNA blocks excitable membrane channels associated with impluse conduction, as do other compounds that induce reversible circumoral and distal paresthesias. However, neurochemical changes seem to revert to normal after the drug leaves the animal.

Unfortunately, results of SNA studies are often inconclusive or even contradictory, and the nature of the exact actions of SNA on chemical characteristics of the brain is still poorly understood. Some of the reasons for the conflicting results might be the use of acute vs. chronic administration, the use of different doses, the use of diverse animal strains and species, and the selection of specific brain areas. In addition, the results of in vitro experiments cannot be easily extrapolated to the in vivo situation, because the high concentrations necessary to produce in vitro effects are not accumulated by the brain in living organisms. However, the main objection to extrapolating these data to man might well be that all studies use healthy, undisturbed rats, whereas, as is well known, SNA produces its most serious effects in people who are emotionally labile, have had psychiatric problems, or are polydrug users.

SNA, an arylcyclohexylamine, belongs to a class of drugs known as dissociative anesthetics. Under the proper conditions of dosage and administration, it can act as a central nervous system (CNS) stimulant, a CNS depressant, a hallucinogen, an analgesic, or combinations of these.[14] Related arylcyclohexylamines share some of SNA's pharmacologic actions. The drug was originally developed as a general anesthetic agent, but it proved unsuitable for human use, because delirium often occurred as patients were emerging from anesthesia.

SNA had advantages as a surgical anesthetic, however: it can produce anesthesia with marked analgesia in the presence of normal pharyngeal and laryngeal reflexes, and it stimulates, rather than depresses, the respiratory and cardiovascular systems. It has therefore been useful in veterinary surgery, although recently its desirability as a drug of abuse has led to termination of its use by most veterinarians. Ketamine, a phencyclidine analogue with briefer duration of action, is currently in wide use as a surgical anesthetic.[79]

The pharmacology of SNA and related components has been reviewed recently.[22,51] Early in the study of SNA, it was learned that its overt actions varied enormously from species to species--thus, it produced stimulation in rats, convulsions in dogs, and tranquility in rhesus monkeys.

SNA has multiple effects on several neural systems, but evidence is increasing that at least some of its effects are mediated via interaction with a specific brain receptor. Two laboratories[65,72] have independently identified SNA binding sites in rat brain membranes. These binding sites show specificity and saturability. Other drugs with affinity for these binding sites have behavioral effects similar to those of SNA, and their binding affinity correlates well with behavioral potency. The distribution of the binding sites is similar to that of the sigma subcategory of opioid receptors, and other sigma agonists have substantial affinity for SNA binding sites. Several laboratories have recently been searching for an endogenous ligand for the binding sites/receptors; however, no positive findings have been published. Although these developments hold great promise for understanding the actions of SNA, further work is needed to clarify its complex actions.

The pharmacologic aspects of SNA that make it desirable as a drug of abuse are unclear.[41] The effects depend on dosage, but usually include sedation (light-headedness, ataxia, and slurred speech), cognitive distortions (disorganized thoughts, feelings of sensory isolation, a sense of unreality, and loss of ability to distinguish self from surroundings), and perceptual disorders (visual and auditory hallucinations and numbness). Many users become appropriately frightened or angry. Delusions of persecution may occur, and violent behavior may be directed at oneself or at another person or an object. At higher doses, SNA's effects may progress from severe agitation through stupor to coma and death. Autonomic effects are striking. SNA produces tachycardia, hypertension, hypersalivation, sweating, and fever. The acute effects persist for 4-6 h.[20]

Hallucinogenic drugs like LSD are not reinforcing in animals, in that they will not self-administer these drugs. SNA, however, is self-administered in large quantities by monkeys equipped with intravenous catheters when access is unlimited. Tolerance of the acute

effects of SNA was shown to develop in monkeys and has been clinically reported in man. After SNA is self-administered by monkeys for 20-30 d, abrupt cessation produces physical and behavioral signs of withdrawal.[7,54] Thus, there is evidence of both tolerance of and physical dependence on SNA, but it is not clear that persistent daily use produces dependence in human subjects.

SNA produces an acute psychosis with some similarity to that seen in schizophrenic disorders.[35] In some users, this schizophrenialike psychosis persists for weeks after the ingestion of the drug.[2] In some cases, a patient's first psychotic episode has been precipitated by SNA and has been followed by long-term schizophrenic symptoms without additional SNA. However, results of predrug examinations of these patients were not available. SNA has been given experimentally to known schizophrenics, who were found to be very sensitive to the induction of long-lasting psychotic symptoms by the drug. It is not known whether single doses of SNA can produce long-lasting psychotic behavior in normal persons or whether this can occur only in persons with pre-existing mental disorders.[42]

Chronic users of large doses of the street drug PCP containing SNA have been reported to undergo personality changes, cognitive impairment, and slurred speech for 6-12 mo after stopping the drug. Clinical reports have included the presence of chronic anxiety, depression, and schizophrenic disorders in former long-term SNA users. Again, these reports were based on users examined after their experience with the drug, and we have no knowledge of their predrug condition.

GENETIC AND REPRODUCTIVE EFFECTS

Mutagenicity

Information on the mutagenic potential of SNA is almost completely lacking. A single abstract reported the cytogenetic effect of chronic use of the drug over 2-3 yr by one male and four females 18-26 yr old. The most frequent route of administration was inhalation of smoke from a blend of SNA and marijuana. No chromosomal abnormalities were found in this limited investigation.[28]

A cytogenetic study was performed on a group of drug-using parents of children with limb-reduction defects or of liveborn triploid infants. SNA as contained in the street drug used was implicated. An increase in ploidy was observed that decreased in time when successive peripheral lymphocyte samples were taken. The published abstract contained no specific data.[67]

Given the lack of critical studies of this chemical, no conclusion can be made as to its mutagenic activity. To determine the mutagenic potential of SNA, a battery of available in vivo and in vitro studies should be performed, including cytogenetic studies in cultured mammalian cell lines and in vivo bone marrow cells from rodents. In vitro microbial assays with and without activation should be undertaken, to determine whether this chemical causes gene mutations. In vivo and in vitro repair studies would also be informative. Additional studies, including the dominant-lethal assay and body-fluid analysis in rodents and in vivo and in vitro adduct formation, could be undertaken. These studies, with cell transformation studies in mammalian cell lines, would also indicate the probability of carcinogenic effects.

Teratogenic-Reproductive Effects

Walker and Seig[67] reported two clusters of anomalies, limb-reduction defects and liveborn triploid infants born to parents involved in the drug culture. Retrospective investigation suggested that the mothers had ingested street drugs containing SNA during the early first trimester of pregnancy.

Acute placental transfer of [^{3}H]SNA was studied in pregnant rabbits and mice; the chemical readily crossed the placental barrier. In mice, peak concentrations were found 2 h after administration, and fetal concentration was 10 times as high as that in maternal tissue. n lactating mice, the chemical was found in breast milk at a concentration 10 times that found in plasma.[50]

Jordan et al.[38] reported significant numbers of gross anomalies--including dysplasia of the extremities, micrognathia, cranial dyplasia, and cleft palate--after SNA was given intraperitoneally to rats on days 6-15 of gestation.

Summary

No definitive studies have been conducted in humans or experimental animals to determine reproductive or genetic effects of SNA. Anecdotal studies (e.g., that of Golden et al.,[30] who, in a case report of an infant whose mother used SNA, speculated that SNA was related to abnormal behavior and development of dysmorphology and spastic quadriparesis in the infant) and the limited investigations reported here indicate that this chemical may be teratogenic. If SNA is shown to be teratogenic, further studies would have to be conducted to determine whether the teratogenic effect has a genetic basis.

DELAYED AND LONG-TERM EFFECTS

SNA, in its brief use as a dissociative anesthetic in humans, produced an excessive number of cases of emergent delirium and post-operative behavioral disruption. It was eventually withdrawn from medical practice and from veterinary practice in most states. Since the mid-1960s, as PCP, a street drug of unknown purity, it has been a drug of abuse and has been used as an adulterant in other street drugs sold as cocaine, tetrahydrocannabinol (THC), and a variety of hallucinogens. Its illicit use and associated mortality rose sharply during 1975-1981. During the last 3 yr, mention of PCP abuse and related mortality has decreased, according to the DAWN system quarterly reports (Drug Abuse Warning Network, NIDA, Rockville, Md., 1982). It must be recalled that most PCP consumers never come to the attention of health-care personnel.

Information about long-term effects of SNA is derived from two sources: its administration as an anesthetic and its chronic use as a street drug. As to the latter, it must be noted that PCP rarely occurs in a pure form and commonly is abused with other substances, such as THC and cocaine.

Data on drug abusers do not permit the construction of a dose-response curve. However, blood and urine concentrations of SNA after emergency hospitalization may permit some extrapolation to the quantities consumed.[24]

Some 30 phencyclidine-like compounds are known to produce psychophysical effects resembling those of SNA. Of these, ketamine (Ketalar, Ketaject), a chlorophenyl methylaminocyclohexanone, is currently used fairly widely as a dissociative anesthetic in humans. Therefore, its use can provide clinical information about undesired effects of single doses of the phencyclidine series.

A number of possible long-term or delayed effects of SNA can be listed on the basis of experience with adverse reactions in drug abusers and in anesthesiology patients.

- • Possible psychotic reactions.
- • Organic brain damage as a residuum of status epilepticus or cerebral hemorrhage secondary to acute hypertension resulting from the toxic effects of SNA use.[10]
- • Residual cerebral or other trauma in connection with the intoxicated state.

Prolonged Psychotic Reactions

A schizophreniform psychosis can follow administration of single or multiple, usually high, doses of SNA. Allen and Young reported on nine patients seen over a 13-mo period at an Army hospital with SNA psychosis within a week of multiple exposures.[2] Despite antipsychotic treatment, three had not recovered from the residua of their psychosis 30, 60, and 90 d later.

Erard et al.[25a,43] believe that the psychosis is a special form of an acute schizophrenic episode activated by the drug in some susceptible persons. Luisada estimated that 1-5% of the population may be susceptible (Luisada, P.V. personal communication). Although the Army volunteers were psychologically screened, preschizophrenic test subjects may have been included. As noted, the psychotic reactions associated with SNA typically occur immediately or soon after consumption of the drug. If serious mental consequences were not observed during the immediate followup period or during the later Army tour of duty, it seems unlikely that a delayed SNA psychosis occurred.

That a low dose was administered only once or a very few times in the Army research does not completely rule out the possibility that a vulnerable subject might have developed a delayed psychotic reation. The dose given by mouth in Edgewood experiments was up to 5 mg--a modest amount, but one that should produce some symptoms of intoxication in most nontolerant subjects.[18] Other subjects inhaled unknown amounts in aerosol form.Table 3-4 lists the clinical effects of various doses of SNA. Doses of 250 mg and more have been ingested by chronic users after the development of tolerance. In comparison, 5-10 mg intravenously can result in delirium, 20 mg in coma, and 50 mg in convulsions.

Organic Brain Damage

Case reports indicate that chronic organic brain impairment has resulted from frequent, intensive SNA use.[3,18,26] Carlin et al.[10] studied 12 chronic users, a like number of polydrug users who did not use SNA, and a group of non-drug-using controls. All the drug users had been abstinent for at least 3 wk. The average length of abstinence of the SNA group was 27 mo. Six in the SNA group, five in the polydrug group, and none in the control group showed mild neuropsychologic impairment. The most frequent effects were difficulties in abstracting and impairment of complex psychomotor skills.

In the amounts given to the experimental subjects at Edgewood, it is highly unlikely that major chronic organic brain syndrome developed, inasmuch as no status epilepticus or cerebral hemorrhage

TABLE 3-4 Clinical Effects of Various Oral Doses of SNAa

Dose, mg	Effects
5-10	Ataxia, nystagmus, mood changes, hallucinations, vomiting, analgesia, paresthesias; onset, 1-2 h; duration, 4-8 h
10-20	Stupor, eyes open, random movements, resting horizontal or vertical nystagmus, hyperreflexia, hypertension; onset, 0.5-1 h; duration, 8-24 h
50	Deep coma, chills, nystagmus, eyes open or closed, hypertension, labored breathing, seizures; duration, up to 4 d
100	Lethal; 3-10 d of respiratory depression, hypertensive crisis, cerebral bleeding, loss of deep tendon reflexes, decreased renal and liver function

[a] Data from Hollister;[33] Vehicle unknown

occurred after drug administration. However, the development of more subtle neuropsychologic impairment, such as difficulties in cognitive functioning and the impairment of complex psychomotor skills, cannot be ruled out, because these were not evaluated.

Residual Trauma in Connection with the Intoxicated State

The behavioral toxicity that can accompany SNA intoxication can lead to head and other injuries and perhaps leave permanent residua. Such injury would occur during intoxication or delrium and would have been recorded in the Edgewood subjects' charts. No such incidents were recorded.

Food and Drug Administration Reports of Adverse Reactions

Only two reports are available on neuropsychiatric complications reported by anesthesiologists to the Food and Drug Administration in connection with SNA. One involved convulsions, and the other,

psychosis. In addition to SNA, ketamine produced untoward reactions. It is clear that psychosis, hallucination, and convulsions are the most frequent immediate complications.

These FDA reports did not include the doses of SNA or the duration of the adverse reactions. Ketamine doses ranged from 80 to 575 mg intravenously or intramuscularly in the cases with postoperative adverse reactions.

Neither the FDA (for proprietary reasons) nor Parke, Davis and Co. provided any other information about SNA despite requests.

Summary

The target organs that may be involved in prolonged or delayed effects of SNA exposure are the brain and cardiovascular system. At high doses taken over a prolonged period, the effects on the brain are immediate and transitory, but occasionally a prolonged psychotic state is recorded. Neuropsycholologic effects include psychosis, a paranoid state, convulsions, and depression. Irreversible effects, such as organic mental disorders, might be encountered after frequent consumption. A prolonged or irreversible effect might be seen when a schizophrenic process is precipitated after exposure to SNA. The cardiovascular effects of acutely administered high doses include hypertension and tachycardia. These are transient and do not leave permanent residua, except after a cerebral hemorrhage.

At the dosages used at Edgewood, it is not expected or likely that any long-term or delayed effects have occurred or will occur, because immediate prolonged mental or cardiovascular effects did not take place within a week of drug administration. However, no followup clinical data are available to confirm this. Therefore, the development of more subtle neuropsychologic impairment, such as difficulties in cognitive functioning and impairment of complex psychomotor skills, cannot be ruled out, as these were not evaluated immediately after administration of the test drug and cannot now be evaluated in the framework of this study. Results from the questionnaire now being analyzed may shed light on some of these matters.

EFFECTS ON VOLUNTEERS

The following summary of acute effects in humans is based primarily on a review of the charts in the clinical files at Edgewood. Like many clinical records, these vary greatly in extent of detail, ranging from sketchy and incomplete notes or one-line summaries to records that could serve as models for research documents. Independent chart reviews by NRC scientists provided some cross-checks on

the completeness of data. In addition, technical reports prepared at the time of the experiments provided summary data on the SNA experiments.

The SNA research going on throughout the world at about the time of the Edgewood studies is relevant to this study. The experiments at Edgewood were not unusual and did not involve extraordinary doses (see Table 3-1) or special populations of volunteers, compared with experiments in the laboratories and clinics of scientists and clinical researchers throughout the world.

When the Army began experiments at Edgewood with SNA in the late 1950s, a fair amount was already known about it, because of the clinical studies sponsored by Parke, Davis. Reports published between 1958 and 1972 described the effects of SNA given to more than 1,500 people. Most were surgical and obstetrical patients given large, generally anesthetic doses ranging from 8 to 70 mg intravenously. A substantial number of children received SNA, sometimes repeatedly, for anesthesia during burn-dressing changes or tonsillectomies. Over 200 psychiatric patients, including over 100 schizophrenics, were given doses averaging about 6 mg (3-18 mg) intravenously, orally, or intramuscularly. Over 100 normal volunteer research subjects representing a variety of student populations were given average doses of 8 mg intravenously or orally. Multiple doses were given to psychiatric patients to facilitate therapeutic sessions. Repeated doses were given chronically to psychiatric patients for periods of 4-6 wk.

Of seven subjects who underwent a water loading test, those treated with SNA at 0.1 mg/kg before a saline infusion showed evidence of inhibition of antidiuretic hormone secretion. Another group of subjects were tested on the treadmill at high temperature (100°F) before and after receiving oral doses of 5 and 10 mg of SNA. SNA at these doses did not significantly add to the exercise-induced heart-rate changes, metabolic rate, evaporative heat losses, or tolerance times and in general did not interact greatly with the temperature stress.

The only studies done at Edgewood that were not similar to those done in many civilian settings involved the administration of SNA by inhalation. SNA was dispersed in a methylene dichloride solution and delivered to the subjects as an aerosol in a small inhalation chamber. Nineteen volunteers were exposed to SNA and nine others to SNB for periods of 0.5-3 min. The concentration varied, but averaged 100 mg·min/m^3, to produce a retained dose of approximately 100 µg/kg. At 25-62 µg/kg, subjects had feelings of unreality--dreamlike states with perceptual size changes. Both horizontal and vertical nystagmus developed. Affect and mood changed and were variable, ranging from euphoria to sullen withdrawal. Some subjects were talkative and uninhibited, others passive and withdrawn. At 100 µg/kg,

the aforementioned symptoms became more intense and were accompanied by visual disturbance, blurred vision, ataxia, limb paresthesias, and memory impairment. Subjects rapidly became noncommunicative. At approximately 100-180 µg/kg, analgesia, nausea, and vomiting developed rapidly. Four subjects were given the largest dose (225 µg/kg); collapse and prostration occurred in three and incapacitation in the fourth, and convulsions did not occur. They recovered over the next few hours. In these aerosol studies, dose was measured in milligrams of a 2- to 5-µm aerosol per minute per cubic meter. It was estimated that the anesthetic dose was about 20-50 mg·min/m^3. Exposures were 250-888 mg/m^3 for the most part over 3-4 min, with Ct's of 249-2,955 mg·min/m^3.

In these experiments, as in most of the others at Edgewood, routine biochemical tests were used to screen for unexpected hepatic or renal toxicity. No clinically significant abnormalities were noted in the records.

In summary, the Edgewood studies of SNA demonstrated a dose-dependent effect on a number of systems. Blood pressure increased, respiratory minute volume increased, and heart rate increased with no evidence of arrhythmia. At moderate doses, reflex activity was not impaired. An outstanding effect of the drug was amnesia, which was one of the properties that first attracted investigators' attention to it. Excitation and arousal occurred in some subjects and sedation, depression, and withdrawal in others at the same dose range--a well-known variability in response to SNA. Body boundaries were altered, feelings of estrangement and loneliness occurred in some, and an alcoholic-like euphoria occurred in others. Thought was generally disorganized, with an inability to maintain set. Some subjects appeared catatonic and reported dream-like experiences at the highest doses. Performance in tasks that involved sequential and organized thinking was impaired. Perceptual discriminations were altered. Psychomotor retardation was evident, with distractibility and slowing in task performance. No subjects became overly assertive, hostile, or unmanageable. In general, the signs and symptoms were relatively short-lived, disappearing at 6-8 h, although at the highest doses in occasional subjects symptoms persisted for 24 or 48 h, or even longer. The variation in response observed from the beginning of SNA research in civilian studies was evident in the Edgewood studies.

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REVIEW OF AVAILABLE INFORMATION ON DIBENZOPYRANS: DIMETHYLHEPTYLPYRAN AND RELATED COMPOUNDS

CHEMISTRY

The structure of the active principle of the cannabis plant (Cannabis sativa), Δ-9-tetrahydrocannabinol, or THC (Figure 3-2), was elucidated in the mid-1960s.[11,22,42] However, Adams and Baker[2] and Todd and co-workers[12] in 1940 independently synthesized a Δ-6a,10a congener that differed from the natural material by the position of the cyclic double bond. A series of homologues of that congener was prepared in which the alkyl side chain on the aromatic ring was varied, and the compounds were tested for their capacity to produce ataxia in dogs.[3,5] The most active compound was dimethylheptylpyran (DMHP), the 1,2-dimethylheptyl analogue (EA 1476). Table 3-5 shows the relative potencies of several natural and synthetic dibenzopyrans.[3,5]

TABLE 3-5 Relative Potencies of Several Dibenzopyrans4

Compound	Potency (Capacity to Produce Ataxia in Dogs)
Synthetic C_5H_{11}	1.00 (standard)
Acetate (natural THC acetate)	14.6
Natural THC by hydrolysis of acetate	7.8
Natural THC from cannabidiol	7.3
(DMHP)	512

Because DMHP contains three asymmetric carbon atoms, it can exist as four diastereoisomeric racemates, each consisting of a (+) and a (−) isomer. These isomers, synthesized by Aaron and Ferguson[1] and isolated as the acetates, have been assigned the designations EA 2233-1 through EA 2233-8; the racemic mixture was designated EA 2233 (Figure 3-2). A mixture of isomers 2 and 4 was designated EA 2233-24. Table 3-6 lists the optical rotations of the isomers.

DMHP is a colorless or pale yellow viscous oil that readily undergoes autoxidation. It is insoluble in water, but soluble in alcohols, benzene, and nonpolar solvents. Because the acetate form (EA 2233) retains the atactic potency of the parent compound in dogs and is more stable, it and its stereoisomers were a focus of attention at Edgewood and were tested on human volunteers.

The DMHP used in these tests was procured from Shell Development Corporation or made at Edgewood. Analytic information on the test material is not available, but some Edgewood-prepared samples were reported to have a purity of 98%.

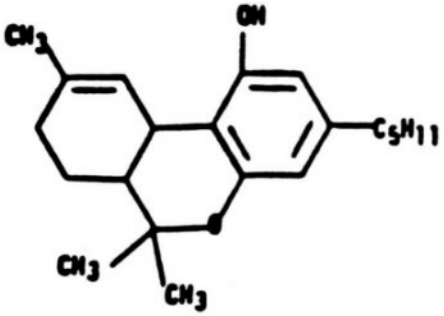
EA 2233 was produced at Edgewood, but no specific analytic information on the material used is available.

The eight optical isomers of EA 2233 were synthesized at Edgewood and analyzed by gas-liquid chromatography. Each of the eight products exhibited three peaks; a primary product in the first peak represents about 85% of the total mixture, a second peak represents about 10-22%, and a third peak represents 3-5%. The impurities were not identified, but they were not isomers.

ABSORPTION, FATE, AND ELIMINATION

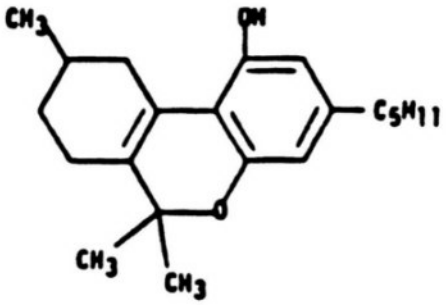
Absorption

Early research on effects of DMHP and its acetate ester (DMHP acetate) was conducted in a variety of animals (mice, rats, rabbits, cats, dogs, and monkeys) with intravenous administration.[44] Rapid onset of such signs as ataxia, mydriasis, generalized weakness, nystagmus, and ptosis was seem with this route. With oral administration, fatigue, thirst, headache, postural hypotension, temporary blurring or loss of vision, and pronounced psychomotor activity were observed in humans.[44] These results indicate that both drugs are well absorbed when administered orally. Efficacies of the drugs when administered parenterally and orally have been confirmed by additional studies in cats,[8] rats and rabbits,[32] mice,[29] and man.[28,31,35,36,38,40]



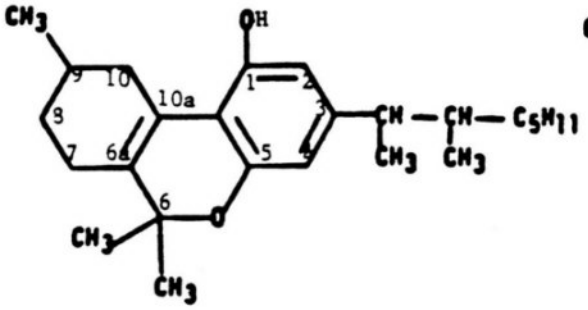
I

Δ-9-tetrahydrocannabinol

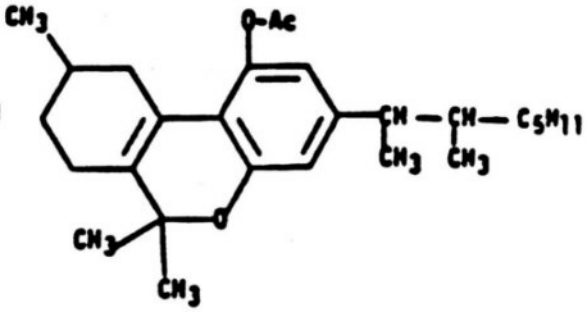


II

Congener of I



III



IV

Δ-6a, 10a-Dimethylheptylpyran (DMHP) EA 1476

EA 2233, Acetate form of EA 1476

FIGURE 3-2 EA 1476 and EA 2233, derivatives of Δ-9-tetrahydrocannabinol tested at Edgewood.

TABLE 3-61 Optical Rotation of Isomers of DMHP Acetatea

Isomer	[α]D, deg (MeOH)
1	+65
2	−130
3	+133
4	−70
5	+94
6	−110
7	+105
8	−93

[a] Isomers 1 and 4, 2 and 3, 5 and 8, and 6 and 7 are enantiomorphic pairs.

Distribution

Tissue concentrations were measured in rats and rabbits sacrificed at selected times after intravenous administration of [^{14}C]DMHP.[32] Radioactivity (from DMHP and its metabolites) in rats at 1 h after administration was highest in lung and liver. Concentrations decreased to approximately 10% of initial values in 24 h and gradually diminished during the next 48 h. Radioactivity was lower in brain than in other tissues assayed. Plasma concentrations of [^{14}C]DMHP decreased from an initial value of approximately 5,000 dpm/ml to about 50 dpm/ml at 72 h. Disappearance was in two phases; the initial one was attributed to tissue distribution (breakpoint, 6 h), and the second to elimination. Rabbit spleen, liver, and lung exhibited their highest radioactivity 3 h after injection; radioactivity in all tissues diminished with time. In this species, initial disappearance from plasma (and, presumably, distribution to other tissues) was more rapid, and the secondary phase slower. The investigators concluded that the tissue distribution of total radioactivity (from both DMHP and its metabolites) is similar to that of THC. The octanol-water partition coefficient was 7,500, compared with 6,000 for THC.

[^{3}H]DMHP was injected intravenously into adult male albino Tuck strain No. 1 mice;[29] 20 min after administration, the blood:brain concentration ratio was 3.7:1. Brain concentration of the drug, on the basis of radioactivity, was approximately 2.5 picograms/gram (pg/g) 15 min after injection and about 1.7 pg/g at 90 min; it slowly decreased to nearly 1.2 pg/g after 16 h.

In three young men who received 200 μg of a racemic mixture of DMHP containing [^{14}C]DMHP,[31] radioactivity disappeared from plasma in a multiphasic manner. The rapid initial disappearance was attributed to uptake and distribution into tissues, and the later disappearance to metabolism and excretion.

Metabolism

Cannabinoids may be metabolized to both hydroxy and dihydroxy metabolites.[9] The hydroxylated compounds are not taken up in the circulation after metabolism by the liver, but are predominantly excreted in bile. It is unlikely, but not known, whether the dihydroxylated compound can be further metabolized or activated to form more reactive compounds.

Metabolism of [^{14}C]DMHP was studied in vitro with 9000 g liver supernatant fractions obtained from rabbits, mice, rats, guinea pigs, or dogs and in vivo in rats and rabbits.[32] Incubation for 1 h with the supernatant fractions in the presence of an NADPH-generating system resulted in metabolism of the following percentages of the total amount of DMHP: rabbit, 68; mouse, 63; rat, 44; guinea pig, 41; and dog, 34. Thus, of the species studied, the rabbit preparation was the most active, and that of the dog the least active. The rabbit preparation gave rise to three radioactive metabolites; one of these appeared to be further metabolized to one of the other metabolites. All seemed to be more polar than the parent drug, according to their behavior in thin-layer chromatographic systems.

When [^{14}C]DMHP was administered intravenously to rats or rabbits, it appeared that DMHP was hydroxylated and then further oxidized to at least one acidic compound, which was recovered from urine. No nonmetabolized DMHP was found in the urine of either species.

A similar in vitro system used [^{3}H] Δ-9-DMHP*; mass spectra of incubation extracts were silylated and subjected to gas chromatography/mass spectrometry. Strong evidence was accumulated that the major metabolite was 11-hydroxy-DMHP. Overall recovery of the metabolite was only 4.7%; this low yield was insufficient for confirmatory analyses by other methods, such as nuclear magnetic resonance. The low recovery indicated to the investigators that DMHP and its metabolites are much more strongly bound to tissue components than are THC and its metabolites. Sixteen hours after injection of [^{3}H]DMHP into mice, their brains were extracted. Gas chromatography of the extracts indicated retention times identical with those of synthetic 11-hydroxy-DMHP, which accounted for 90% of the radioactivity; two

* This DMHP is a Δ-9 form, in contrast with the DMHP used at Edgewood and by Lemberger, which was a Δ-6a,10a DMHP.

other fractions were noted, which the investigators referred to as "non-extractable material" and "polar metabolite."

Lemberger et al.[30] used gas chromatography/mass spectrometry and reported that the hydroxymethyl metabolite represents only two minor metabolites produced by rat liver microsomes; this suggests that with DMHP the methyl group at C-11 is not active and therefore that the 11-hydroxy DMHP would not be a major metabolite. Lemberger et al. have given evidence that the hydroxylation occurs primarily on one of the methyl groups of the side chain.

Elimination

Kinetic data on the cannabinoids are limited, but it appears that they are eliminated slowly from the body. Reported THC half-lives in human plasma have ranged from 19 to 57 h. Because of their high lipid solubility, cannabinoids and their metabolites can be sequestered in fatty tissues, and traces may be detectable for over a week. The pharmacologic significance of these persistent cannabinoids and their metabolites is unknown.[26]

Very slow elimination (or at least displacement or metabolism at bioactive sites) was suspected in early studies of the effects of DMHP and DMHP acetate on human volunteers, because such signs and symptoms as sluggishness, inability to concentrate, dimness and blurring of vision, and orthostatic hypotension occurred up to 48 h after drug administration.[44] At high doses of DMHP, cats "lay in plastic-like poses for hours or days against the side of the container in which they were placed."[8]

DMHP in plasma appears to have a half-life of 20 h in both rat and rabbit;[32] the half-life of total radioactivity ([^{14}C]DMHP plus C-labeled metabolites) in the slower phase of elimination was approximately 24 h. In the rat, 70% of the total radioactivity of the intravenous dose was recovered in urine and feces during 72 h; 4% was excreted in urine and 66% was found in feces. A 7-d collection of urine and feces of rabbits given [^{14}C]DMHP resulted in recovery of 87% of the total radioactivity--24% in urine and 63% in feces. In mouse brain,[29] the half-life of [^{3}H]Δ-9-DMHP appeared to be about 20 h, whereas 11-hydroxy-DMHP and the "polar metabolite" seem to have (by extrapolation) half-lives in excess of 48 h. All three values were calculated on the basis of the slower phase of elimination.

Studies on human volunteers given DMHP intravenously indicated a half-life of the terminal phase of [^{14}C] DMHP elimination of 39 h; 58% of radioactivity was excreted in the feces and 11% in the urine during a 7-d collection period.[31] The investigators concluded that

the plasma disappearance curve in humans obtained with DMHP is similar to that obtained with THC.

In summary, both DMHP and DMHP acetate are well absorbed after oral and parenteral administration, have a relatively long half-life, are extensively metabolized, and are excreted mainly via the feces.

ANIMAL TOXICOLOGY

The animal-toxicity data in this section, taken from several sources,[18,20,35,36,40,44] show that the compounds most studied in animals and humans were DMHP and DMHP acetate (more light- and air-stable than DMHP). Although eight optical isomers of the acetate were tested in humans, no specific toxicology data were found.

Route of Administration and Vehicle

The intravenous route of administration was usually used for testing these compounds in animals. Because they are insoluble in aqueous solvents, they were dissolved in small amounts of alcohol or emulsified with an oil-lecithin mixture or polyethylene glycol.

Toxicity of DMHP

DMHP was chosen as the prototype of the various cannabinol test compounds used at Edgewood and was studied more thoroughly than its congeners.

Acute toxicity studies have been performed in various animals: mice, rats, rabbits, cats, dogs, and macaques.[18,20,35,36,40,44] Lethal doses of DMHP are extremely high, in comparison with the small doses required to produce its pharmacodynamic effects. For instance, the intravenous LD_{50} in mice is 63 mg/kg, whereas the minimal effective dose in 50% of the animals (MED_{50}) is 0.075 mg/kg, for a safety factor of 840. The dose required to produce tranquilization in the unanesthetized dog is 0.05 mg/kg, and the minimal lethal dose is 10 mg/kg by the same route. The margin of safety in the dog is about 200. By comparison, the margin of safety of reserpine is 5.0.

The major signs of toxicity are ataxia, analgesia, mydriasis (less prominent in monkeys at lower doses), and profound central nervous system (CNS) depression lasting from several hours to several days, depending on dose. At higher doses, the CNS depression may be preceded by CNS stimulation and convulsions. Marked hypothermia at an intravenous dose of 1 mg/kg, hypotension, and respiratory depression are other significant effects of this compound. DMHP produces a

marked decrease in mean arterial pressure in the anesthetized dog. This effect occurs slowly at small doses, but the latent period is considerably shortened at large doses. In the monkey, the minimal effective intravenous dose to produce ptosis and to decrease activity was 0.0316 mg/kg, whereas the minimal lethal dose was 10 mg/kg by the same route, for a safety factor of about 300.

Death after intravenous administration of DMHP in dogs is preceded by ventricular fibrillation, which may be secondary to hypothermia. However, there are no fatalities if the animals' rectal temperature is kept from falling.

Studies of the interaction of DMHP with other drugs were performed with dogs. If such compounds as cocaine, caffeine, d-amphetamine, and nalorphine are used to antagonize the CNS depression produced by DMHP, they also can produce a marked increase in toxicity and death. Death occurred during the depressed state that followed the stimulation induced by these agents.

Subchronic toxicity was studied in rats and dogs by administering DMHP intravenously over a period of a month.[18] DMHP at 0.1, 1.0, and 10 mg/kg-d was given intravenously to male albino rats (10 per dosage) for 20 daily injections over a 4-wk period. No effects were seen at 0.1 mg/kg. However, rats at both 1.0 and 10 mg/kg-d showed the following effects: gross signs of toxicity, decreased body weight gain, decreased liver and kidney weights, and decreased ratios of liver and kidney weights to body weight. In addition, the high-dosage group showed decreased food consumption and histologic lesions of the lungs. Histologic examination revealed of mild to severe pneumonitis, fatty metamorphosis of the liver, arrest of spermatogenesis, and cellular alterations of the ovaries among all dogs. No hematologic changes occurred at any dosage. In dogs, repeated intravenous doses at 0.1 and 1.0 mg/kg-d were given to mongrel dogs (three per dosage) for 10 daily injections during a 2-wk period. Transient signs of effect at 0.1 mg/kg-d consisted of moderate hyperpnea and a slight decrease in activity, which were noted after each injection. Similar signs of toxicity were seen at 1 mg/kg-d, and these generally persisted throughout the course of the study. Terminal body weights were significantly lower in all high-dosage dogs, and significant changes in ratios of organ weights to body weight occurred among both groups. Although hematuria had been observed in earlier studies in mice and cats, it was not observed in these long-term studies.

Studies with dogs and monkeys have shown that tolerance to the toxic effects of DMHP develops.[20,35,36,40,44]

Toxicity of DMHP Acetate (EA 2233)

The same types of toxicity data were gathered on DMHP acetate as on DMHP.[18]

In mice, the intravenous LD_{50} was greater than 25 mg/kg, and the MED_{50} (median effective dose) was 0.1 mg/kg, for a safety margin greater than 250. In the dog, slight hypertension was seen at 0.1 and 1 mg/kg. In monkeys trained on a visual discrimination test, minimal behavioral effects were seen at 0.316 mg/kg, and moderate to marked effects at 1.0 mg/kg.

Repeated intravenous administrations of DMHP acetate at 0.01, 0.1, and 1.0 mg/kg-d to male albino rats (10 per dosage) were carried out for 20 daily injections during a 4-wk period. Miosis was observed at the intermediate and high dosages, and lacrimation only at the high dosage. The high-dosage animals exhibited significantly lower food consumption, growth rates, liver and kidney weights, and ratios of liver to body weight and significantly higher ratios of adrenal to body weight. No changes attributable to the compound were seen in blood chemical tests or in gross or microscopic pathologic tests.

When dogs were treated with DMHP acetate at 0.01 or 0.1 mg/kg-d for 10 daily injections, no significant effect was seen at the low dosage. Marked defensive hostility developed at the high dosage--perhaps a sign of a cumulative effect. At this dosage, there was histologic evidence of glycogen storage in the liver.

MECHANISM OF ACTION

The mechanism of action of cannabinoids is not understood. Gill and Lawrence[13] have shown that some active cannabinoid derivatives produce disordering of artificial lipid membranes, whereas inactive compounds either do not have this action or actually increase membrane ordering, as determined by electron spin resonance. This suggests that the cannabinoids resemble anesthetics in their mode of action. Cannabinoids do, however, appear to exhibit selectivity. Thus, DMHP, which is active in animals and produces marked vasomotor changes in humans, does not induce subjective effects;[31] but nabilone, which is structurally related to DMHP, causes both subjective and vasomotor changes. Burstein and Hunter[7] have suggested that cannabinoids exhibit specificity in interacting with such enzymes as phospholipase A_2 and cholesterol esterase, which may mediate some of their actions.

NEUROPHARMACOLOGY

Little is known about the pharmacology of DMHP and DMHP acetate, but the reference compound, THC, has been extensively described both in humans and in animals. Many reviews and symposia have discussed marijuana and the cannabinoids.[6,10,19,26,39] The National Institute on Drug Abuse has reported to the Congress annually on research on marijuana. And the National Research Council[34] and the Institute of Medicine[23] issued recent reports on marijuana. Thus, we will briefly describe marijuana before commenting on the two compounds tested at Edgewood.

The pharmacology of THC in humans has been carefully explored. THC intake at 50 μg/kg by smoking produces feelings of giddiness and changes in time sense and the perception of visual and auditory stimuli. At this dose, subjects may behave in a "silly" manner. Higher doses (over 200 μg/kg orally) may produce nausea, marked visual and auditory distortions, feelings of unreality and depersonalization, and auditory and visual hallucinations. The perceptual changes may be associated with panic reactions. Pulse rate is increased in a dose-related manner; blood pressure decreases, particularly on standing (orthostatic hypotension); and conjunctival injection (reddening) is observed.[24] Although some subjects liken marijuana and THC to LSD, the effects of these drugs can be clearly differentiated by subjects in double-blind comparisons.[25] The cannabinoids may also increase blood pressure, particularly diastolic pressure.[15] Hepler and Frank[21] have shown that smoking marijuana reduces intraocular pressure, and Adams et al.[4] demonstrated that this property was shared by THC. This effect is thought to be due to vasoconstriction of the afferent blood vessels to the ciliary body, which causes a decrease in perfusion pressure.[14] THC also has an antiemetic action.[37] Marijuana and THC also produce bronchodilatation.[41]

The effects of THC and other cannabinoids on psychomotor performance are not easily summarized. There is clear impairment in most psychomotor tasks at high doses. The major conclusions that can be drawn are probably that moderate doses of THC have little effect on attention or on performance of very simple and well-practiced tasks. Furthermore, performance of complex tasks and tasks requiring complex processing of information is significantly impaired by THC and other centrally active cannabinoids.

Of the specific cannabinoids studied at Edgewood, pharmacologic data are available on DMHP. It was effective in producing sedation in animals at oral doses lower than 0.5 mg/kg. Several doses and routes of administration were studied, up to 1 mg/kg given intravenously. In rats, sedation occurred without initial stimulation; in dogs and monkeys, hyperactivity was followed by depression and then, at higher doses, by coma. Motor effects were dramatic, in that animals developed spastic ataxia and showed extremely active deep tendon

reflexes. If the animals were handled while sedated by the drug, extensor seizures often occurred. In contrast with these results in intact animals, animals subjected to spinal transection showed mild depression of deep tendon reflexes.[17]

Autonomic changes were quite prominent in DMHP-treated animals--hypothermia, mydriasis, hypersalivation, bradycardia, and reduced respiratory rate. Several experimental manipulations were undertaken to determine the mechanism of these autonomic changes. The tentative conclusion was that the drug was acting centrally to reduce sympathetic tone.

DMHP was also found to have anticonvulsant properties. Studies conducted in mice showed that it was 8 times as potent as THC.[27]

DMHP was found to be 20 times as potent as THC in prolonging hexobarbital sleep time in mice. The drug produced EEG changes similar to those produced by morphine. In an experiment that might link some of DMHP's effects to opiate receptors, DMHP-induced sedation, ataxia, and analgesia were significantly reversed by the mixed-opioid antagonist nalorphine.[16]

DMHP appeared to have a wide margin of safety for acute toxic effects. One investigator calculated the therapeutic ratio for producing tranquilization in nonanesthetized dogs.[16] The ratio of LD_{50} to ED_{50} was 2,000:1, compared with only 5:1 for reserpine, a clinically approved drug. No long-term effects in animals after single doses were looked for, and no repeated-dose or chronictoxicity studies of DMHP were reported. DMHP has more potent and more prolonged hypotensive effects than THC, but far fewer psychologic effects.

MUTAGENICITY, TERATOGENICITY, AND CARCINOGENICITY

Although the literature on cannabinoids is large, no information is available with regard to the capacity of DMHP and the eight acetate isomers to produce mutagenesis, teratogenesis, or carcinogenesis in animals or man.

DELAYED AND LONG-TERM EFFECTS

DMHP and a series of optical isomers of DMHP acetate, studied at Edgewood in humans, produce similar symptoms, but vary greatly in potency. The more potent isomers appear to produce postural hypotension and fewer psychologic effects than equivalent doses of THC. However, they all evoke redness of the eyes, dryness of the mouth, a

sense of hunger, tachycardia, and drowsiness.[31,38] Postural hypotension is immediately reversed and blood pressure returned to normal by lying down.

Death due to inhalation or ingestion of marijuana has not been reported. Nor are there lasting ill effects from the acute use of marijuana,[45] except that the acute or chronic use of marijuana occasionally precipitates or exacerbates a schizophrenic state.[43] Isomer 2 is the most potent of the DMHP acetate isomers, being active at intravenous doses of 0.5-2.8 μg/kg. Postural hypotension was regularly noted, but euphoric responses were infrequent. DMHP has been the most extensively studied of these analogues.[44] At toxic doses μof 50 μg/kg or more, postural hypotension, tachycardia, hypothermia, and lethargy were noted. Fatigue, thirst, and headaches were associated symptoms. Prolonged or delayed effects of a small number of acute doses were not mentioned in the literature.

The literature on THC is much more voluminous and may be used in this evaluation, because DMHP and the DMHP acetate isomers and THC are related chemically and pharmacologically. This cannabinoid also produces no known long-term or delayed effects, except when administered chronically in large doses.[33]

The doses of the dibenzopyrans used at Edgewood were similar to those used by other investigators. Lemberger et a1.[30] used DMHP at 200 μg per 70 kg intravenously, for example. The severe postural hypotension that occurs when the drug is taken intravenously, intramuscularly, or orally is a limiting factor in giving hallucinogenic amounts of DMHP isomers.

Two long-term effects are theoretical considerations. One is that exposure to the cannabinoids may somehow have caused a chronic or delayed posttraumatic stress disorder. In the dosage and frequency used, this is unlikely. The postexperimental effect that was most undesirable was postural hypotension. This resulted in dizziness and faintness, from which all subjects recovered. Such a stress is insufficient to provoke a delayed or chronic posttraumatic stress syndrome, nor is there any evidence that any such syndrome occurred. A second consideration is that exposure to DMHP at Edgewood may have produced a tendency toward abuse of cannabinoids in later years. This is not possible to assess.

The target organs that may be involved in prolonged or delayed effects are the brain and the cardiovascular system. The mental effect consists of a transient or reversible psychosis, which may in rare instances result in activation of a schizophrenic process. The cardiovascular effects are postural hypotension and tachycardia. These are transitory and leave no permanent residua.

Given the absence of followup information on the effects of DMHP, the Committee cannot evaluate the possibility that the exposures at Edgewood produced delayed or long-term effects. However, information on THC suggests that such effects are unlikely to be associated with the exposures tested. In addition, clinical evaluations immediately after test administration did not indicate any acute effects likely to presage future complications or long-term sequelae.

A review of the epidemiologic aspects of DMHP is in Appendix C.

EFFECTS ON VOLUNTEERS

This review of acute effects on volunteers is based on clinical records at Edgewood. When the cannabinoid studies began at Edgewood in November 1958, much less was known about the pharmacology of DMHP than about the pharmacology of phencyclidine (SNA). The studies of the DMHP series in humans spanned the period from 1958 through 1968, with concentration in 1963-1966.

Although they are generally more potent, the DMHP derivatives had effects in the normal volunteers at Edgewood that were very similar to those later described over the last 15 yr by many research laboratories working with cannabis and THC. After administration of DMHP, there was more orthostatic hypotension than with THC or cannabis and possibly fewer subjective and mood effects. The time course appeared more variable, and DMHP's effects were often slower or more erratic in onset, particularly when it was given orally, than were those of THC. DMHP's effects also persisted longer.

In some of the earliest studies, beginning about November 1958, racemic mixtures of DMHP were given to approximately 35 volunteers at 0.5-4 mg per 70 kg of body weight. At 0.5 mg per 70 kg, fatigue, drowsiness, mild headache, and occasionally increased thirst developed. At 1 and 2.5 mg per 70 kg, postural hypotension was common, and faintness on standing was observed often. Blood pressure in a supine or prone position was normal or slightly increased. Weakness, ataxia, a feeling of giddiness, and general slowing of motor activity were common. At the highest doses, the subjects often showed marked psychomotor retardation, sluggishness, difficulty in concentrating, and blurred vision lasting for as long as 48 h after a single dose. Fewer comments were made about postural hypotension, probably because at this dose volunteers were unwilling or unable to get out of bed. Volunteers given over 2 mg of DMHP were judged to be incapable of performing their regular military duties. The intensity and duration of the hypotension, tachycardia, decrease in oral temperature, visual disturbance, subjective symptoms of thirst and dry mouth, and decreases in motor performance were generally dose-related, but their intensity varied among subjects. Thus, many of the signs and symptoms of DMHP intoxication were similar to those reported in recent

years in many cannabis and THC studies of volunteers, except that DMHP was more potent and probably had more effects on the cardiovascular system.

The most extensive experiments at Edgewood were done in 1963-1966 with DMHP acetate. Approximately 100 volunteers were given doses of a DMHP acetate racemic mixture during this period. Oral, intramuscular, and intravenous routes of administration were used. Oral doses ranged from 3 to about 60 μg/kg. Intravenous doses ranged from 0.5 μg/kg to (in a few subjects) 5 μg/kg. Intramuscular doses were between 0.5 and 5 μg/kg. Most subjects received only one drug exposure, and a few had multiple exposures, but rarely more than two.

Cardiovascular effects were most notable. Tachycardia and orthostatic hypotension were seen in some subjects at almost all doses. ECGs occasionally showed such nonspecific changes as inverted T waves. ECGs documented the 6- to 10-s lag in heart-rate increase caused by DMHP acetate after standing. Many subjects felt light-headed and faint on standing. As the studies progressed and the relationship between dose and orthostatic hypotension was better appreciated, this effect was less likely to occur. In general, oral doses produced changes in heart rate and blood pressure at 1 or 2 h and peak effects at 6-10 h. Major effects on the cardiovascular system disappeared in most subjects after 24 h, but persisted for several days in a few subjects in whom hypotension and increased heart rate occurred.

As is often observed with cannabis, conjunctival blood vessel injection was common. Body temperatures decreased, sometimes by 3-4°F. These changes were generally dose-dependent. Dryness of the mouth and throat, nasal stuffiness, apathy, and nausea were common, and their intensity was dose-related.

Psychomotor impairments were measured by such test batteries as the numerical facility, speed of closure, Purdue pegboard, and Stromberg manual dexterity tests. Anecdotal reports, both by subjects and by staff, of changes in behavior and mood generally paralleled the other symptoms. The spectrum of the effects and their intensity is similar to that commonly reported in the recent literature on cannabis studies in other volunteer populations. However, DMHP acetate seemed to elicit more orthostatic hypotension, and cannabis, a greater degree of mental effects.

The lack of evidence of severe mental or emotional disturbances, even in volunteers who were observed to experience intense and persistent cardiovascular effects, is noteworthy. Although DMHP acetate elicits far greater cardiovascular consequences than other cannabinoids, it appears to induce less severe mental impairment. It is possible that careful screening and a supportive test milieu tend to minimize the occurrence of adverse mental effects.

The acute effects of eight optical isomers of DMHP acetate given singly or in combination were assessed in about 125 volunteers. Several of these subjects had participated or were participating concurrently in other DMHP experiments. The isomers were given intramuscularly or intravenously. Some of the intravenous injections were given with the isomer diluted in propylene glycol and others with alcohol as the vehicle. Isomers 1, 3, 5, 6, 7, and 8 appeared to have little biologic activity (generally at about 0.5-10 µg/kg). Apart from nonspecific symptoms, such as pain at the injection site, subjects appeared unaffected subjectively and objectively. In one sense, this series of experiments provides some index of placebo responsiveness--minimal. Many subjects commented (as recorded in the charts) that they generally enjoyed the experiments, thought well of the staff support, and, in general, had few complaints other than about the food.

Isomers 2 and 4 and mixtures thereof had significant biologic activity. Intravenous doses of 1-2 mg of isomer 2 produced fairly intense tachycardia and orthostatic hypotension in the volunteers, as already described. The postural hypotension was marked, increases in heart rate were present but less intense, and feelings of impaired cognition and concentration and altered mood were present and dose-dependent. The volunteers seemed able to function reasonably well--if they were able to get out of bed and walk around. However, during the first few hours of intoxication, this was virtually impossible in many cases, because of hypotension. Dryness of the mouth, increased thirst and hunger, mild sleepiness, injected conjunctivas, and mild to severe hypotension are consistent with the effects of cannabis. Some\charts contain comments about such observations as skin pallor on standing. These are understandable in the light of the circumstances.

During the DMHP studies, hepatic function and renal function were assessed. Although occasional borderline-abnormal results were noted after exposure, these were generally followed up and did not appear to be clinically significant. Some attention was given to EEG and ECG assessments to follow the intensity and duration of any drug-induced changes in cardiovascular and brain functions. In no instances of followup, did the effects appear to be particularly specific or clinically significant for acute or long-term toxicity.

In summary, DMHP and some of its acetate isomers produced various degrees of physical incapacitation due largely to the moderate to marked and prolonged orthostatic hypotension. Blood pressure was normal in the supine position. Mental effects of DMHP were much less severe than those of THC or cannabis at doses that produced similar degrees of orthostatic hypotension. Individual differences in intensity of response were considerable: some subjects showed little or no response at doses that produced intense symptoms in other subjects.

This pattern of variability has been commented on in the extensive civilian literature on cannabinoid research. Duration of effects also varied. With most doses and subjects, the majority of measurable effects disappeared in 24 h, although in a few instances they persisted for 2 or 3 d. DMHP and biologically active isomers of its acetate cause greater and longer-lasting orthostatic hypotension and fewer psychologic effects than THC; otherwise, they are very similar on the measures recorded during these experiments. The potencies of DMHP acetate and DMHP itself seemed relatively similar. The eight isomers of DMHP acetate varied greatly in potency. Those with biologic activity seemed similar to DMHP in their effects.

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CONCLUSIONS

The Committee found the evidence on the long-term health effects of the tested psychochemicals to be sparse.

The target organs that may be involved in prolonged or delayed effects of phencyclidine are the brain and cardiovascular system. Mental or cardiovascular effects were not observed, however, within one week of exposure to the drug at Edgewood.

One measure of the margin of safety of a drug can be estimated by considering the ratio of the lethal dose to the pharmacologically effective dose (the dose at which some detectable biologic effect occurs). On this basis, the margin of safety is large for acute intravenous, intragastric, intraperitoneal, and subcutaneous administration of phencyclidine in animals. It is somewhat smaller for inhalation of the aerosolized form.

On the basis of the scientific literature alone, it is not possible to predict whether any long-term effects would be associated with the exposures to phencyclidine used. However, at the small doses and low frequencies of administration used at Edgewood in a small number of test subjects, it is not likely that any detectable long-term or delayed effects have occurred.

Acute administration of the dibenzopyrans (dimethylheptylpyran and congeners) produced various degrees of physical incapacitation in Edgewood subjects, mainly because of moderate to marked and prolonged orthostatic hypotension. The duration and intensity of effects varied among doses and subjects. Despite these variations, there is a large pharmacologic margin of safety in the use of these compounds in animals. The dibenzopyrans produced more potent long-lasting orthostatic hypotension and weaker (but otherwise similar) psychologic effects than Δ-^{9}-tetrahydrocannabinol during the Edgewood experiments. There is no information on chronic effects of dibenzopyrans.

Evaluation of the toxicity literature and the Edgewood studies led the Committee to conclude that at the doses and frequencies of administration of phencyclidine and dibenzopyrans used at Edgewood, it is not likely that detectable long-term or delayed effects have occurred or will occur. Specific information to support this conclusion is, however, lacking.

4

IRRITANTS AND VESICANTS

BACKGROUND

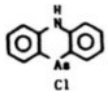
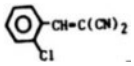
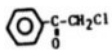
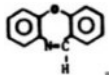
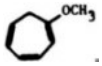
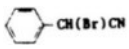
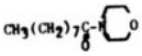
The compounds discussed in this chapter (see Table 4-1) are mustard gas (H), chloroacetophenone (CN), dibenz[b,f][1,4]oxazepine (CR), o-chlorobenzylidene malononitrile (CS), brombenzyl cyanide (CA), diphenylaminochlorarsine (DM), chloropicrin (PS), nonanoyl morpholide (EA 1778), a cycloheptatriene (EA 4923), and 123 compounds involved in limited testing. H is a vesicant, pulmonary irritant, and systemic poison, depending on dose and route of entry into the body. The other compounds are irritants. Of the irritants, DM is a sternutator (a substance that causes sneezing), whereas the others are lacrimators. All are called gases in warfare use, although they may be administered as vapors, liquid droplets, smokes, or mixtures thereof. Mustard gas and irritant chemical agents, the latter often called harassing agents, were introduced to the battlefield in World War I to confuse, harass, and disable enemy troops. Since the 1920s, irritants have been used as riot-control agents by civil authorities. Of these, CN came into widespread use and is now commonly supplied in a formulation under the name Mace. Although CS was synthesized in 1928, it became known as a riot-control agent only in the 1960s, when it appeared safer than CN.

For military use, harassing agents are intended to reduce or destroy the effectiveness of enemy troops. For this purpose, rapid onset of effects is usually, but not always, desired. Rapid recovery facilitates the handling of prisoners, whereas men injured by mustard gas require intensive care and weeks for recovery. There were no plans for studying long-term effects of World War I harassing agents.

Riot-control agents are also designed to have a rapid onset of effects, produce a high degree of immediate disability, and require a short recovery time as soon as the rioters are dispersed from the area. With the increasing use of such agents as CN and CA in recent years, their possible long-term effects have aroused concern.

At the end of World War I, medical thought was turning to the possibility that soldiers who had been gassed with mustard, chlorine, phosgene, and other agents would develop tuberculosis. In the early postwar years, publications described efforts to identify cases of tuberculosis among gas casualties. The expected epidemic failed to appear, and attention subsided. More extensive studies, such as that of Beebe, were initiated.[1] Gradually, mustard gas became the

TABLE 4-1 IRRITANT AND VESICANT COMPOUNDS

Military or Common Name	Toxicity No.[a]	CAS No.	Chemical Name	Structural Formula	Type of Chemical	No. Subjects Evaluated/ Treated
H, Mustard gas	E-133	505-60-2	Bis(2-chloroethyl)sulphide	S $(CH_2CH_2Cl)_2$	Vesicant	147/152
DM, adamnite	E-131	578-94-9	Diphenylaminechlorarsine		Sternutator	67/67
CS, EA 1779	E-108	2698-41-1	o-Chlorobenzylidene malononitrile		Lacrimator	1,366[b]/1,372
CH	E-129	532-27-4	Chloroacetophenone		Lacrimator	9/99
CR, EA 3547	E-124	257-07-8	Dibenz [b,f][1,4] oxazepine		Lacrimator	97/97
EA 4923, CHT	E-126	1728-32-1	1-Methoxy-1,3,5-cycloheptatriene		Lacrimator	16/16
PS, chloropicrin	E-132	76-06-2	Trichloronitromethane	CCl_3NO_2	Lacrimator	136/138
CA	E-128	5798-79-8	Brombenzyl cyanide		Lacrimator	13/13
EA 1778	E-110	5299-64-9	Nonanoyl morpholide		Pungent	32/32

[a] Arbitrary designation by Committee staff.

center of interest; the discovery of its mutagenic properties stimulated further research. Sufficient time has now passed for long-term effects to become clear. Mustard gas has become the subject of a large body of reports, which have disclosed a multifaceted problem.

On the other agents discussed here, there is no such volume of information. Their effects have generally been regarded as transient, and only in the Himsworth reports[2,3] on the use of CS in the Londonderry riots of 1969 is there even a proposal to study the long-term effects of the riot-control agents. Indeed, on several compounds, practically no useful information is available, except with regard to chemistry, acute toxicology, and pathology.

Because information on possible long-term effects of the other irritant chemicals used in the Edgewood tests is sparse, this chapter focuses on the effects of mustard gas and two lacrimators, CS and CN. Information on the potential long-term adverse effects of these chemicals is derived from several sources: first, observation of long-term disabilities in soldiers who were exposed to a single (in most cases) toxic concentration of irritant during World War I and in persons exposed in peacetime accidents or riot-control procedures; second, studies of morbidity in workers chronically exposed to chemical irritants during their manufacture; and third, studies in which experimental laboratory animals were exposed to selected chemicals by topical application, injection, or aerosol inhalation.

A review of the literature on experiments to assess possible chronic effects, especially mutagenic activity and carcinogenicity, of the irritant and vesicant agents reveals that these effects have not been studied systematically by current standards and techniques.

The current view is that a carefully selected battery of tests involving prokaryotic and eukaryotic organisms can be used to assess the mutagenicity or carcinogenicity of a chemical. Mutagenicity tests should include assays for gene mutations and chromosomal aberrations. No such systematic investigation has been conducted of any of the agents reviewed in this chapter, except possibly mustard gas. In the case of any agent for which a risk assessment is desired with respect to past human exposure or that continues to be used for riot control or related purposes, it seems desirable to use a battery of tests, such as those recommended in the National Research Council report on chemical environmental mutagens.[4]

Under the sponsorship of the National Cancer Institute and the National Toxicology Program, two chronic tests of CN and CS administered by inhalation are under way.[5,6] Preliminary data obtained in the subchronic studies preparatory to the definitive inhalation carcinogenicity tests are presented in the sections of this chapter dealing with CS and CN. One probable adverse effect, to judge from

observation of the subchronic tests, is irritation of the upper respiratory tract and lungs. A potential consequence of inhalation of these chemicals by humans is pulmonary damage that might readily develop into pneumonia. Prediction of any other chronic effects, including carcinogenesis, awaits the generation of appropriate data, including in vitro and in vivo bioassays.

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MUSTARD GAS

CHARACTERISTICS

Mustard gas (H)--also known as yellow cross, yperite, sulfur mustard, Schwefellost, bis(2-chloroethyl) sulfide, and dichlordiethylsulfide--is a chemical-warfare agent with both vesicant and systemic affects. H is colorless and almost odorless and is an oily liquid at 14-215°C with a molecular weight of 159.08. Except in extremely cold weather, the low vapor pressure (0.072 mm Hg at 20°C) and low volatility of H are sufficient to make contaminated surfaces a source of danger to anyone nearby. H is slightly soluble

in water and soluble in oils, fats, and organic solvents. It penetrates clothing readily. Moist skin of armpits, groin, and inner surfaces of thighs is especially vulnerable.[15,47,77]

Its garlicky odor, faint at first, is soon imperceptible. Exposure to H does not cause immediate discomfort; rather, the onset of effects is delayed and insidious. Troops have been known to remain in contaminated areas until their eyes, skin, and respiratory organs were affected. Exposure of skin produces erythema, then blisters that are painful and slow to heal. Such eye injuries as conjunctivitis, keratitis, and corneal ulcers cause temporary or permanent blindness. The respiratory effects of H include rhinitis, laryngitis, bronchitis, and, in severe cases, destruction of mucous membranes. The bone marrow and digestive system are affected by systemic administration of H. The multiple effects of this insidious agent make it among the most potent used on the battlefield.

TOXICOLOGY

Mutagenicity

Mutations are heritable changes in genes or chromosomes. Although mutations occur spontaneously as rare events in all organisms, their rates of occurrence can be markedly increased by exposure to mutagenic agents. Geneticists generally agree that the effects of human exposure to mutagens are deleterious and that such exposure should be minimized.[54,55] The basis for concern about human exposure to mutagens is that increases in the rates of mutation in human germ cells and somatic cells may lead to an increased incidence of genetic diseases and cancer, respectively.

After H. J. Muller showed in 1927 that x rays induce sex-linked recessive lethal mutations in the fruit fly Drosophila melanogaster,[51] efforts were made to determine whether chemicals can also be mutagenic. Unequivocal evidence of chemical mutagenesis was not obtained until the 1940s. Among the first demonstrations of chemical mutagenesis was a report by Auerbach and Robson[6] that mustard gas induces mutations in Drosophila. Over several years, Auerbach and her colleagues found that mustard gas causes genetic alterations ranging from gene mutations to chromosomal breaks and rearrangements.[5,19]

The mechanism of mutagenesis by sulfur mustard (and other mustards) involves the alkylation of DNA. As a bifunctional alkylating agent, sulfur mustard causes cross-linkage of DNA strands, as well as monofunctional alkylation products.[19] Sulfur mustard and nitrogen mustard have been used in mutation studies in a variety of organisms, but data on the relative frequencies of induction of different alky

lation products in DNA by the two agents are limited. Nevertheless, sulfur mustard seems to exhibit greater S_N1 character[50] as an alkylating agent than does nitrogen mustard.[19] Because agents involved in S_N1 reactions attack oxygen sites in DNA more readily than do agents whose reactions are almost exclusively of the S_N2 type, sulfur mustard may differ substantially from nitrogen mustard in the spectrum of alkylation products formed in DNA. Such differences in mechanisms of alkylation and in alkylation products can lead to considerable differences in mutagenicity.[30,71] Therefore, although nitrogen mustard and sulfur mustard are both alkylating agents, one must be cautious in assuming they are comparably mutagenic.

A comprehensive review of the mutagenicity of sulfur mustard and nitrogen mustard has been published by Fox and Scott.[19] The mutagenicity data base on nitrogen mustard is more extensive than that on sulfur mustard. Nevertheless, results have been reported regarding the genetic activity of sulfur mustard in tests for forward mutations and reversions in bacteria;[12] differential killing of DNA-repairdeficient strains of microorganisms and their repair-proficient counterparts;[34,38] reversions in fungi;[38,68] chlorophyll mutations in vascular plants;[19] gene mutations and chromosomal aberrations in Drosophila;[5,19] reversions in cultured L5178Y mouse lymphoma cells;[12,19] host-mediated assays involving bacteria or mammalian cells in mice;[12] and dominant lethal mutations in mice.[62] Clastogenic effects of sulfur mustard have been studied in plant root tips and microsporocytes[19] and in cultured mammalian cells.[65]

Data from a mouse dominant-lethal test suggest that sulfur mustard reaches germinal tissue and induces dominant lethal mutations.[62] However, the data are inadequate for prediction of genetic risk to human germ cells. Uncertainties stem from the lack of data on defined genetic events induced by sulfur mustard in mammalian spermatogonia or oocytes and from the variation in mutagenic potency that has been observed for the mustards in various assay systems. Nevertheless, the possibility that sulfur mustard is a human germ cell mutagen cannot be disregarded, particularly because it is mutagenic in diverse assays, including tests for germ cell mutations in Drosophila and dominant lethals in mice; moreover, other directacting alkylating agents are known to induce mutations[63] and chromosomal alterations[21] in mammalian germ cells.

Pathogenesis of Skin Lesions

Vogt et al.[72] recently studied the pathogenesis of lesions caused by the application of H to the skin of guinea pigs and rabbits; their methods included light and electron microscopy, histo

chemistry, and the use of contrasting Evans blue dye. Cutaneous applications of H at 25-250 μg/cm^2 caused severe injury to the skin of both species and resulted in nonvesicating, necrotic, encrusted inflammatory lesions.

Cutaneous response to H had immediate and delayed phases. Within the first hour of exposure, injury to superficial capillaries and venules, vascular leakage, and infiltration by granulocytes with a high percentage of basophils were observed. The delayed phase was evident after 8 h; was manifested by nuclear pyknosis, an increase in lysosomal enzymes, and autophagic vacuoles in basal epidermal cells; and was accompanied by diffuse vascular leakage, infiltration by polymorphonuclear leukocytes, and ulceration. After the peak of inflammatory reaction at 24-72 h, the superficial, encrusted ulcers healed in about 10 d. Topical and systemic administration of glucocorticosteroids decreased the extent of edema during the immediate phase, but did not affect the rate of healing.

Carcinogenicity

Because of the correlation between mutagenicity and carcinogenicity, one would expect sulfur mustard to be carcinogenic on the basis of mutagenicity data alone. This expectation is borne out by carcinogenicity tests in experimental animals and by data from human exposures. The International Agency for Research on Cancer classifies sulfur mustard as one of relatively few chemical agents on which the data are adequate to show an association with the induction of cancer in humans.[7]

H-induced carcinogenic effects have been demonstrated in mammals. Heston[26] reported that in two experiments the intravenous injection of aqueous H into strain A mice resulted in pulmonary tumors in 93% and 68% of the surviving mice. In 1953, Heston[27] documented the appearance of a variety of tumors in strain A, C3H, and C3Hf mice after the subcutaneous injection of H in olive oil. At the injection site (middorsal area), there were sarcomas. At other sites, there were mammary and pulmonary tumors and hepatomas, and one mouse developed lymphocytic leukemia. Heston[28] found significant increases in lung tumors after inhalation exposure of mice. Heston[26,27 and28] reported many separate experiments, some of which were performed with nitrogen mustard. Heston concluded that both mustard compounds were mutagenic and carcinogenic.

Chronic exposure of rats to H (at 1 or 100 μg/m^3, 6.5 h/d, 5 d/wk) at Edgewood produced an increase in epithelial cell tumors, but no evidence of systemic injury.[45]

The investigations discussed above show a clear progression from the discovery of a mutagenic action of H in Drosophila, through the studies of alkylation reactions of H with DNA, to the experimental production of tumors in mammals, including humans.

INDUSTRIAL AND MILITARY EXPOSURES

A few clinical observations may be instructive before discussion of the major studies of H carcinogenicity in humans.

Jackson and Adams[37] studied 33 cases of extensive basal cell carcinoma, two of which involved mustard-gas burns sustained during World War I. One of those developed 35 yr after the burn, but 2 yr after irradiation with cobalt-60. In the other, basal cell carcinoma developed at the site of three separate burns, 3 yr after exposure. Some of the mustard burns did not lead to basal cell cancer.

Illig et al.[35] treated nineteen patients suffering from psoriasis vulgaris once or twice with 50 g of 0.005% H in petrolatum. They concluded that whole-body inunction with H presents a low carcinogenic risk. That is likely to be erroneous, in view of the low dose and brief treatment used.

When examining reports of exposures to chemical agents, one should note the different conditions involved. After July 1917, during World War I, H was used often by both Germany and the Allies. Some areas of French battlefields became so badly contaminated that they were abandoned by both sides.[13] Thorpe[69] estimated atmospheric concentrations of H during gas shelling as averaging 3 ppm, with a maximum of 5 ppm (about 19 and 32 mg/m^3, respectively). Prentiss estimated that exposure at 23 ppm (150 mg/m^3) for 10 min, giving a Ct (product of concentration and duration of exposure) of about 1,500 mg·min/m^3, would be lethal for an unprotected man.[60]

In military and riot-control situations, exposure to agents is acute, but relatively brief. The clinical cases cited[17,40] involved “long-term” exposure, meaning a few months to a few years.

Nakamura,[53] in a 1956 paper, reported working conditions in a Japanese mustard-gas factory operated secretly in Hiroshima from 1930 to 1945. Workers alternately worked 1 h in gas production and 2 h in a gas-free environment over a 10-h workday. They wore gasmasks and complete protective clothing, including rubber boots, and were often rotated. Nevertheless, many workers showed a darkening of skin; some developed ulcers, diarrhea, and jaundice and later coughed blood and developed tuberculosis. The concentration of mustard gas[78] may have reached 50-70 mg/m^3, as determined by bioassay. The bioassay involved exposure of unprotected birds in the work areas that resul

ted in death in 12-15 h. Exposed rabbits refused food, coughed, and died within a 3-d period. Some adverse health effects experienced by workers may have been due to the inadequacy of protective equipment.

Yamada et al.[79] found 97 deaths during the period 1946-1957 among workers exposed to H in a Japanese factory before and after the war. Of the 97 deaths, 20 were from malignant tumors, 13 of them in the respiratory system. In 1963, Yamada[78] further reported 172 deaths, extending the survey to 1933-1962. Yamada gave no figure on the total number of men, but Wada et al.[73] stated that there were 495. Forty-eight deaths (28%) were caused by cancer, including 28 (16% of the 172) involving the respiratory tract. Among 5,030 deaths in the nonexposed general population, 406 (8%) were from cancer, including 25 (0.5% of the 5,030) involving the respiratory tract (Table 4-2).

Wada et al. extended Yamada's observations on the same men,[73] finding 33 deaths from respiratory tract cancers for 1952-1967, compared with an expected 0.9, a relative risk of nearly 37. For 960 employees not exposed to H, Wada et al. found only three deaths from respiratory tract cancer, compared with 1.8 expected. These data point to a connection between long, low-dose exposure to H and later cancer, especially in the respiratory tract.

Weiss and Weiss[75] found a statistically significant increase in malignant tumors, especially bronchial and bladder carcinomas and leukemia, among 245 German workmen employed in the manufacture of H and nitrogen mustard (HN) in the period 1935-1945. The 245 men, studied over 20 yr, all had verified case histories. By 1974, 114 of these men had died, 40 of malignant tumors and 38 of chronic respiratory ailments. From 1951 to 1972, there were 32 deaths from various cancers among the exposed workmen, more than expected in a comparable nonexposed population; only bronchial carcinoma showed a statistically significant difference: 11 observed vs. 5 expected, a relative risk of more than 2.

Hellmann[25] studied German munitions workers and reported 20 deaths from cancer among 157 former workmen. It is not clear whether these were included in the 245 of Weiss and Weiss.

Morgenstern et al.[48] reported on over 200 workmen in an American chemical plant making H during World War II, focusing on 10 case histories that illustrated the immediate and delayed effects of daily exposure to small quantities of H vapor. Exposure for 3 wk to 6 mo led these men to the dispensary for treatment of respiratory distress. Typically, a man developed some or all of the following symptoms: red eyes, photophobia, lacrimation, impaired vision, blepharospasm, loss of taste and smell, nosebleed, sore throat, chest pain, wheezing, and dyspnea. After several such occurrences, a worker was removed from further contact with H.

TABLE 4-2 Characteristics of Male Mustard-Gas Workers Who Died from Cancer (1955-1967)a

Case	Exposure Duration,		Interval from Employment to Death,		Age at Death,	Site of Neoplasm	Histologic Type
	yr	mo	yr	mo	yr		
3	7	2	22	4	62	Pharynx	Undifferentiated
4	5	3	22	0	40	Pharynx	Squamous cell
5	8	0	24	5	57	Paranasal sinus	Squamous cell
6	4	11	23	2	44	Paranasal sinus	Squamous cell
7	7	5	28	1	65	Paranasal sinus	Squamous cell
10	8	0	16	10	62	Larynx	Squamous cell
11	10	0	19	2	52	Larynx	Squamous cell
12	12	10	22	11	58	Larynx	Squamous cell
13	7	11	20	10	58	Larynx	Squamous cell
15	8	0	25	5	48	Larynx	Squamous cell
16	7	0	23	4	59	Trachea	Squamous cell
18	1	4	10	11	30	Bronchus	Squamous cell
21	6	1	23	1	62	Bronchus	Squamous cell
22	8	1	17	4	62	Lung	Squamous cell
23	2	0	33	0	54	Bronchus	Undifferentiated
24	17	0	27	0	54	Bronchus	Undifferentiated
26	16	4	27	10	61	Bronchus	Undifferentiated
27	4	10	17	9	61	Bronchus	Squamous cell
29	2	2	19	3	58	Bronchus	Undifferentiated
30	8	5	22	5	63	Bronchus	Undifferentiated
32	5	8	20	8	50	Bronchus	Undifferentiated
38	7	11	27	7	74	Lung	Squamous cell
40	2	0	27	6	55	Bronchus	Squamous cell
43	0	3	26	1	47	Lung	Undifferentiated
44	7	3	29	0	63	Bronchus	Squamous cell

[a] Data from Wada et al.[73]

Büscher,[11] Morgenstern et al.,[48] and others emphasized the lingering bronchitis, bronchial asthma, hoarseness, aphonia, and hypersensitivity to smoke, dust, and fumes that develop especially in men working in industrial situations that expose them to mustard at constant low concentrations. Even after discontinuing such work, they may be subject to continuing respiratory and systemic disabilities with a general deterioration of health that leaves them partial or complete invalids. These men recovered partially after leaving the mustard plant, but were subject to bronchitis, were susceptible to respiratory infections, and were likely to develop bronchiectasis. Many men were partially or completely disabled by 1945, when these observations ended. The implication of the observed disabilities is that complete recovery would probably not occur. It should be emphasized that the sequelae outlined here resulted from the chronic, long-term exposure to H in the working environment. Most important, these long-term sequelae (except the malignancies) generally constituted extensions or continuations of acute problems experienced during exposure to H; they did not suddenly appear years after exposure.

MEDICAL EFFECTS

Immediate Effects

An unprotected person exposed to H vapor will suffer simultaneously from skin burns, eye injury, and irritation of the respiratory tract.[20] The acute effects of H depend on the concentration of the gas, the duration of exposure, the ambient temperature, the extent of protection, and the susceptibility of the person.[23] Clothing will be contaminated and become a secondary source of poisoning even after a gas cloud has blown away.

Onset of action may occur within several hours of exposure or after a latent period of up to 24 h. The immediate effects of H within 0.5-3 h of exposure include sneezing, acute conjunctivitis, lacrimation, rhinorrhea or nasal bleeding, soreness and burning of the the throat, hoarseness and dry, hacking cough, and erythema of exposed skin. Within 4-16 h of exposure, the effects of H include eye pain with acute conjunctivitis, corneal edema, lacrimation, photophobia, blepharospasm, and edematous erythema of the eyelids; nausea, vomiting, diarrhea, and epigastric pain; and erythema and vesication of the skin with coalescence of vesicles to form bullae.[2,47,77] Between 24 and 48 h, effects on the eyes and skin progress and are manifested by eyelid pain and edema with blepharospasm, lacrimation, photophobia, and blindness; erythema, vesication, and edema of the skin; persistent cough and hoarseness or aphonia due to membranous laryngotracheobronchitis; increased temperature, pulse, and respiratory rate, as well as granulocytosis resulting from sec

ondary infection or bronchopneumonia; persistent nausea and vomiting; and marked apathy, depression, and despair.[11,15,23,24,47,77] If death occurs, it is usually after 48 h and as the result of the systemic effects of H with evidence of shock or from secondary infection, depletion of hemopoietic cells in the bone marrow, and leukopenia.[2,77]

The acute pathologic effects of H on the eye include edematous clouding of the cornea and necrosis of corneal stroma. About 5 h later, infiltration by segmented leukocytes is noted at the sclerocorneal junction and in the corneal stroma. Healing usually occurs in several weeks, but the injury may result in persistent or recurrent corneal ulceration and blindness.[33]

Burns of the skin by H may range from erythematous subvesicating injuries to large annular blisters. Experimental studies[61] of humans exposed to H have shown the following pathologic effects between 12-18 h: epidermal nuclear pyknosis, lysis of cytoplasm, liquefactive necrosis in the malpighian layer above the dermal papillae, and hyperemia, edema, and perivascular mononuclear infiltration of the dermal papillae. After 12 h, the foci of liquefactive necrosis may coalesce and enlarge to form vesicles, and there the dermis is infiltrated by mononuclear cells and polymorphonuclear leukocytes. By 72 h, there is ingrowth of epithelium at the margin of vesicles with coagulation necrosis of the corium. Healing is complete in 4-5 wk.

Postmortem examinations of persons who died as a result of exposure to H have shown depletion of lymphoid cells in the spleen, thymus, and other lymphatic organs; depletion of hematopoietic cells of the bone marrow; necrosis and desquamation of epithelium in the small intestine; acute ulceration of the duodenum; membranous laryngotracheobronchitis; and pulmonary edema, congestion, and patchy emphysema that may be complicated by bronchopneumonia or other evidence of pulmonary infection.[2,47]

The acute effects of H were observed in three children, as well as in the rescuer and attending medical and nursing personnel, after they were injured by the accidental explosion of a 40-yr-old mustard-gas shell.[29] Two children died within 4 h after experiencing congestion and swelling of the eyes, continuous vomiting, edema of the skin, pulmonary edema, pain, and shock. The older child survived, but full recovery took 5 wk. Vomiting, patchy erythema of the skin, and edema of the face were noted for 3.5 h after injury. At 5.5 h, there were vesicles on the face, puffy eyelids, irritated conjunctivae, and evidence of photophobia. Coma and agitation occurred between 6 h and 5 d. The rescuer and attending personnel had delayed onset of symptoms, including drowsiness, coughing, nausea, vomiting, hoarseness, ocular pain, photophobia, lacrimation, blepharospasm, conjunctivitis, headache, dyspnea, and burns of the skin.

A German text on noxious gases[18] updates approximate exposure conditions that may result in injury from mustard gas (Table 4-3).

Injury to Skin

Except with high concentrations of H, the initial vesicating reaction may take hours, so that more of the agent is absorbed before its presence is recognized. Cullumbine,[15] describing the action of an experimental droplet on human skin, said, "The first macroscopic sign of the action of mustard gas appears under temperate climatic conditions about two hours later" (after application). Penetration of the skin is rapid, but only about 12% remains in the skin, the rest moving into the circulatory system.[15]

In 1919, vast quantities of German chemical munitions were stored in the Luneburg Heath, awaiting destruction. On October 24, an explosion destroyed buildings, tank cars of chemicals, stacked artillery shells, and other material. When workmen began the task of cleaning up, chemical burns were inevitable. Hermann Büscher,[11] a young physician just out of the German Army, practiced only a few kilometers away in Münster. At first a few, then a flood of injured workmen were sent to Büscher. Knowing almost nothing about the chemicals and their effects, he worked empirically, gradually developing systematic therapies for the results of exposure to mustard, Lewisite, and other agents.

Eventually, Büscher experimented on human volunteers. Like Cullumbine, he found that, when a drop of H is placed on skin, the initial reaction appears in about 2 h. Vesication begins in about 24 h, but healing does not begin until after about 4 wk, and later for severe burns. Büscher wrote: "There are also irreparable, permanent injuries. . . . Cicatricial contractures are very frequent sequelae. . . . Following severe wounds this scarring is very extensive so that there can be no question of complete recovery." In all cases of extensive burns from mustard gas, careful and extended treatment is required to prevent infection and other complications.

Eye Injuries

Of the long-term complications of wartime exposure to mustard gas, perhaps the best documented and one of the most serious is recurring corneal ulcers, with eventual opacification and blindness.[32] NO exact figures are available for predicting the eventual development of such long-term corneal lesions, but it has been reported that a Ct of 100 mg min/m^3 will cause acute blindness for 24-48 h.[20] Permanent blindness typically occurred about 14 yr

TABLE 4-3 Effects of Various Concentrations of Mustard Gas on Humansa

Concentration		Duration of Exposure	Effects
mg/m^3	ppm (approx.)		
0.5	0.08	10-25 min	Eye and skin damage
1.0	0.15	1-2 h	No serious damage
1.0	0.15	8-10 h	Incapable of combat
1.2	0.18	45 min	Damage to eyes after 12 h; damage to skin after 2 d
2.5-5.0	0.4-0.8	30-60 min	Slight irritation of sensitive skin
6.5	1.0	60 min	Occasional serious lung injury
70	11	30 min	Apparently lethal
100-200	15-31	Few seconds	Itching of sensitive skin for many weeks

[a] Data from Flury and Zernick.[18]

after acute necrotizing injury to the corneal epithelium and basement membrane, with but little loss of visual acuity in the intervening years.[32]

The human eye is sensitive to H vapor, and liquid drops will produce severe burns leading to blindness. Inflammation, conjunctivitis, iritis, and keratitis are terms used to describe eye injuries. Temporary or permanent blindness results from light to moderate exposures to the vapor. Efforts to measure the effects of H vapor on animal eyes have yielded values cited by Hughes.[32] The human eye has been estimated to be 4 times as sensitive as the rabbit eye:[20] a Ct of 100 mg·min/m^3 causes impairment of vision for 24-48 h, and it is estimated that a Ct of 200 mg·min/m^3 would produce blindness for a week or more (Table 4-4).

Buscher encountered relatively few cases of liquid mustard in workmen's eyes, because gasmasks were usually worn. Of men exposed to H vapor, he wrote, “Even in cases of severe keratitis in which there are various opacities of the corneal epithelium, the disease usually terminated fairly favorably, without leaving too much permanent damage.” This was written in 1931 before the delayed and relapsing keratitis recorded by later writers occurred.

Mann[43] examined the records of 84 men described as suffering from “delayed mustard gas keratitis.” This group had been treated at the Contact Lenses Clinic at Moorfields, England. The eye injuries were described as “typical mustard gas scars with corneal degeneration.” Mann found a low incidence of onset of trouble in the early postwar years, with a sharp rise in 1931 and peaks in 1934 and 1937 (Figure 4-1). Most, 19-23 yr old when gassed, were about 33-37 when the eye trouble peaked. The onset of symptoms was commonly provoked by minor eye injuries and followed by ulcers that tended to recur spontaneously and cause steady diminution in visual acuity. These men were all fitted with contact lenses. About half were able to wear them with improved vision; the others varied from partial success to total failure. Even those helped most, however, suffered slow deterioration of visual acuity.

Scholz and Woods[66] reviewed 136 cases of mustard injuries of the eye, including the 84 described by Mann. They found no essential difference between “chronic” and “recurring” cases. Although a number of injured men retained “fair visual acuity” after the initial burn, vision degenerated later; the average loss of vision (excluding five minor injuries) was about 88%. The only effective treatment for these patients was contact lenses.

TABLE 4-4 Dosages of H Vapor for Production of Injuries in Man

Protection	H Dosage, mg·min/m^3 Hot and Humid Weather, Temp. above 80°F, Sweating Skin	Warm Weather, Temp. 60-80°F, Skin not Wet with Sweat	Cool Weather, Dry, Temp. 40-60°F, Skin Cool and Dry	Effect	Disability	Time to Onset	Duration of Effect
None (no mask or protective clothing)	50	50	50	No significant injury, maximal safe dosage	--	--	--
	100	100	100	Eye damage of threshold military significance	Partial	6-24 h	1-3 d
				Temporary blindness	Total	3-12 h	2-7 d
Mask (no protective clothing)	100	150	400	No significant injury, maximal safe dosage	--	--	--
	200	300	1,000	Skin burns of threshold military significance	Partial	2-12 d	1-2 wk
	500	1,000	2,000-4,000	Severe genital burns	Partial	2-7 d	1-4 wk
	750	2,000-4,000	4,000-10,000	Severe generalized burns	Total, (Partial)	24 h (4-12 h)	1-2 wk (3-6 wk)

[a] Data from Gates and Moore.[20]

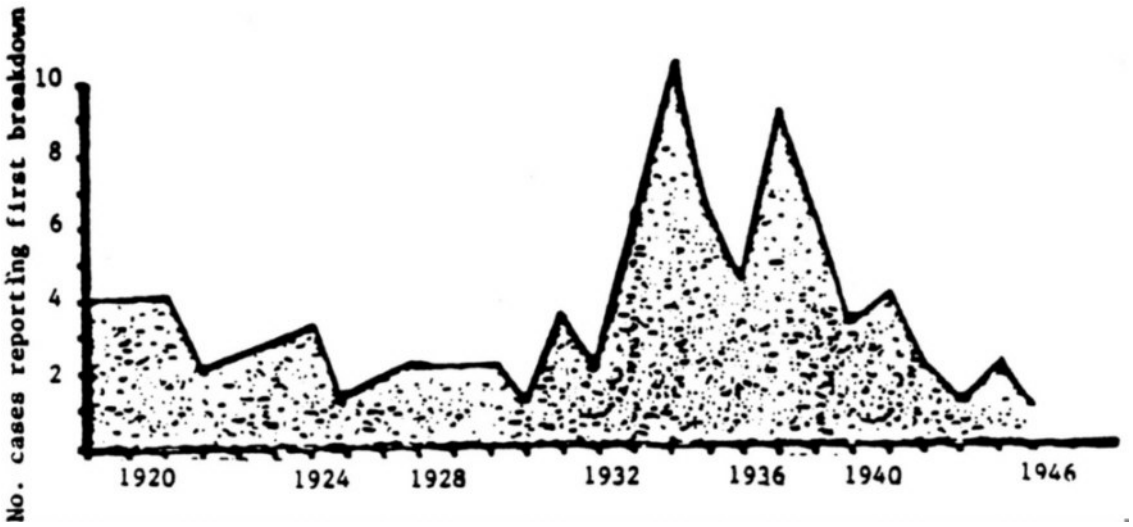

FIGURE 4-1 Distribution of onset of delayed keratitis in 82 of 84 cases. Two of the 84 did not remember the date of the first onset of trouble after apparent cure. Four stated that they had had continuous trouble from the time of gassing, and four had had a slight breakdown within a year. Redrawn from Mann.[28]

Hughes[32] reviewed mainly British and European reports and cited official British data estimating that 75-90% of mustard gas casualties had some degree of ocular injury. A rough estimate, based on information reported by Case and Lea,[13] indicates a little over 100,000 cases of eye injury. Hughes stated that about 10% of these injuries resulted in corneal erosion, which he considered predictive of visual degeneration. Corneal transplants or contact lenses could be expected to help many patients.

Hughes[33] cited these doses of H found by various experimenters to produce eye lesions:

- Destructive corneal lesions (rabbit?)--0.0004 ml.
- Ocular lesion (rabbit)--70 mg/m^3 (as vapor) for 30 min.
- Conjunctivitis (dog)--1 mg/m^3 (as vapor) for 2 h.
- Visible reaction (man)--0.5 mg/m^3 (as vapor) for 1 h.

Atkinson[4] provided details on two of his patients who had suffered mustard poisoning from the vapor; both eyes and lungs were affected. He found no decrease in visual acuity (both men being practically perfect in acuity) between prewar and 20-yr-postwar examinations. Both had some corneal haziness, which proved the precursor of progressive deterioration of vision.

Injury to the Respiratory System

Studies of fatal and near-fatal human exposures to mustard gas, with animal studies, provide compelling evidence in the aggregate that exposure to H can cause injury to the respiratory system, particularly to the bronchial tree, and that lethal doses can be associated with a superimposed bronchopneumonia with abscess formation (reviewed by Gilchrist and Matz[23]). Moreover, as many as 19,000 British soldiers, or approximately 12% of those exposed to mustard gas in combat, were pensioned during 1920 for disability believed to be caused by gas exposure during combat.[22] The predominant type of pulmonary injury reported is acute and chronic bronchitis. A factual basis for such injury is provided by repeated demonstrations in both man and experimental animals that high exposures to mustard gas cause necrosis of airway epithelium, often with pseudomembrane formation and secondary bacterial infection.[23]

Berghoff[9] noted that, whereas a single exposure to mustard gas primarily affects the respiratory tree acutely, long-term (5 mo) effects of such exposure most notably involved the skin and eyes, although as many as 30% of gas victims also appeared to manifest chronic bronchitis. Similarly, Gilchrist and Matz[23] reported that gas victims could develop chronic bronchitis, emphysema, and asthma--again, as long-term (10 yr) consequences of mustard exposure.

Less clear, however, is the exact incidence of pulmonary sequelae after exposure to mustard gas, because it was often difficult to document whether a person had been exposed to mustard alone, to mustard in combination with another agent, or to an entirely different agent.[22,42] In yet other cases, influenza or other respiratory diseases were misdiagnosed as mustard-gas injury.[8,22] It was particularly difficult to determine with any accuracy the dosage or extent of exposure under wartime conditions, particularly where people received multiple exposures. Finally, early long-term studies of chronic pulmonary effects often failed to control or correct adequately for other causes of chronic pulmonary disease (particularly bronchitis), such as cigarette-smoking and long-term exposure to polluted urban environments. In summary, although there is compelling reason to believe that high-dose exposure to mustard gas can result in acute and chronic injury to the respiratory airways (bronchitis), the data do not permit one to calculate the dose-response relationships or the incidence of pulmonary sequelae with any degree of certainty.

Injury to the Nervous System and Other Organ Systems

Injury to the nervous system, although often reported, is somewhat more subjective, and the reports do not appear to have been based on objective data gathered in a scientific fashion. The general malaise, apathy, depression (reviewed by Lohs[41]), loss of

libido,[74] and other psychopathologic effects are difficult to interpret in the absence of carefully controlled studies. Other reported effects include brain abscesses, periodontal disease, osteoporosis, and liver damage, but, again, substantive epidemiologic data are lacking.

Systemic Effects

Lohs[41] reviewed the literature and came to highly disputed conclusions. The panel felt that information was misquoted, distorted, scientifically inaccurate, or politically flavored, because authors hold strong personal views opposing chemical warfare.

Cullumbine[15] postulated the production of a "capillary permeability factor" caused by the reaction of H with tissues. Experiments that he and co-workers performed supported the appearance of such a factor. Figure 4-2 shows what he suggested occurs after H contaminates the skin.

In severe poisoning, shock, vomiting, and diarrhea develop. Cullumbine's estimate of 12% retention of mustard in the skin means that nearly all the agent attacks the body generally. Citing contradictory results obtained by others, he came to no conclusions as to precisely how the mustard is carried in the circulation, opining that both formed elements and plasma are carriers and that the circulating mustard is taken up rapidly by the body tissues. Leukopenia occurred in World War I gas casualties, facilitating secondary infections and delaying recovery.

In discussing the effect of mustard on the intestinal tract, Cullumbine pointed out that severely poisoned men suffered loss of water and electrolytes because of vomiting, diarrhea, and vesication. Oligemia and irreversible circulatory failure follow, much as in thermal burns. He showed the importance of the loss of water and electrolytes by experiments in which the mortality rate of poisoned rats and rabbits was greatly reduced by the administration of physiologic saline solution either by mouth or by injection.

Long-Term Morbidity

The long-term morbidity associated with exposure of soldiers to H at toxic concentrations is well established. Although it was not possible, in most cases, to determine the Ct for each individual exposure, it has been estimated that battlefield concentrations ranged from 19 to 32 mg/m^3.[69] This estimate seems reasonable, inasmuch as approximately 1% of exposures proved fatal[23] and short exposures at 150 mg/m^3 are generally lethal for man.[60] Long-term sequelae can be further evaluated from the fact that approximately 20,000 of 150,000 British gas victims were pensioned after the war; these figures include all forms of gas used, and the proportions for mustard gas are likely to be larger.

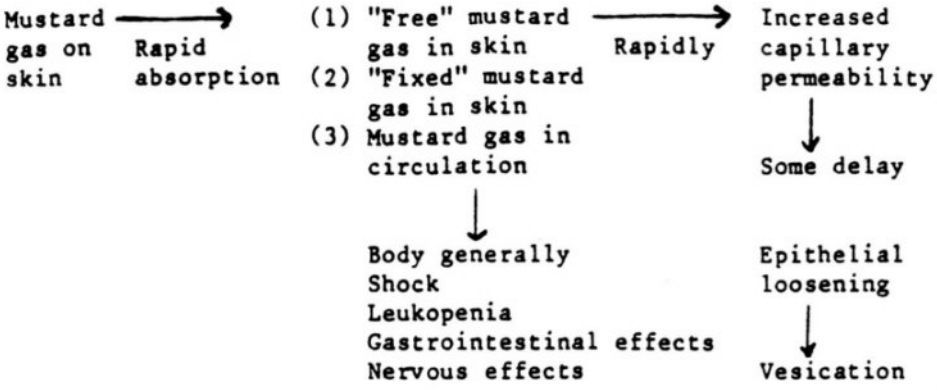

FIGURE 4-2 Physiologic processes that occur after mustard-gas contamination of skin.

WORLD WAR I CASUALTIES: SEQUELAE

Tuberculosis

During the latter half of World War I and the early postwar years, there was serious concern that men poisoned by inhalation of chlorine, phosgene, and mustard (the three most common and deadly agents) would develop tuberculosis and cancer. Limited studies were begun, but it proved difficult to produce evidence to support the idea. Achard,[1] Wilson and Mackintosh,[76] Sergent and Haas,[67] Dennis,[16] Cowen,[14] Morris,[49] Meade,[46] Atkins and Klotz,[3] Berghoff,[9] Sandall,[64] Hueper,[31] Gilchrist,[22] Gilchrist and Matz,[23] and the U.S. War Department[70] all reported on this. No increased incidence of tuberculosis was found.

About 10 yr after men had been gassed, Gilchrist and Matz[23] found residual disabilities, such as chronic bronchitis (usually accompanied by emphysema), bronchial asthma, chronic conjunctivitis, blepharitis, keratitis, and corneal opacities.

Beebe,[8] in a later study mainly on cancer, included some data on tuberculosis. These data (Table 4-5), although suggestive of a residual effect from mustard-gas exposures, were not statistically significant.

Cancer

The work of Boyland and Horning,[10] Heston,[26,27 and 28] and others stimulated interest in the mutagenic and carcinogenic potential of mustard gas. This was followed by the work of Case and Lea,[13] who examined the possible carcinogenic effects of H on exposed British soldiers. They cited total British gas casualties as 160,970, 80% of whom were estimated to have been H casualties. As of January 1, 1930, 1,267 men in England and Wales were receiving pensions for the effects of H in 1917-1918. Almost all were suffering from bronchitis. This group was studied. A group of 1,421 men who were pensioned because of bronchitis but had not served overseas after the first use of mustard gas were controls. A second control group of 1,114 was selected at random from men not exposed to mustard gas and pensioned because of single-leg amputation. The results of this study, summarized in Table 4-6, showed unusually high mortality in both the mustard and bronchitis series. In the mustard series, the high mortality involved excessive deaths from bronchitis and, to a lesser extent, excessive deaths from cancer of the lung and pleura. In both series, deaths from cancer of the lung and pleura occurred at twice the rate for the general population. The amputation series did not deviate from the rate of the general population. Case and Lea[13] concluded that chronic bronchitis from H led to cancer of the lung and pleura, but that H was not a direct carcinogen.

Beebe,[8] like Case and Lea, set up three groups: mustard gas casualties, pneumonia cases, and those with leg wounds; the latter two series excluded any who might possibly have had contact with H. Sample size was set at 2,500 each, but the restrictions left Beebe with groups of 2,718 H casualties, 1,855 cases of pneumonia, and 2,578 men with leg wounds. All in the H group had evidence of H injury to skin, eyes, and respiratory tract. All were born between 1888 and 1893 and were alive on January 1, 1919. Records in the Veterans' Administration files enabled Beebe to follow the men up to January 1956. In a followup study of lung-cancer mortality, Norman[56] extended Beebe's work to 1965. Because Norman studied the same men, his data are presented here in combination with Beebe's.

Table 4-7 shows observed deaths in the three categories compared with age-specific expected numbers based on mortality data on U.S. white males born in 1891. In general, the observed:expected ratios are lower than 1, apparently because the preinduction medical examinations excluded men not in good health. Deaths did increase sharply in the mustard-gas roster for 1930-1939.

During the last 7 yr of the Norman study (except 1956-1958), all three rosters showed mortality lower than the standard values for all U.S. white males. Mortality from cancer of the respiratory system

TABLE 4-5 Numbers of Deaths and Deaths per 1,000 Men per Year for Respiratory Tuberculosis, by Roster and by Period of Followupa

	Roster					
	Mustard Gas		Pneumonia		Wounded Controls	
Followup Period	No. Deaths	Rate	No. Deaths	Rate	No. Deaths	Rate
1919-1928	17	0.64	23	1.28	15	0.59
1929-1938	41	1.63	12	0.70	13	0.54
1939-1948	27	1.20	15	0.96	19	0.86
1949-1955	14	1.03	4	0.43	7	0.53
Total	99	1.13	54	0.89	54	0.63

[a] Data From Beebe.[8]

		Disease Class							
All Causes		All Neoplasms		Cancer of Lung and Pleura		Neoplasms other than Cancer Cancer of Lung and Pleura			
Series	Deaths	No. Deaths	Mortality Ratio	No. Deaths	Mortality Ratio	No. Deaths	Mortality Ratio	No. Deaths	Mortality Ratio
Mustard gas	Found	547	1.53	79	1.30	29	2.07	50	1.07
	Expected	357.3	(VHS)	60.8	(S)	14.0	(HS)	46.8	(NS)
Bronchitis	Found	932	1.38	104	1.09	29	2.01	75	0.93
	Expected	673.8	(VHS)	95.0	(NS)	14.4	(HS)	8.06	(NS)
Amputation	Found	383	1.05	72	1.00	13	0.84	59	1.04
	Expected	365.7	(NS)	72.2	(NS)	15.5	(NS)	56.7	(NS)

[a] Data from Case and Lea.[13] VHS = very highly significant ($p < 0.0001$). HS = highly significant ($0.017 > p > 0.0001$). S = significant ($0.05 > p > 0.01$). NS = not significant ($p > 0.05$). Mortality ratios based on theoretical numbers of years lived.

TABLE 4-7 Observed and Expected Deaths, by Roster and Timea

Series and Period	No. Deaths		
	Observed	Expected	Mortality Ratio
Mustard-gas			
1919-1929	122	163	0.75
1930-1939	248	190	1.31
1940-1949	314	323	0.97
1950-1959	519	534	0.97
1960-1965	413	426	0.97
Total	1,616	1,636	0.99
Pneumonia			
1919-1929	97	112	0.87
1930-1939	121	130	0.93
1940-1949	224	221	1.01
1950-1959	346	372	0.93
1960-1965	269	300	0.90
Total	1,057	1,135	0.93
Control (Wounded)			
1919-1929	99	155	0.64
1930-1939	169	180	0.94
1940-1949	305	307	0.99
1950-1959	507	527	0.96
1960-1965	383	421	0.91
Total	1,463	1,590	0.92

[a] Data from Norman.[56] Expected deaths based on U.S. white age-specific male mortality data for 1919-1965.

was greater among men exposed to mustard gas than among the pneumonia or wounded cohorts (Table 4-8). These data suggest that gassed men experienced a 40% excess of lung-cancer mortality. The risk relative to controls, 1.4 (95% confidence limits, 0.9-1.9), was less than that required for statistical significance. Beebe and Norman discussed the possible influence of cigarette-smoking as an additional risk of lung cancer. Statistically, the differences were insignificant. However, direct observations on smoking were not generally available. Furthermore mortality among men in the mustard roster was generally greater than that in the other rosters--except for 1959-1960, but these exceptions were not great.

Although Case and Lea[13] concluded that exposure to H tended to increase the development of bronchitis and might therefore be a cause of respiratory cancer, even indirectly, Beebe[8] and Norman[56] failed to find a significant association between bronchitis and cancer. In contrasting the lack of a statistically significant proof of military H exposure as a cause of respiratory cancer with the highly significant excess of lung-cancer mortality in Japanese H factory workers, Norman suggested that the usual single exposure in military service was insufficient to be carcinogenic. The carcinogenicity of prolonged exposure to H was convincingly shown by the Japanese data.

Experimental and epidemiologic support for the carcinogenic potential of mustard gas has been confirmed by a World Health Organization review of data from all sources and periods of time and not merely World War I.[36]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

Between 1955 and 1965, 147 human subjects underwent exposure to H at Edgewood. One hundred sixteen masked subjects had aerosol chamber exposures to test the effectiveness of various protective garments. Equipment was tested for leaks with chloropicrin exposures before H exposures. Subjects underwent up to 14 exposures to H on different days and were removed from the tests when dermal erythema indicated garment leakage. Some tests simulated tropical or windy conditions, and others simulated battlefield functions. Thirty-one subjects had cutaneous exposures to test the effectiveness of antidotes or treated cloths or for sensitization.

In 1955, 11 subjects underwent up to 10 aerosol exposures lasting 17-22 min each. The maximal cumulative Ct was 6,000 mg min/m^3. Five subjects sustained dermal effects and discontinued the test after three to eight exposures. Erythema occurred on the upper chest and flexor surfaces of the arms. Eight subjects had normal post-exposure blood counts and urinalyses.[44]

TABLE 4-8 Observed and Expected Deaths Due to Respiratory Cancer, by Age at Death and by Roster, 1919-1965a

Age at Death, Yr	No. Deaths							
	Mustard Gas		Pneumonia		Wounded		Total	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
26-29	0	0.03	1	0.02	0	0.03	1	0.08
30-34	0	0.13	0	0.09	0	0.12	0	0.34
35-39	0	0.27	0	0.19	0	0.26	0	0.72
40-44	1	0.66	0	0.45	0	0.64	1	1.75
45-49	5	1.85	0	1.28	2	1.80	7	4.93
50-54	3	3.80	5	2.66	1	3.73	9	10.19
55-59	13	7.69	5	5.36	12	7.55	30	20.60
60-64	15	12.46	3	8.51	14	12.07	32	33.04
65-69	14	16.47	10	11.44	13	16.11	37	44.02
70-74	14	13.71	8	9.92	5	13.86	27	37.49
Total	65	57.07	32	39.92	47	56.17	144	153.16

[a] Data from Norman.[56] Expected deaths based on U.S white age-specific male mortality data for 1919-1965.

In 1955, 12 subjects underwent up to 14 aerosol exposures of 45 min each. The maximal cumulative Ct was 30,800 mg·min/m^3. Ten subjects sustained dermal effects and discontinued the test after eight to twelve exposures. Erythema occurred on trunks and extremities. Three subjects had blisters. One subject, whose total Ct was 17,700 mg·min/m^3, was hospitalized 5 d after his last exposure because of diffuse erythema and bullae.[44]

In 1957, two subjects had three daily aerosol exposures of 499, 1,000, and 2,031 mg·min/m^3. No leaks occurred.

In 1963, 18 subjects underwent up to 10 exposures of 14-60 min each. The maximal cumulative Ct was 9,504 mg·min/m^3. Five subjects sustained dermal effects and discontinued the tests after six to eight exposures. Erythema occurred on backs and extremities. One subject developed marked vesication of his extremities 7 d after the last exposure. His total Ct was 5,898 mg·min/m^3.[57]

In another 1963 experiment, 13 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 16,000 mg·min/m^3. There were no injuries.

In 1964, 12 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 10,800 mg·min/m^3. Eight subjects had dermal effects, and three discontinued the test after six to eight exposures. Erythema occurred on backs and flexor surfaces of the extremities.[52]

In another 1964 experiment, 10 subjects had two applications of 4 mg of H on their forearms. Each drop was treated with an experimental decontaminant (Decon 10 and M-5 ointment). All exposure sites developed erythema and five vesicated.

In another 1964 experiment, 12 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 10,400 mg·min/m^3. Nine subjects developed dermal effects, and seven discontinued tests after four to eight exposures. Erythema occurred on anterior trunks, genitalia, and groin. One subject developed a vesicle.[58]

In another 1964 experiment, 10 subjects had H at 2 g/10 cm^2 on three separate swatches of protective cloth taped to their arms. The protocol notes that the cloth was protective in nine subjects. One subject was hospitalized when the exposed area vesicated with later eschar formation.

In 1965, 36 subjects underwent up to 10 exposures of 15-60 min each. The maximal cumulative Ct was 10,300 mg·min/m^3. Thirty-four subjects had dermal effects and discontinued the tests after two to

nine exposures. There was mild to moderate erythema on the necks.[59]

In another 1965 experiment, 11 subjects had percutaneous H exposures to test for skin sensitivity. Ten of these subjects had previous aerosol H exposures. Each subject had seven daily exposures of moving H vapor at the same site for a total Ct of 1,100 mg·min/m^3. There were then three daily exposures, at a different site, to static H vapor with a Ct of 200 mg·min/m^3 each day. Two subjects had mild erythema on the latter sites at the completion of the tests.

In 1966, 10 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 10,250 mg·rain/m^3. Four subjects developed burns, and two discontinued tests after 6 and 7 d. Erythema occurred on necks.[39]

In summary, the records of the 147 men who participated in tests involving H were reviewed. Many subjects sustained dermal injuries. No reactions to exposures were observed in 59 men. An additional 77 men experienced erythema without skin blistering. Blistering was seen in 11 men, two of whom were hospitalized, one for 5 d. None of these subjects sustained ocular or respiratory tract injury. This indicates that the ocular and respiratory systems were adequately protected during these tests. A few of the skin injuries might have been severe enough to cause permanent scarring. Although subjects entered the H vapor several times, they were protected by special garments, and exposures were discontinued after dermal injuries indicated equipment failure (leaks). Subjects' actual exposures to H were therefore limited, and there was no evidence of acute pulmonary or ocular injuries. Given the absence of followup data, it is not possible to predict long-term health effects, except scarring from acute injuries.

SUMMARY

Mustard gas is mutagenic in various organisms and test systems. One cannot readily predict the degree of genetic risk that it poses for man, however, because data on its mutagenicity in mammalian germ cells are very limited, and the mutagenic potency of mustards varies considerably among assay systems. Nevertheless, the available evidence suggests that the possibility of mutagenic effects of mustard gas in human germ cells should not be disregarded. The clear mutagenicity of mustard gas in various assays is consistent with its carcinogenic potential.

Mustard gas is not only a vesicant, but also a systemic poison. Its acute effects have been demonstrated in bone marrow, intestinal tract, and respiratory tract. It can cause blindness and permanent

skin scarring with a potential for skin tumors. It probably can also cause acute and chronic bronchitis. Other nonmalignant chronic effects have not been adequately documented.

Single exposures, even if severe, as in military service, are not associated with statistically verifiable increases in mortality from tuberculosis and cancer; but repeated small exposures, such as occur in industrial operations, do increase cancer deaths significantly.

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O-CHLOROBENZYLIDENE MALONONITRILE

CHARACTERISTICS

o-Chlorobenzylidene malononitrile (CS)--also called o-chlorobenzalmalon-nitrile and o-chlorobenzylidene malononitrile (see Table 4-10) is a white, crystalline solid that melts at 95°C, boils at 310-315°C, and has a faint, peppery odor. It is almost insoluble in water, but breaks down rapidly in water and body fluids, forming less active compounds. At a pH of 7.4 in water, the half-time for breakdown is 14 min.[3] CS is moderately soluble in alcohol and soluble in acetone, dioxane, methylene dichloride, ethyl acetate, and benzene.[32] CS, first prepared by Corson and Stoughton[12] in 1928, is a strong sensory irritant. When it came into use in the 1960s as a riot-control agent, the long chemical name was replaced with the initials of the chemists.

CS is a lacrimator that is more effective, less toxic, and 10 times more potent than CN.[3,42] The vapors of CS are extremely irritating to the eyes and respiratory tract. For these reasons, it has largely replaced Mace (CN) as a riot-control agent. It is disseminated by spraying in powder form, sometimes mixed with an anti-caking silicate (Cab-o-Sil), spraying in a liquid carrier, or incorporating in various types of grenades as a pyrotechnic mixture.

BIOCHEMISTRY AND PHYSIOLOGY

CS causes alkylation of sulfhydryl-containing enzymes and inhibits lactic dehydrogenase, glutamic dehydrogenase, pyruvic decarboxylase, and alpha-glycerophosphate dehydrogenase.[24,40] It reacts with a number of nucleophilic compounds, such as glutathione, plasma protein, and lipoic acid.[24]

Estimates of the incapacitating and lethal concentration-time products (ICt_{50} and LCt_{50}) of CS in man are 0.1-10.0 and 52,000-61,000 mg·min/m^3, respectively.[28] As an aerosol, it has a toxic

concentration (TC_{50}) for the eye of 4 x 10^{-3} mg/m^3 and an IC_{50} of 3.6 mg/m^3.[3] A concentration of 4 mg/m^3 is effective in riot-control conditions for harassment,[3,20] but 10 mg/m^3 may be used in field conditions to disperse troops.[20] Small particles of CS are more effective than large particles to produce rapid ocular and respiratory irritation, as well as rapid recovery after exposure.[3,16] The minimal detectable irritant concentration of CS is 0.004 mg/m^3,[20] and lacrimation occurs at 1 mg/m^3.[49] Although the human lethal dose of CS by inhalation is estimated as 25,000-150,000 mg·min/m^3, no deaths from use of CS aerosols have been documented.[3]

Cucinell et al.[13] reversed the CS inhibition of lactic dehydrogenase in dogs by injecting sodium thiosulfate. Rats poisoned by CS at 80 mg/kg (more than the LD_{50}) were saved by injections of thiosulfate. Cucinell et al. speculated that the toxic effects of CS were caused by inhibition of sulfhydryl-containing enzymes.

Cuclnell et al. examined some physiologic reactions to CS in the dog. A spray containing CS at 25 µg/L caused an increase in blood pressure, tachycardia, and changes in respiratory pattern. The dogs yelped and might have been in pain. Release of bradykinin in rabbits tested with CS may have been related to the pain caused by CS.[13]

TOXICOLOGY IN IN VITRO AND ANIMAL STUDIES

Mutagenicity

CS has been tested for its capacity to induce mutations in bacteria and in the fruit fly Drosophila melanogaster and for its capacity to cause chromosomal damage, as measured by a micronucleus test, in mice. Its capacity to bind to DNA in mammalian liver and kidney has also been studied. The results have been predominantly negative.

Von Däniken et al.[47] tested CS for mutagenicity in the Salmonella/microsome test in the standard tester strains TA1535, TA1537, TA1538, TA98, and TA100 of Salmonella typhimurium. Tests were conducted both with and without an in vitro metabolic activation system and were performed both by the standard Salmonella/microsome plate-test procedure and by a preincubation modification of that procedure. Only strain TA100 showed evidence of mutagenicity, and its response was weak. The maximal increase in numbers of revertants per plate was by a factor of about 2, and that increase occurred at the high chemical concentration of 1 mg/plate.

In two recent studies of CS, the weak mutagenicity reported by von Däniken et al.[47] has not been confirmed. Rietveld et al.[37] (1983) described CS as nonmutagenic in S. typhimurium strain TA100

both in the presence and in the absence of a rat liver metabolic activation system. The compound was tested at concentrations up to 1 mg/plate; at higher concentrations, bacterial toxicity was observed. Unfortunately, no data were presented to support the conclusion of nonmutagenicity, and an independent evaluation of the results is therefore not possible.

Wild et al.[48] also reported CS to be nonmutagenic in the Salmonella/microsome test. Tests were conducted in strains TA1535, TA1537, TA1538, TA98, and TA100 both with and without a rat liver metabolic activation system. Concentrations up to 1.5 mg/plate were tested, and toxicity was noted at the high concentrations. Data were presented only on strain TA100 in the absence of metabolic activation; the data on a negative control, a positive control, and eight concentrations of CS supported the authors' conclusion of nonmutagenicity. Although the result would be more convincing if data were available on all strains and on tests with metabolic activation, it is noteworthy that the negative data presented were on the same strain in which von Däniken et al. reported a weak positive response.

Von Däniken et al.[47] (1981) also tested CS for its ability to bind to DNA and protein in rat liver and kidney. When CS labeled with 14C was administered to rats by intraperitoneal injection, it bound readily to liver and kidney nuclear protein, but not to DNA in these organs. From these results, von Daniken et al. concluded that mutagenesis and carcinogenesis from CS exposures would be unlikely in humans. It should be noted that binding studies do not measure defined genetic events and therefore do not provide a strong basis for regarding a substance as nonmutagenic. Because other tests for genetic damage have been negative, however, the failure to detect binding to DNA can be regarded as consistent with the negative results.

In a test for sex-linked recessive lethal mutations in Drosophila, Wild et al.[48] found no evidence of mutagenicity of CS. More than 9,000 chromosomes were tested, and the frequencies of mutations in the treated groups did not differ from those in the concurrent negative controls or the historical negative controls. The available information on the toxicity of CS under the treatment conditions is minimal. The actual dosages received by the flies are also uncertain, particularly because CS breaks down rapidly in water. Nevertheless, the available data give no indication of mutagenicity of CS in Drosophila.

CS has been tested for its ability to cause chromosomal damage in a micronucleus test in mice. Micronucleus tests detect small nuclei that arise from chromosomal fragments or chromosomes that fail to be incorporated into normal daughter nuclei when cells divide. An increased frequency of micronuclei is evidence of chromosomal break

age or loss. No increase in the frequency of micronuclei was observed in polychromatic erythrocytes in the bone marrow of mice treated with CS. Treatments were given either by oral administration or by intraperitoneal injection, and they included appropriately toxic dosages.

Taken in their totality, the tests of CS for gene mutations and chromosomal damage in several organisms provide no clear evidence of mutagenicity. In fact, most of the evidence is consistent with non-mutagenicity. The available data are not sufficient to preclude mutagenicity absolutely. However, in the Committee's judgment, it is unlikely that CS poses a mutagenic hazard to humans.

Teratogenicity

In 1973, Upshall[46] reported tests of CS for teratogenicity in female Porton strain rats and New Zealand White rabbits. He attempted to simulate conditions that exist in riot-control situations, looking for teratogenesis and changes in numbers of offspring in response to aerosol exposure of both species. Control rats were subjected to handling stress and aerosols without CS.

Aerosol exposures were at 6, 20, and 60 mg/m^3 for 5 min on days 6-15 of pregnancy in rats and days 6-18 of pregnancy in rabbits. Control aerosols consisted of water or suspensions of Neosil (silica dust), and the experiments were conducted with 12-24 animals. The adults were killed a day before parturition; rat fetuses were examined for 18 abnormalities and rabbit fetuses for eight abnormalities. In addition, rats were studied for teratogenic effects after exposure to CS by intraperitoneal injection at 20 mg/kg on day 6, 8, 10, 12, or 14 of pregnancy.

The results led Upshall to conclude that CS is neither teratogenic nor lethal to embryos. No significant increases in numbers of abnormal fetuses or in resorptions were noted. No dose-related effects were observed, except in one experiment in which rats had marginally lower fetal weights. A high incidence of abnormalities occurred in two control groups of rats.

There is no evidence of developmental toxicity of CS in the Upshall study, but the exposure conditions were limited. The short (5 min/d) exposures and seemingly low dosages did not fully test the potential teratogenicity of CS. In riot-control situations, humans may be exposed to CS at 4-10 mg/m^3,[46] and the absolute concentrations used in this study were 6, 20, and 60 mg/m^3. Without considering time of exposure, the sixfold difference in concentrations (60 vs. 10 mg/m^3) between humans and test animals may not constitute a sufficient margin for an adequate assessment of terato

genic risk. No data were presented on maternal systemic toxicity or mortality at those dosages. Teratology studies are routinely performed at dosages up to those which cause maternal mortality, so that the relative sensitivity of the pups and mothers can be compared. A possible source of misinterpretation in the study is that the exposures appear to have been whole-body exposures, rather than "nose-only"; this means that an animal could bury its eyes and nose in its fur to minimize irritation and thereby lower the effective exposure during the 5-min exposure period. Although those problems do not apply to the injection study, the lack of toxicity data makes it impossible to conclude definitively that CS would not be teratogenic under other exposuure conditions. On balance, one can conclude only that under the conditions of this study CS did not exhibit teratogenic or fetotoxic activity.

Carcinogenicity

The National Toxicology Program in the United States performed a subchronic study of CS to generate data on the maximally tolerated dose (MTD) of this agent preparatory to launching a full-scale chronic toxicity and carcinogenicity bioassay.[45] These tests were conducted in Fischer 344 male and female rats and in $B6C3F_1$ hybrid male and female mice. Six groups of 20 rats and mice divided equally between sexes were exposed to an aerosol of CS by inhalation at concentrations of 0 (control), 0.40, 0.75, 1.5, 3.0, and 6.0 mg/m^3 for 6 h/d, 5 d/wk, for 13 wk, for a total of 65 exposures of 6 h each. In both species, exposures at the two highest doses (3.0 and 6.0 mg/m^3) led to listlessness and mouth breathing. The clinical signs included a hunched appearance, squinting, and closure of eyes at all concentrations of CS. However, at the end of 13 wk, there were no gross lesions that could be definitely attributed to exposure to CS. Microscopically, in both species there was extensive inflammation with erosions of the nasal epithelium in animals exposed at 0.75 mg/m^3 or higher. Rats exposed at 1.5 mg/m^3 or higher had epithelial hyperplasia and squamous metaplasia of the nasolacrimal duct and squamous metaplasia of the trachea and larynx. Females exhibited the same picture as males, but somewhat less severe. On the basis of the combined data, it was recommended that the chronic study could be conducted at 1.5 mg/m^3.

McNamara et al.[29] tested CS for carcinogenicity in mice and rats. Groups of 100 (50 male and 50 female) A/J strain (tumorsensitive) mice and Sprague-Dawley-Wistar rats were exposed to CS at high and low doses in methylene chloride, to methylene chloride alone, and to urethane in methylene chloride. Controls were unexposed mice and rats in the same numbers. During the 24-mo experiment, groups of animals were killed and examined for tumors (Table 4-9). All groups gained weight with no significant differences for

the first 12 wk, after which weights were not checked. During the exposure period (5 d/wk for 4 wk), one control and 14 experimental mice died; one control rat and 10 exposed rats died. Urethane was used as a positive control, because it induces tumors under some conditions. In this experiment, urethane caused a significantly greater incidence of tumors than the methylene chloride controls (38% vs. 22%). No tumorigenic effects were found in the CS-exposed animals.

There were no statistically significant differences among the other groups. It was concluded that, because the high Ct's used were not significantly tumorigenic, the Ct's to be expected in a riot-control situation (1.0-10.0 mg·min/m^3) would not be dangerous. Data on small-animal toxicity have been collected by Ballantyne[3] (Table 4-10).

Lethal Effects

The Himsworth report[19,20] recommended that any chemical agent that might be used as a riot-control agent be studied in the same way as a new therapeutic drug. To a considerable extent, this has been done with CS.

Cucinell et al.[13] reported an LCt_{50} of 57,000 mg·min/m^3 for the anesthetized dog. McNamara et al.[28] compared LCt_{50} values for six animal species exposed to aerosols of CS generated by melting and spraying the agent with smokes from M7A3 grenades (Table 4-11).

Striker[43] exposed 32 immature Macaca mulatta monkeys to CS aerosols in a chamber at four concentrations in groups of eight (Table 4-12 and Table 4-13). At 80,000 mg·min/m^3, the highest Ct, five monkeys died soon after exposure. The others, including controls, were killed for examination at 12 h, 72 h, 1 wk, and 30 d. Only mild symptoms of pulmonary congestion were seen at Ct's of 2,700 and 8,500 mg·min/m^3. At a Ct of 28,500 mg·min/m^3, severe symptoms developed and pneumonia occurred. At a Ct of 80,000, mg·min/m^3, three survivors had important lesions (edema, emphysema, and bronchiolitis) at autopsy. Striker[44] similarly exposed monkeys to lower Ct's, with five animals at each (Table 4-13). Only mild coughing and nasal discharges were observed after these exposures. No lesions were found on necropsy. They concluded that no cumulative or systemic injuries follow long exposures at low concentrations.

TABLE 4-9 Pulmonary Tumors in Animals after 20 Inhalation Exposures to Various Chemicals[a]

Chemical	Average Daily Ct, mg·min/m^3	No. Animals with Pulmonary Tumors/ No. Animals Tested									
		A/J Mice					SDW Rats				
		6 mo	12 mo	18 mo[b]	24 mo	Total	6 mo	12 mo	18 mo[c]	24 mo	Total
Controls	--	2/24	0/25	15/25	2/7	19/81	0/26	0/23	1/25	0/12	1/86
Methylene chloride	500	2/24	2/23	4/25	5/12	13/84	0/24	0/26	0/25	0/20	0/95
Urethane	500	5/24	7/25	16/29	3/3	31/81	0/23	2/25	0/26	0/17	2/91
CS	50	0/24	3/25	14/25	7/8	24/82	0/23	0/24	0/25	0/17	0/89
CS	500	2/22	5/25	11/25	0/4	18/76	0/25	1/29	2/25	0/16	3/95

[a] Data from McNamara[9]

[b] Subcutaneous tumors: 14 of various types; 7 in controls, none in CS-exposed mice. Internal organ tumors: 1 uterine tumor in a CS-exposed rat. 4 tumors in controls: 2 in liver, 1 in kidney, 1 in spleen.

[c] Other tumors: 16 mammary tumors: 2 adenomas, 10 fibroadenomas, 3 adenocarcinomas, 1 fibroma. In skin: 1 inclusion cyst.

[d] One rat had a fibroadenoma in the inguinal region.

[e] One rat had an ovarian granulosa cell tumor.

TABLE 4-10 Comparative Acute Mammalian Toxicity of 10-Chloro-5,10-dihydrophenarsazine (DM), Chloroacetophenone (CN), 2-Chlorobenzylidene Malononitrile (CS), and Dibenz[b,f][1,4]oxazepine (CR)a

		LD_{50}, mg/kg, or LCt_{50}, mg·min/m^3[c]			
Route	Species	DM	CN	CS	CR
Intravenous	Rabbit	6-26	20-31	23-27	47
	Rat	26	35-41	28-35	68
Intraperitoneal	Rat	164	36-56	48-66	766-817
Oral	Rat	563	52-258	178-1,366	
	Rabbit		118	142-401	l,760
Inhalation[b] (pure material)	Rat	3,700-12,710	3,700-18,800	88,480	425,000
	Mouse	22,400-46,245	18,200-73,500	67,200	169,500
	Guinea pig	6,599-7,900	3,500-13,140	50,000	169,500
	Rabbit		5,842-11,480	54,100	169,500
Inhalation[b] (smoke)	Rat	48,217	23,330	68,000	139,000
	Mouse			76,000	203,000
	Guinea pig	29,888	15,400	35,000	169,000
	Rabbit	46,959	15,800	63,000	169,000

[a] Derived from Ballantyne.[3] Ranges cover results from various authors.
[b] LCt_{50}, values for exposures at high concentration for short duration.
[c] LCt_{50} for inhalation, LD_{50} for other routes.

TABLE 4-11 Lethal Effects of CS in Animals Exposed by Inhalationa

	Molten CS		M7A3 Grenade	
Species	No. Animals	LCt_{50}, mg·min/m^3	No. Animals	LCt_{50}, mg·min/m^3
Mouse	120	41,790	--	--
Rat	70	32,293	160	94,378
Guinea pig	70	8,410	220	65,573
Rabbit	20	17,452	66	37,683
Dog	36	33,551	42	29,748
Monkey	31	50,089	30	123,195

[a] Data from McNamara et al.[28]

TABLE 4-12 Exposures of Monkeys to CS at High Ct'sa

Duration of Exposure, min	Average Concentration, mg/m^3	Ct, mg·min/m^3
3	900	2,700
5	1,700	8,500
10	2,850	28,500
32	2,500	80,000

[a] Data from Striker.[43]

TABLE 4-13 Exposures of Monkeys to CS at Low Ct'sa

Duration of Exposure, min	Average Concentration, mg/m^3	Ct, mg·min/m^3
5	53	265
10	55	550
30	50	1,500
5	305	1,525
10	307	3,070
30	304	9,120

[a] Data from Striker.[44]

Ballantyne and Callaway[4] exposed four species of animals to CS smoke generated from a standard grenade. In the first experiment, groups of animals were exposed for 5, 10, 15, and 20 min at a mean concentration of 4 g/m^3. LCt_{50} values were estimated as follows:

	LCt_{50}, mg·min/m^3	
Species	Mean	Range
Guinea pig	35 x 10^3	25-45 x 10^3
Rabbit	63 x 10^3	50-80 x 10^3
Rat	68 x 10^3	61-77 x 10^3
Mouse	76 x 10^3	61-119 x 10^3

The experiment was repeated, but with a mean CS concentration of about 38.3 mg/m^3 and longer exposure times (5-35 h). The corresponding LCt_{50} values were as follows:

	LCt_{50}, mg·min/m^3	
Species	Mean	Range
Guinea pig	48.6 x 10^3	22.4-98.4 x 10^3
Rabbit	54.1 x 10^3	18.8-243.0 x 10^3
Rat	25.2 x 10^3	8.7-52.9 x 10^3
Mouse	36.1 x 10^3	20.9-61.0 x 10^3

No animals died during exposure. Deaths occurred later, and examination revealed mainly damage to the pulmonary system. Survivors showed no residual pathologic effects when sacrificed and examined 14 d later. The authors commented that, although guinea pigs and rabbits seem to be equally susceptible to CS under both sets of conditions, rats and mice are more susceptible to long exposures at low concentrations.

A second series of experiments compared rats and hamsters under three exposure conditions: 150 mg/m^3 for 2 h (Ct, 18,000 mg·min/m^3), 480 mg/m^3 for 1 h (Ct, 28,800 mg·min/m^3), and 750 mg/m^3 for 30 min (Ct, 22,500 mg·min/m^3). At the lowest Ct, two animals died, but survivors had only negligible effects. At a Ct of 22,500, there were no deaths; five survivors had minor lesions in lungs and kidneys. At a Ct of 28,800, 40 deaths were caused by extensive damage to lungs and kidneys; among survivors, 13 of 70 had minor lesions in lungs, kidneys, and liver.

In a third series, 56 rats were exposed to pure CS at 1-2 g/m^3 for 5 min on 5 successive days. Another 50 rats were exposed to CS at 12-15 mg/m^3, 80 min/d for 9 successive days. There were no deaths among animals exposed at the high concentration; a few survivors had minor lesions. Five rats examined 2-3 wk later had developed bronchopneumonia. There were five deaths from bronchopneumonia among the low-concentration animals and nine similar infections among the survivors. It was concluded that animals exposed repeatedly to CS may become susceptible to pulmonary infections.

Care must be taken in comparing LCt_{50} values, as was shown by Ballantyne and Swanston,[8] who described toxicities of CS and CN given by various routes. The CS was pure, thermally generated, rather than from grenades. They measured LCt_{50} values for the inhaled agent. In comparison with the values of the first experiment of Ballantyne and Callaway (above), they estimated the following:

Species	Mean LCt_{50}, mg·min/m^3
Guinea pig	67.2 x 10^3
Rabbit	54.1 x 10^3
Rat	88.5 x 10^3
Mouse	50.0 x 10^3

The chief variable appears to have been the change from grenadegenerated CS to pure CS, but there were also differences in exposure times and concentrations. It is assumed that the general procedures and equipment were the same.

Ocular Effects

Owens and Punte[30] tested CS solutions in rabbits and monkeys by dropping 0.2 ml of 1% CS in dipropylene glycol into one eye of each rabbit or monkey, the other eye serving as a control. Six animals were used in each test. Conjunctival redness and swelling lasted for 1-3 d. The experiment was repeated, but with applications of 0.2 and 0.05 ml on 5 successive days. The 0.2-ml applications produced conjunctivitis, iritis, severe chemosis, and corneal ulceration. The 0.05-ml applications resulted in conjunctivitis, moderate chemosis, and iritis. These conditions had all cleared 7-10 d after the last dose. Rabbits and monkeys had qualitatively similar symptoms, but they were less severe in the monkeys.

Aqueous sprays of 0.1 and 0.25% CS into the human eye caused no histologic ocular changes.[35] Spray of 0.5% into the rabbit eye caused transitory corneal changes.[36]

Ballantyne et al.[6] examined the ocular effects of CS in polyethylene glycol (PEG) solutions, as a powder, and as smokes. Test solutions were made up in concentrations of 0.5, 1, 2, 5, and 10% CS in PEG. Each was tested on 10 rabbits with 0.1-ml drops instilled into one eye. Four groups of 20 rabbits were tested at 0.5, 1, 2, and 5 mg of CS as a powder. Ten rabbits were exposed to CS grenade smoke for 15 min; the average CS concentration was 6 g/m^3. The effects of the hydrolysis products of CS were also examined. Ten animals were tested with o-chlorobenzaldehyde and 10 with malononitrile. The amounts of the two compounds were determined as the ratio obtained by the hydrolysis of a 5% solution of CS. This was a

3.72% solution of o-chlorobenzaldehyde and a 1.75% solution of malononitrile. Again, 0.1 ml was instilled into a rabbit eye.

The effects of the CS solutions ranged from mild, transient lacrimation, blepharitis, chemosis, and congestion at 0.5%, which cleared in 24 h, to iritis lasting up to 7 d and keratitis lasting over 45 d, at 10%. Powdered CS had milder effects than solutions. Lacrimation was mild and lasted 24 h at all doses. Blepharitis ranged from just detectable to mild as the amount of CS increased; it cleared in a week. A just-detectable iritis and keratitis lasting 24 h occurred in two of 10 rabbits given 5 mg; all showed mild chemosis. Exposure to 6-g/m^3 smoke for 15 min caused transient lacrimation and just-noticeable blepharitis lasting 24 h. The breakdown products of CS, which were estimated to be equivalent to the hydrolysis of a 5% solution of CS, caused mild lacrimation, blepharitis, and chemosis of short duration.

The authors concluded that direct application of CS-PEG solutions over the surface of the eye caused increased absorption. Ocular contact with CS powder or smoke may have permitted removal of the agent by excessive lacrimation. Damage to the eye can occur with CS solutions of 1% or more. Smokes and powders do little harm, even in relatively high amounts.

Rengstorff[33] measured the effects of CS in a wind tunnel on visual acuity of 10 young human volunteers with 20-20 vision--six with CS at 0.1 min/m^3, one at 1.3 mg·min/m^3, two at 1.6 mg·min/m^3, and one at 1.7 mg·min/m^3. Rengstorff also exposed 34 men to CS in a chamber, seven at 0.4, 17 at 0.6, six at 0.9, and four at 1.0 mg·min/m^3. The men were free to leave the chamber at will; a few left in less than 1 min, but half stayed for a full 10 min. Rengstorff and Mershon[35] tested 10 men by instilling one drop of 0.1% CS (five men) or 0.25% CS (five men). The water contained 0.5% polysorbate 20 as a carrier. A second group of men received the same CS mixture as a 2-s spray. Four men received the 0.1% CS and two the 0.25% CS. Rengstorff and Mershon[34] studied the safety of trioctyl phosphate (TOF) as a vehicle for CS. As in the preceding experiment, some men received CS in TOF by the instillation of a drop into one eye; two men each were tested with 0, 0.05, 0.1, 0.25, 0.5, and 1.0% CS. Eight other men were sprayed in one eye with various concentrations of CS: two at 0.1%, one at 0.25%, one at 0.5%, and four at 1.0%.

In all these tests, the men were asked to read Snellen chart numbers as soon as possible after opening their eyes. In the chamber test with CS aerosols, an Orthorater (Bausch and Lomb vision tester) was used to measure near and far visual acuity. All the treated eyes were then examined by ultraviolet and slitlamp procedures to detect corneal injury. The results of all three experiments may be summarized as follows: There were fairly wide variations in the times

after exposure to eye-opening, but normal vision was restored in all subjects a few minutes after the eyes were opened. Postexposure corneal inspections revealed no injuries either immediately or later. CS aerosols, CS in water with polysorbate 20, and CS in TOF presented no harmful effects on visual acuity.

Ballantyne and Swanston[9] developed a laboratory procedure to measure the threshold concentrations of CS that produced sensation in the human eye and tongue, to compare various irritant agents. Threshold concentrations of CS were also measured in the rabbit and guinea pig with blepharospasm as the criterion of ocular response. CS at various concentrations was dispensed into the eyes and onto the tongue in 0.01-ml droplets. The effective concentrations for 50% of the subjects (EC_{50}s) for blepharospasm were as follows: guinea pig, 2.2×10^{-5} M; rabbit, 5.9×10^{-5} M; and man, 3.2×10^{-6} M. The human eye (just-noticeable) sensation threshold was 7.3×10^{-7} M (0.14 mg/L), and that for the tongue, 6.8×10^{-6} M.

On the basis of comparison of their figure for CS in solution with an EC_{50} for CS aerosols (unpublished) of 4.0×10^{-3} mg/m^3 of air, the human eye was much more sensitive to CS aerosol than to CS in solution. This can be explained by the great dilution of molecular CS in solution compared with the concentrated action on a sensory nerve ending of a micrometer-sized particle in an aerosol. Humans are more sensitive than the test animals, so caution isrequired in extrapolating animal data to humans. The authors estimated a 6,650-fold safety factor between the EC_{50} for these threshold values and the CS concentration likely to cause the least

Cutaneous Effects

Single applications of 1.0 ml of 1% CS solution were administered to the clipped backs of rabbits.[29] They developed mild to moderate erythema that cleared in 3 d. Monkeys showed no signs of irritation. A 5-d test of the 1% solution caused only moderate erythema that cleared in 8 d. A 0.2-ml application caused only moderate irritation in the rabbits. In monkeys, 1-ml and 0.2-ml applications caused no irritation. Patch tests on rabbits and monkeys with similar doses caused somewhat more skin irritation, but generally the lesions were reversible; no systemic toxicity was demonstrated.[30]

One study[7] prompted by the Himsworth report examined the extent of burns inflicted by pellets from CS grenades and the possibility of their interference with the healing of wounds suffered by rioters. Anesthetized guinea pigs were subjected to light skin abrasions, deeper skin wounds, skin burns, and effects produced by allowing an ignited CS grenade pellet to burn on the skin (10-15 s). Controls

were provided. Wounds and burns not inflicted by pellets were contaminated by CS, both dry and dissolved in diethyl ether. Healing of CS-contaminated animals and controls did not differ significantly.

Tests of CS-Loaded Pen Guns

Ayers and Stahl[1] studied the ballistics of pen guns loaded with CS. When the gun was fired from a distance of 7.5 cm, no damage to the skin or underlying tissues of rabbits occured. At a distance of 2.5 cm, contusions and small lacerations of the skin were, observed. On direct contact, one test resulted in a fractured femur. In another, over the chest, the rabbit was killed. The wad in the cartridge was the cause of the damage.

In a second experiment, Ayers and Stahl[2] studied the effects of discharging a CS pen-gun cartridge into a rabbit eye at a distance of 20 cm. At that distance, the wad caused less damage than the blast and the particles of CS. Only three of 10 animals suffered severe eye lesions, which appeared to combine mechanical damage (lacerations) from the wad and conjunctivitis, intraocular hemorrhage, keratitis, and corneal edema probably from the blast and CS particles driven into the eye. The other animals showed only mild conjunctivitis, which cleared after 3 d.

TOXICOLOGY IN HUMANS

Immediate Effects

The effects of CS are immediate and self-limiting. Recovery usually occurs within 30 min after exposure ceases.[3] The effects include a burning, pricking, or peppery sensation in the eyes, nose, mouth, throat, and skin; lacrimation, rhinorrhea, and salivation; blepharospasm and injection of the conjunctivas and margins of the eyelids; photophobia lasting up to 1 h in 10% of subjects; tightness of the chest associated with gripping pain, breathholding, dyspnea, coughing, and sneezing; erythema and occasionally vesiculation of exposed skin; and nausea, vomiting, headache, and apprehension.[3,19,20,28] Moisture worsens the skin effects.[11,22]

Tolerance to CS may develop from repeated exposures at low concentrations,[20] but it is reduced by hyperventilation, as well as by increased environmental temperature and humidity.[3] Whole-body exposure to CS solutions may result in a transient increase in blood pressure, but not to the extent observed with CR.[3,20] CS is a hapten and may cause allergic contact dermatitis, with erythema, edema, and vesication, which is less severe than the effects of CN.[3]

The effects of CS on persons exposed in confined spaces are similar to those described above, and recovery occurs quickly after exposure ceases. During the Londonderry riots, exposure of a child in a bedroom resulted in crying, gasping for breath, pallor, and lacrimation, but the child recovered promptly.[19]

A causal relationship between exposure to CS and asthmatic attacks has not been established, but irritant CS smoke may pre-dispose susceptible persons to asthmatic attacks.[3,19,20] Similarly, persons with pre-existing chronic bronchitis may have superimposed acute bronchitis or bronchopneumonia after exposure to CS.[3,20]

Experimental Human Exposures

In some experiments, volunteers were exposed to CS aerosolsthrough gasmasks arranged for air-agent passage in and out. Typically, men were exposed in a large wind tunnel in which CS concentration, air speed, and temperature were controlled. Table 4-14 shows test conditions.

The estimation of the incapacitating dose for humans is usually for a 1-min period, so the ICt_{50} is the concentration at which half the exposed population is affected.[28] Typically, men leave the exposure with tears, nasal secretions, and saliva pouring out, and towels rather than handkerchiefs are needed to cope with the fluids. In 5-15 min, the irritation ceases. Punte et al.[32] noted that men with a history of sinusitis felt such relief that, after the effects of CS subsided, they asked to take part in further tests. One man claimed to have improved hearing after exposure to CS.

Determining the ICt_{50} for humans entails some difficulty due to differences in motivation and tolerance. The official ICt_{50} for humans was therefore estimated by the Research Laboratories at Edgewood as a range: 0.1-10.0 mg·min/m^3. The LCt_{50} for humans was derived from extensive animal data and was expressed for two conditions:

	LCt_{50}, mg·min/m^3	Safety Factor
Molten CS	52,000	5,200-520,000
M743 grenade	61,000	6,100-610,000

The safety factor is obtained from the ratio LCt_{50}:ICt_{50}.[27] Table 4-15 shows animal data on which these values were based.

TABLE 4-14 Comparative Effectiveness of CS in Men Exposed under Various Conditions (Individual Experiments)a

Study	Year	Dissemination Method	Exposure Conditions	Concn., mg/m^3	No. Men	ICt_{50}[b] mg/m^3
1	1959	Sprayed acetone solutions	Wind tunnel--5 mph; group exposures--total body; no specific psychologic motivation techniques used--men briefed before exposure and asked to resist agent to best of their ability	5-442	78	3.0 4.7
2	1967	Sprayed acetone solutions	Wind tunnel--5 mph; individual exposures--head only; no motivation--as for study 1	0.03-8.0	35	0.1 0.07
3	1968	Sprayed acetone solutions	As for study 1	0.02-5.4	30	0.3 0.2
4	1968	CS-methylene dichloride solutions dropped into heated cup	20-m^3 chamber; group exposures--total body; no motivation--as for study 1	0.40-0.90	21	0.7 0.6
5	1968	CS-methylene dichloride solutions dropped into heated cup	20-m^3 chamber; group exposures--total body; subjects motivated in groups by psychologic techniques	0.50-28.0	130	12.4 6.9
6	1968	CS2[c] dry powder disseminated	As for study 1	0.3-6.7	30	1.4 0.5

[a] Data from McNamara et al.[28]
[b] 60 s tolerance time; Bliss analysis on left, curvilinear regression line analysis on right.
[c] CS2 = 95% CS, 4.75% Cab-O-Sil, and 0.25% hexamethyldisilazane.

TABLE 4-15 Munition (M7A3) Inhalation Toxicity[a]

Species	Ct, mg·min/m^3	Concen., mg/m^3	Exposure Time, min	Mortality
Monkey	246,400	4,265	62	6/6
	149,425	3,558	42	4/6
	119,600	3,739	32	2/6
	62,400	1,950	32	1/6
	55,950	3,730	15	0/6
Dog	72,160	2,488	29	6/6
	62,400	1,950	32	6/6
	50,050	1,925	26	3/6
	33,760	1,688	20	2/6
	27,880	1,991	14	1/6
	12,975	2,595	5	3/6
	4,216	1,054	4	0/6
Swine	72,160	2,488	29	6/6
	67,300	1,819	37	6/6
	34,070	3,786	9	5/6
	21,660	3,094	7	5/5
	13,975	2,595	5	1/6
	4,216	1,054	4	0/6
Goat	82,930	2,592	32	6/6
	67,300	1,819	37	4/6
	62,400	1,950	32	4/6
	43,900	2,927	15	1/6
	34,070	3,786	9	2/6
	21,660	3,094	7	1/5
	17,400	2,486	7	0/6
Rabbit	100,950	2,148	47	6/6
	86,360	1,661	52	6/6
80,260	2,508	32	6/6	
76,800	1,829	42	4/6	
62,400	1,950	32	6/6	
55,950	3,730	15	6/6	
52,080	3,472	15	6/6	
50,050	1,925	26	5/6	
34,070	3,786	9	0/6	
21,660	3,094	7	1/6	
4,216	1,054	4	0/6	
Rat	165,000	3,173	52	20/20
	123,200	1,987	62	16/20
100,950	2,148	47	10/20	
86,360	1,661	52	7/20	
79,250	991	80	7/20	
76,800	1,829	42	4/20	
62,400	1,950	32	3/20	
21,660	3,094	7	0/20	

[a] Data from McNamara et al.[28]

Punte et al.[32] exposed volunteers to aerosol particles of 0.5-1.0 μm. The windspeed was 5 mph. Figure 4-3 shows the variability in response times, especially at low concentrations. These experiments were continued with exposures at various temperatures, with exercise, and with repeated exposures and long low-concentration exposures to develop tolerance. High temperatures and humidity reduce the response time, as does exercise. After tolerance was developed, men given simple problems required more time to complete them, but accuracy was not impaired. Airway resistance did not increase during exposure to CS. One group exposed 10 times over 2 wk at up to 13 mg/m^3 had normal blood electrolytes. Only minor adverse effects were observed in 75 men exposed in these experiments.

Owens and Punte[30] tested six of 50 volunteer subjects best able to tolerate CS, to compare the effects of small and large CS particles on the eye and respiratory system. The mass-median diameter of small particles was 0.9 μm, and that of large particles, 60 μm. Tolerance was defined as the time that the subject could remain in a wind tunnel spray (2% CS in methylene dichloride). Recovery was the time after exposure when they could sort 24 playing cards from which the corner numbers were removed. Small particles produced eye and respiratory irritation more rapidly. Large particles had longer-lasting eye effects. Small particles had a predominantly respiratory effect, whereas large particles had predominantly ocular effects (Table 4-16 and Table 4-17).

Gutentag et al.[17] showed that CS, either dry or in solution, produced erythema and vesicles in the skin of human subjects. Covering the CS-treated area increased severity, and airtight compresses were more damaging than gauze pads.

Hellreich et al.[18] studied simulated tropical exposure conditions. CS aerosols were generated in an aerosol chamber (97°F, 100% relative humidity, windspeed of 5 mph) so as to expose the right forearm and hand of each subject. As controls, the left arms were exposed similarly, but without CS. Four subjects were exposed in each group, as follows:

Group	Time, min	Avg. Concentration, mg/m^3	Ct, mg·min/m^3
I	15	296	4,440
II	30	316	9,480
III	45	312	14,040
IV	60	295	17,700

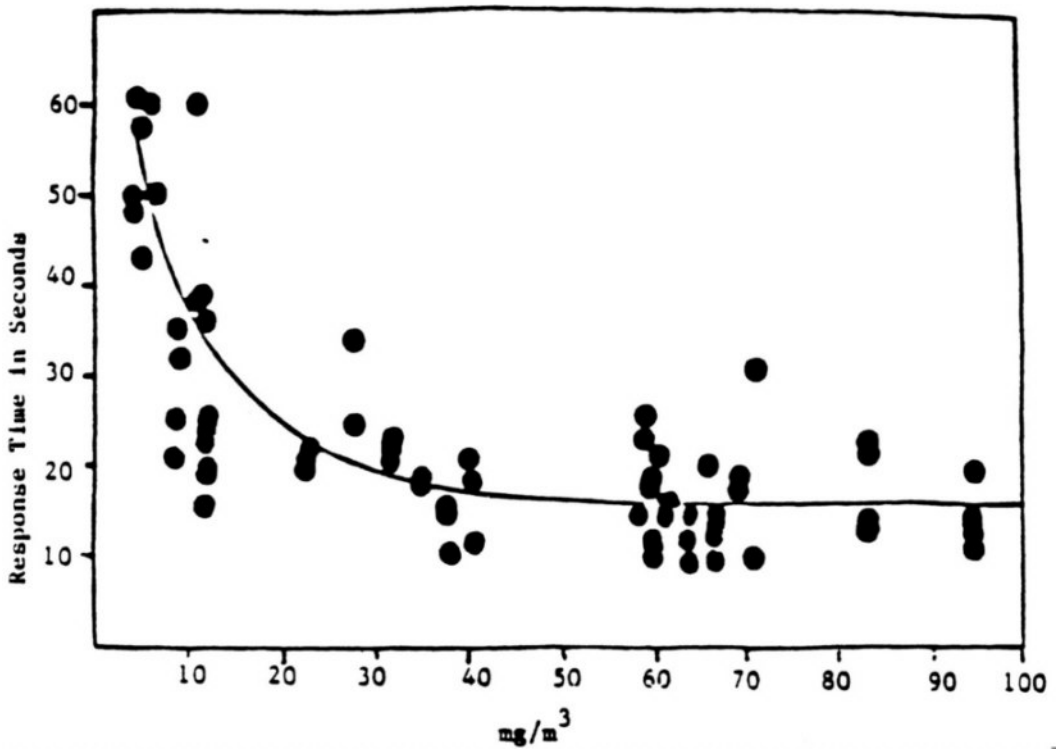

FIGURE 4-3 Response (tolerance time) of subjects exposed to CS. Reprinted with permission from Punte et al.[32]

Subjects in Groups I and II immediately developed erythema, which persisted up to 30 min after exposure and left no after-effects; all subjects reported a stinging sensation soon after the exposure began. In Groups III and IV, the immediate erythematous reaction was more severe, but subsided in 3 h; over the next 12-24 h, all subjects developed moderate to severe first-degree burns. One subject in Group III and three in Group IV progressed to second-degree burns. Medical care was provided, and the burns healed with no after-effects. (Effective medical care would not always be available in field situations.)

Holland and White[21] applied measured quantities of CS to the forearms of subjects under 4-cm-diameter sealed glass covers. The dry powder produced erythema in 30 min when 20 mg or more was used. When 2 drops of saline solution was added, the amount required was 10 mg. Erythema was transient, fading out in 1 or 2 days with no after-effects.

The indiscriminate effects of CS smokes in riot-control use prompted consideration of whole-body CS water spray as a more accurate way of delivering CS to desired targets.[5] Subjects were drenched in cubicles like shower stalls, receiving 15 L of spray in 15 s, with CS concentrations of 0.001, 0.002, 0.003, and 0.005%. Groups of 12 were sprayed outdoors for a minute. During the spraying, the subjects exercised in various ways; some were kept in wet clothing for an hour to investigate the effects of protracted contact with the solution. The subjects reported that stinging of the eyes was followed by stinging of the skin, first of the face, then the neck, the back, and the lower body. Irritation was mild and disappeared in about 10 min. Before and after the drenches, blood pressure was measured. The control drenches with water alone caused a transitory rise in blood pressure, but the effect of the 0.005% CS spray was a mean rise of 31 ± 2.7 mm Hg systolic and 19 ± 2 mm Hg diastolic. It took an average of about 6.3 min for the pressure to decrease to nearly normal--close to the time it took for skin sensation to end. When the CS drench was followed by exercise, the systolic pressure rose as before, but the diastolic pressure decreased. The authors concluded that, although a cold-water drench alone provoked a brief rise in pressure, it was only partly responsible for the rise seen during the CS drench. A direct hypertensive effect of CS was ruled out, inasmuch as the rise in blood pressure came too rapidly to be accounted for by the absorption of a substantial amount of the agent. The rise must therefore have been caused by the intense irritant effect of CS.

Tolerance

In an atmosphere containing CS at low concentrations, men can remain without discomfort for long periods. Punte et at.[32] reported that men can tolerate CS at 1.5 mg/m^3 for at least 90 min; men can tolerate 6.6 mg/m^3 if the concentration is built up over 30 min. In an atmosphere of 6 mg/m^3, built up in 10 min, three men were forced to leave the chamber in 18, 20, and 29 min; the fourth stayed in for 40 min, at which time the test was ended. The Himsworth committee[20] reported a similar experiment in which 35 men were exposed to CS gradually built up to 2.30 mg/m^3 over an hour; all but two remained. Such tolerance is lost quickly. An adjusted person who leaves the chamber for a short time is strongly affected on returning.

The ability to remain in a contaminated atmosphere depends on will power and motivation, as well as the dosage tested. McNamara et al.[28] measured ICt_{50}s for men in a variety of conditions. Subjects were instructed to remain in a wind tunnel facing a CS spray (5% in acetone) until they could no longer tolerate it, at which time

TABLE 4-16 Ability of Subjects to Tolerate Exposures to 1-μm and 60-μm Particles of CS Aerosolsa

	Percentage of Subjects Tolerating CS for 1 min	
Exposure of:	Small Particles	Large Particles
Eyes	40	100
Respiratory system	0	67

[a] Data from Owens and Punte.[30]

TABLE 4-17 Mean Recovery Time of Subjects after Exposure to 1-μm and 60-μm Particles of CS Aerosolsa

	Mean Recovery Time, s	
Exposure of:	Small Particles	Large Particles
Eyes	91	280
Respiratory system	51	9

[a] Data from Owens and Punte.[30]

they emerged. Incapacitating concentrations recorded for two experiments were as follows:

		ICt_{50}, mg/m^3
a.	78 men, merely asked to remain in the wind tunnel as long as possible	3.0, 4.7
b.	35 men, no motivation	0.1, 0.07
c.	30 men, same as a	0.3, 0.2

d.	21 men, same as b	0.7, 0.6
e.	130 men, motivated by special psychologic techniques	12.4, 6.9
f.	30 men, same as a	1.4, 0.5

Money can be effective motivation. At the British Chemical Defense Establishment at Porton Down, Wilts., volunteers were told that a 5-pound note could be found in a hut.[10] A CS cartridge was exploded in the hut and the men were allowed to enter to look for the money. The men could tolerate concentrations of agent probably in the range of several hundred milligrams per cubic meter. With sufficient motivation, then, men can endure exposure to CS at high concentrations. No harmful after-effects occurred in the tests described or in rioters who may heve been exposed long enough to accumulate rather high doses of CS.

Beswick et al.[10] exposed 35 men in small groups to steady and increasing concentrations of CS to study tolerance. In two trials, there were eight men in the chamber. Four wore masks until the last 5 min of the hour and then unmasked to demonstrate the difference between their reactions and those of the four who had become accustomed to the agent. In 10 trials, only two men were forced to leave the chamber because of nausea. After about 5 min, men reported that symptoms were more bearable; some were able to play cards. When the CS concentration was increased, most reported a temporary increase in symptoms. The highest exposures (Ct's between 60 and 90 mg·min/m^3) were higher than would be experienced by rioters.

Sensitization

Guinea pig tests indicated that CS had a potential for producing human skin irritation and sensitization.[38,39]

Fisher,[14] discussing tear gases and their effect on human skin, remarked that CS is a sensitizer and a primary irritant capable of causing first- and second-degree burns and even ulcers if not washed off the skin. In experimentally sensitized subjects, CS elicited a skin response in one of nine when tested at 0.1%, but none reacted at 0.01%.[27] Concentration is a factor in the elicitation of a skin response to a sensitizer, as well as an irritant.

Thus, it is not surprising that munitions plant workers in both England and the United States developed rashes, dermatitis, and blisters from contact with CS powder.[11,41] Protection of these workmen was achieved by the use of air-supplied suits and rigorous attention to bathing and changing of clothing after work. Bowers et

al.[11] noted that no one seemed immune to the effects of CS, although some appeared more sensitive than others. Bowers et al. reported case histories of 11 men who developed contact dermatitis after exposures to CS from 2 d to 2 mo. There appeared to be a wide range of sensitization potential, greater in white than in black workers. Five men developed hypersensitivity after repeated exposures. Others suffered various degrees of dermatitis, pruritus, and vesiculation in proportion to exposure. High heat and relative humidity intensified these effects.

Shmunes and Taylor[41] reported contact dermatitis in a plant manufacturing CS. Of 28 workmen, 25 (89%) had dermatitis of some degree in one or several episodes. Nearly all began to suffer from dermatitis 2 wk to 6 mo after the original exposure to CS. Although protective clothing was worn, carelessness in sealing neck and wrist junctions and in changing clothing at the end of the workday seemed to cause the dermatitis. Most of the lesions were on the neck and wrists. Patch tests to detect allergic sensitiztion to CS showed that only two of the 25 subjects reacted.

Accidental Exposures

Park and Giammona[31] reported the effects of a 2- to 3-h exposure to CS on a 4-mo-old child. The infant was in a house into which police fired several canisters of CS. No estimate of concentration was available. On admission to the hospital, the child was suffering from severe respiratory distress and first-degree burns on the cheeks. Despite a week of treatment, pneumonia developed. The child was released after 28 d of hospital care.

USE IN NORTHERN IRELAND

Himsworth Report, Part I

On August 13 and 14, 1969, extensive rioting broke out in Londonderry, Northern Ireland. CS was used in large quantities (a total of some 14 kg). An official inquiry into the medical hazards of such riot-control measures was instituted. The findings of the committee were published in September 1969[19] and September 1971.[20] Part I of the "Himsworth report" is devoted to an account of a 3-d investigation (September 1-3, 1969) in the area of the riots. A three-member committee, later expanded to eight members, interviewed the local public-health authorities, hospital officials, physicians, and many inhabitants of the district. Given the chaotic situation, no exact information on exposure conditions or immediate after-effects could be expected. A decision was made to gather information on two groups of exposed people: those who had previously appeared healthy

and those who had some illness that had been exacerbated by the exposure to CS. Part I concluded:

> The conclusions that we have been able to reach in respect of the effects of CS in the circumstances, and under the conditions existing in Londonderry during the incidents of the 13th and 14th August 1969, necessarily vary in their firmness. We feel reasonably confident in the conclusions that we have drawn in regard to effects of exposure to CS in previously healthy persons. We feel that our conclusions in regard to the effects on persons who had certain previously established illness are more open to question, although we believe that they could be rated as strong possibilities. Our conclusions in respect of the future course of illnesses that had been present before exposure are necessarily more tentative. With these provisos we summarise our main conclusions below.
>
> We have found no evidence even among those most heavily exposed to CS of incapacitation as a result of exposure such as to prevent their moving away to a clearer atmosphere where the acute symptoms rapidly abated.
>
> We have similarly found no evidence in previously healthy persons that, following exposure, any illness developed in the following three weeks that could clearly be attributed to the effects of CS. The most common complaint was of mild diarrhea but there are certain features about the incidence of this that make us hesitate to ascribe it to the effects of CS.
>
> In respect of persons who were in ill health previous to exposure, we have paid particular attention to cases of asthma, chronic bronchitis and emphysema. We believe that it must be accepted in principle that exposure to CS may precipitate an acute asthmatic attack, but we found no evidence that such attacks differed in kind or degrees from those attributable to natural causes and, in the cases that we either saw or heard of, we were unable to exclude the possibility that the attacks in mid-August were due to such causes. We must accept the possibility that exposure to CS of a patient with chronic bronchitis and emphysema may result in an acute bronchitis being superimposed on the chronic condition. Again this does not appear to differ from the situation when such an acute exa

cerbation occurs from natural causes. In reaching an assessment of the possible effects of the present acute episodes of illness associated with exposure to CS upon the future course of the previously established chronic disease, we feel, therefore, that it would be reasonable to suggest that these are not likely to differ from the effects of comparable acute episodes produced by natural causes.

Finally, we would express a general opinion to which we have come in respect of chemical agents that might be used for civil purposes. In our opinion, the point of view from which the effects of any such agent should be studied should be more akin to that from which we regard the effects of a new drug than to that from which we might regard a weapon. We are aware that over recent years opinion has tended increasingly towards this view and that investigations have been made with such safeguards in mind. But we feel that this view should now graduate from the status of a tacit understanding to that of an explicit requirement. To that end, the effects of any such agent should be appropriately investigated, not only in respect of the healthy persons against whom it may be directed, but also in relation to the possible effects on the young, the elderly and those with impaired health, who may inadvertently be exposed to the agent in question. It is primarily for this reason that we have recommended and it has already been agreed by the Home Secretary that our membership should be expanded and the evidence in regard to CS assessed in the widest possible way.

Himsworth Report, Part II

Part II of the Himsworth report dealt with the toxicity of CS in healthy subjects and with regard to pre-existing disease. Because exposures to CS in riot-control situations, as in Londonderry, are brief but often intense, special attention was given to such exposures.

The lowest concentration detectable by man is about 0.004 mg/m^3. The concentration that would disperse a crowd of rioters was estimated at about 4.0 mg/m^3, and the amount to deter trained troops, at 10 mg/m^3. Above the latter concentration, no increase in the severity of symptoms was detectable.

Animals are less sensitive than humans. When exposed to CS, animals pass through an excitatory phase, after which they usually lie passively. Animals exposed to CS show severe congestion of the blood vessels in the lungs. "With concentrations approaching those that might occur in riots, neither illness nor lung damage is demonstrable."

The report on the effects of CS on healthy people considered ordinarily healthy persons (such as would most likely join in riots) and special groups. No indication had been found during the visit to Londonderry that rioters had suffered marked after-effects, on the basis of interviews with area residents and medical authorities. To obtain reliable information on CS effects, a test was arranged in which 34 human volunteers were exposed to CS for an hour, accumulating a total dose of "90 mg·min/m^3," which is interpreted as 1.5 mg/m^3 for 60 min. Blood samples were taken before and immediately after the exposure and 24 h later, so that hematologic and biochemical postexposure values could be compared with the normal.

Some changes were registered in the hematologic and biochemical values, but they were not regarded as deleterious to the subjects.

Among the special categories of healthy persons were the young and the aged. No cases of illness attributable to CS were found among infants, even among those who were mildly exposed by accident. Similarly, old age as such seemed not to be predisposing.

Effects on pregnancy were sought by examining statistics for abortions, stillbirths, and congenital abnormalities. These statistics gave no indication of any increase in abortions, still-births, or congenital abnormalities.

The Himsworth committee visited Londonderry in July 1970 and investigated the health of those known to have been suffering from various illnesses before the riots, who might have been affected by exposure to CS. A primary concern was for adverse reactions in people with respiratory diseases, such as chronic bronchitis and asthma. Although exposure to CS had exacerbated effects in patients with chronic bronchitis, a followup visit showed that they had returned to their preriot health status. Much the same results were found with asthma patients. No increase in the frequency of attacks had been noticed.

The committee examined other diseases that might have been increased by exposure to CS. Most remarkable were the data on new tuberculosis cases for the year after the riots: the rate had increased for all Londonderry districts except the Bogside, where the riots had occurred.

Although exposure to CS causes a temporary rise in blood pressure,[17] no increase in cases of stroke or heart conditions was reported. The committee speculated that this might have been because exposure to CS does not increase airway resistance[32] --increased airway resistance increases the strain on the heart--and because people with known heart problems were unlikely to take part in rioting.

For a short time after the riots, men who had been suffering from epilepsy and were being controlled by treatment began to have attacks. The committee speculated that, in the excitement of the riots, epileptics had neglected to maintain their medication. No unusual occurrences of psychiatric or other mental disorders seem to have been recorded (see the following section, on sequelae of the Belfast riots).

The committee devoted much effort to the likelihood of deaths in riots as a result of high local concentrations of CS either outdoors during combat or indoors by accidental penetration of a house window. It concluded that in the open air it would be impossible for a healthy human to receive a lethal dose, because even light winds disperse CS and the burning time is short for cartridges (10-15 s) and grenades (25 s). The exposure concentration for a man at whose feet a missile exploded was estimated as 100-300 mg/m^3. Indoors, in a 20-m^3 room, the explosion of a standard cartridge (12.5 g of CS) produced a concentration of 500 mg/m^3 when the room was closed. Assuming a broken window or an open door, the concentration would diminish rapidly, so that even someone unable to leave the room would not be exposed to a dangerous amount of CS.

Jones,[23] in a critique of the Himsworth report, recommended abandonment of CS as a riot-control agent, because lethal amounts could be inhaled under “the most exceptional circumstances.”

Mental Effects of Riots

Fraser[15] studied institutional admission rates and outpatient referral rates for Belfast during the riots of 1969 and compared the rates for psychoses and neuroses with those of the previous year. The city was divided into three areas: Area 1 was the district in which the rioting had occurred; Area 2 was the adjacent territory, in which little violence occurred, but which exhibited signs of tension, such as boarded-up windows and barricades; and Area 3 had been free of disturbance and was small relative to the rest of Belfast.

The data are remarkable. The rates for Area 1 remained almost constant, but the rates for male psychotics and for male and female neurotics increased in Area 2. Statistically, the changes were mar

ginal, but Fraser believed that late referrals might have increased the differences. He suggested that the anticipation of violence in Area 2 provided stress that caused increases. He compared this with similar data compiled for wartime England, in which admission rates rose in the provincial areas, but not in London under the bombing. Fraser examined the numbers of prescriptions for tranquilizers issued in Belfast. Increases were noted in Area 1, but not in the other areas.

Lyons[25] studied the psychiatric sequelae of the Belfast riots, focusing on the period August 15-September 30, 1969. He concentrated on 257 patients from three general medical practices in the riot area of West Belfast and on patients referred to psychiatrists from these general practitioners. He concluded that during civil disturbances:

- There is no increase in acute psychiatric illness.
- Very young and very old people are less likely to develop mental symptoms than young to middle-aged adults.
- Unemployed people are at more risk than working people.
- Women predominate (about 75%) among the patients.
- People living alone are at less risk than others.

Neither Fraser nor Lyons correlated his study with exposure to CS, but the widespread use of CS and the public clamor resulting from this new aspect of riot control must have been an important factor in the stress that operated on the inhabitants of Belfast, both within and outside the combat zone. It seems worth while, therefore, to include the record of psychiatric effects, even without a direct connection to the use of CS.

LONG-TERM FOLLOWUP

Marrs et al.[26] exposed 300 mice, 200 rats, and 200 guinea pigs, all males, to CS at high, medium, and low concentrations in aerosol form 1 h/d, 5 d/wk for 120 d. The aerosols were generated from pure CS and had a mass median diameter of 3-4 μm. Mice were exposed 55 times (11 wk), rats and guinea pigs, 120 times (24 wk). The high-dose animals began to die after a few exposures, so that series was terminated. All survivors in other groups were killed at the end of a year and necropsied.

Because of deaths at the high concentration, the middle-dose animals received higher total doses, and there was no significant mortality. Low- and medium-dose animals showed no greater mortality

than controls. No indications of a dose-response relation between tumors in any particular site and the total dose of CS were found. Marrs et al. concluded that CS below 30 mg/m^3 is not harmful to mice, rats, and guinea pigs. This is more than 8 times the concentration (IC_{50}) intolerable to 50% of an exposed human population in 1 min.

Little is known about possible long-term effects of CS inhalation. This is due in part to the fact that short-term experiments with experimental animals, carried out for from several days to a month and using much higher (in some cases, nearly lethal) concentrations of CS, showed that ocular, respiratory, and cutaneous alterations were mild and readily reversible, whereas necropsy findings failed to reveal any evidence of systemic alterations. Retrospective studies performed by the Himsworth committee at the request of the British Parliament after the extensive use of CS in Northern Ireland showed that no adverse effects of CS use were observed, with respect to eye burns, residual respiratory tract injury, increased death rate in the elderly, exacerbations of mental illness, increased incidence of strokes or heart attacks, or incidence of tuberculosis. At exposure concentrations reported by the Himsworth committee (about 90 mg·min/m^3), no persistent or notably adverse health effects were observed.

Experimental studies carried out in animals and human volunteers exposed to CS aerosols revealed no lasting changes in hematologic or biochemical measures. CS exacerbates symptoms of pre-existing chronic bronchitis and bronchial asthma, but these rapidly return to a pre-exposure state after removal of CS.

Two instances of accidental massive exposure have been recorded, one of a healthy 43-yr-old man, and another of a 4-mo-old child. Both experienced severe respiratory symptoms (pulmonary edema in the first, and later pneumonia in the second), but they reportedly recovered. No long-term followup data are available.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

From 1958 to 1973, at least 1,366 human subjects underwent experimental exposures to CS at Edgewood. For 1,073 subjects, there was some type of aerosol CS exposure, 180 subjects had skin applications, 82 subjects had both skin applications and aerosol exposures, and 31 underwent CS applications to their eyes.

Earlier subjects underwent up to 10 CS exposures in the wind tunnel on different days, but later experiments involved a maximum of three exposures. Most of the CS exposures involved tests of equipment or of subjects' abilities to perform military tasks during expo

sure. Other protocols combined effects of stress, motivation, and other substances and used tests of effectiveness of samples of CS. There was a wide range of exposure Ct's--from 0.03 to 345 mg·min/$m^{3.}$ The highest Ct's were used in equipment studies. (Protocol and Ct information is not available on all subjects.)

Complete experiment records are available on 105 CS subjects (Table 4-18); a summary is available on an additional 86 subjects who participated in CS skin-sensitization experiments in 1972. The records represent a cross-section of many of the CS protocols. The amount of information in each record varies with the protocol. Ct's, where mentioned in the 105 records, ranged from 7 to 345 mg·min/m^3. Exposure times, where mentioned in the 105 records, ranged from 18 s to 10 min. In motivation experiments, subjects attempted to remain in CS for up to 200 s, but many left before test completion. Dosages in skin tests were 0.01 or 0.025 ml of 1% CS applied to bare or clothed arms.

Among the 105 subjects, signs and symptoms due to CS exposure were most marked in the eyes and respiratory tract. All effects were temporary. Frequent symptoms, all temporary, were lacrimation, eye irritation, upper respiratory passage irritation, chest constriction, and dyspnea. The main objective findings mentioned were conjunctivitis and rhinorrhea. Pulmonary examinations were generally not mentioned. Other symptoms included headache, nausea, and diarrhea. The eight skin applications on which records are available reportedly caused no effects.

Postexposure (generally 1 wk) laboratory analyses (complete blood count, complete urinanalysis, blood urea nitrogen, alkaline phosphatase, and serum glutamic oxalacetic transaminase) are available on 50 of the 105 subjects. An additional 22 earlier subjects' records contain results from a less complete series of postexposure laboratory analyses. Pre-exposure laboratory analyses were used for comparison on subjects with abnormal postexposure laboratory results. Eleven subjects had laboratory abnormalities that were not seen before exposure to CS. There were seven subjects with urinary sediment containing 2-10 white cells per high-power field. There are no reports of a lower urinary tract source of white cells in these seven subjects. Results of their other postexposure renal-function tests were normal. Three subjects had abnormally high serum glutamic-oxalacetic transaminase (112, 38, and 31 IU). Leukocytosis accompanied the marked increase in transaminase in one subject. One subject had leukopenia (2,800 cells with a normal differential count). None of the subjects with abnormal postexposure laboratory results had earlier experimental exposures at Edgewood, although some participated in later experiments.

In 1969, 31 subjects had ocular instillations of either 0.1% or 0.25% CS in water with 0.5% polysorbate 20 or 0.05- 1.0% CS in tri-octyl phosphate in their right eyes. The subjects experienced intense ocular irritation and lacrimation. Acute conjunctival injection lasted 1 h. No fluorescein staining of the cornea was seen under ultraviolet illumination. In one subject, corneal staining, visualized with the slit lamp, resolved in 24 h.[34,35]

In 1972, skin applications of 0.01% and 0.1% CS were used to determine the effects of skin pigment on susceptibility to sensitization, sensitizing and irritating concentrations of CS, and cross-sensitization with CN. Eighty-six tests were summarized, and results are available on 45 cases. Seven of 15 subjects were sensitized in skin-pigment experiments; subjects with lighter skin seemed more susceptible to sensitization. Twenty previously CS-sensitive subjects showed no cross-reactivity to CN, although 18 developed primary irritation dermatitis when exposed to 0.2% CN. Four of 20 subjects developed primary irritation dermatitis when exposed to 0.1% CS, but not to 0.01% CS.

There are no data to suggest that the low dose CS-exposure of 105 subjects at Edgewood would give rise to long-term health effects in the primary target organs, the eyes and respiratory tract. CS is a known skin sensitizer, causing allergic contact dermatitis after repeated exposures in a high percentage of subjects. Many Edgewood CS exposed subjects were probably sensitized to CS; in fact, many known sensitized persons were chosen for some protocols. Sensitizing effects of CS on other organs are not known, but the risk of allergic pneumonitis on exposure of a sensitized person to CS is a possibility.

Hepatic dysfunction and urinary abnormalities were seen in some subjects after CS exposure at Edgewood. Little is known of the effects of CS on the kidneys and liver. The small proportion of subjects who had abnormal urinalysis (7 of 50; 14%) and high transaminase (3 of 50; 6%) indicates idiosyncratic reactions, if the abnormalities were indeed due to CS exposure. The most likely course of idiosyncratic drug-induced, nonfulminant hepatitis is complete recovery after removal of the agent. Recurrence of hepatic reactions would be expected on re-exposure to CS if the original transaminase increases were due to CS.

In summary, the available data on Edgewood CS-exposed subjects lead to speculation about several possible health effects. Repeat exposures to CS may cause allergic contact dermatitis in many of the Edgewood subjects. One could speculate that repeat exposures to CS may also induce idiosyncratic hepatitis or allergic pneumonitis in some persons, although no evidence of this exists with the Edgewood subjects.

SUMMARY

CS has been widely adopted, especially in the United Kingdom, as a replacement for CN for use as a tear gas or riot-control agent. This has come about because careful investigation has shown that CS is less toxic than CN, acts faster, and has after-effects of shorter duration and less severity. Both humans and animals have been subjected to extensive investigation. There is a wide safety margin between the lowest concentration that is effective in disabling rioters and the concentration that could cause life-threatening harm to the exposed. There is virtually no evidence that CS poses a mutagenic or carcinogenic hazard. There are no reports of death from CS exposure, as there have been for CN. Thousands of men have been exposed to CS in the course of military training, with few after-effects. The Himsworth committee was unable to locate anyone exposed to the effects of CS during the Londonderry riots who suffered important sequelae, except for temporary exacerbation of chronic bronchitis or asthma. CS has one lasting effect: In addition to being a primary skin irritant capable of causing first- and second-degree burns, it is a skin sensitizer; once exposed to CS, many develop allergic reactions on later contact. This is a problem particularly for industrial workers who are in contact with CS daily.

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CHLOROACETOPHENONE

CHARACTERISTICS

Chloroacetophenone (Mace, 1-chloroacetophenone, o-chloroacetophenone, CAP, phenyl chloromethyl ketone), a lacrimator, is a white volatile crystalline solid with an odor like that of apple blossoms. It is commonly referred to as CN. It melts at 54°C, boils at 245°C, is slightly soluble in water, and is soluble in ethanol, benzene, acetone, and benzyl chloride.[30] Its vapors are extremely irritating to the eyes and skin and may cause permanent injury to eyes.

CN causes alkylation of sulfhydryl-containing enzymes, acts as an enzyme inhibitor, and has a denaturing effect on tissue proteins.[10,20,22] It is an alkylating agent of the S_N2 type (substitution nucleophilic, second order).

CN is generally considered to be moderately toxic, and more toxic than CS. It is used in much the same way as CS--sprayed in a liquid carrier, as a micropulverized powder, or in pyrotechnical grenades. In recent years, pen guns and small pistols carrying CN cartridges have become popular as personal defense weapons. Injuries have resulted from their careless use. As of June 1, 1975, pen guns were classified as firearms and placed under the controls and restrictions of the Gun Control Act of 1968 by the Bureau of Alcohol, Tobacco, and Firearms, Department of the Treasury.

TOXICOLOGY IN IN VITRO AND ANIMAL STUDIES

Lee and Webber[19] treated HeLa cells with CN and studied effects on cell morphology. CN at concentrations of 1.5×10^{-5}, 3.8×10^{-5}, and 7.6×10^{-4} mol/L was added to HeLa cell cultures for 1.75 and 3.5 h and then washed out. The cultures were incubated for 7 d. Growth was measured in terms of total purines and pyrimidines, and the concentration required to cause inhibition of growth by 50% was estimated as 10^{-5} mol/L. Variations from the normal cell form, as well as early degenerative changes, were found. Lee and Webber attributed these effects to inhibition of cellular enzymes through interactions with sulfhydryl groups.

Lakshmi[17,18] studied the effects of CN on chick embryos. Embryos at the primitive-streak stage (18 h) and at the head-process stage (22 h) were treated with CN at 5×10^{-4} M for 15 min and examined after further incubation for 21 h. Primitive-streak embryos had malformations of the brain (81.4%) and of the neural tube (48.7%). Those treated at the head-process stage were unaffected by the CN treatment. Lakshmi attributed the injuries to inhibition of

sulfhydryl-containing proteins during morphogenesis. Apparently, no injury resulted from CN treatment in the head-process stage, because the invagination of the chorda-mesoderm and induction of the nervous system had taken place.

Lakshmi treated primitive-streak stage embryos with CN to study the effect on Hensen's node (related to the organization of the embryo). The embryos were incubated for 3 h after treatment; the nodes were then excised, washed, and grafted to host chick embryos at the same stage. The results showed an inverse relation between the capacity for induction of Hensen's node and exposure to CN.

McNamara et al.[25] summarized the LCt_{50}s for three animal species exposed to CN as a dry dust and in sprayed solutions (Table 4-19). For a comparison of single and repeated exposures, 20 guinea pigs and eight monkeys were exposed to CN at a Ct of 2,300-4,000 mg·min/m^3 on each of 10 consecutive days. The cumulative Ct was 31,445 mg·min/m^3. In a single exposure, this Ct would have killed most of the animals. Only five guinea pigs died. Eight dogs were exposed to CN at a Ct of 3,000-7,000 mg·min/m^3 on each of 10 consecutive days. The cumulative Ct was 60,000 mg·min/m^3, enough to kill all the dogs if it had been a single exposure. Only one died. In another test, 20 guinea pigs, eight dogs, and eight monkeys were given a cumulative Ct of 88,000 mg·min/m^3 in 10 daily exposures; few animals died. These tests showed that CN is detoxified rapidly and has little cumulative toxicity.

TABLE 4-19 Lethal Effects of CN in Three Animal Species

Species	No. Animals	LCt_{50} mg·min/m^3
Rats	190	8,878
Guinea pigs	106	7,984
Dogs	62	7,033
Total	358	6,189

[a] Data from McNamara et al.[25]

LD_{50} data for several species of animals and several irritants are in Table 4-10.

Striker et al.[36] exposed 30 Macaca mulatta monkeys to CN, 10 at each of three exposure conditions, as shown in Table 4-20. Two monkeys at each Ct were killed and autopsied at 12 h, 24 h, 72 h, 7 d and 30 d. At the low dose, pathologic changes were greatest from 24 to 72 h; animals autopsied at 30 d had no lesions. The middle dose, however, caused increased pulmonary edema; the high dose resulted in hemorrhages. Permanent damage was caused at the two higher doses; three spontaneous deaths occurred 24 h after exposure at the high dose. These doses were far higher than would be encountered in an open-air situation, such as a riot; but they might be approached in a closed space if several grenades were exploded.

Ballantyne and Swanston[4] examined the acute toxicity of CN administered by several routes in mice, rats, guinea pigs, and rabbits. Table 4-21 and Table 4-22 present the results for oral, intravenous, intraperitoneal, and inhalation exposures. The animals that died from oral administration usually did so 2-18 h after dosing. They showed congestion of the lungs, stomach, and intestine (with erosion of the mucosa) and congestion and hemorrhages of the thymus. Kidney and liver necrosis was seen in many animals. Intravenous administration resulted in congestion of liver, kidneys, lungs, spleen, thymus, and eyes. Surviving animals recovered in about 3 wk and showed no abnormalities when autopsied. Intraperitoneal CN was more toxic in guinea pigs, but about as toxic as intravenous CN in rats. Congestion in the viscera was much the same as that caused by other routes of administration. Animals that died after inhalation of CN had congestion of the alveolar capillaries and intrapulmonary veins, alveolar hemorrhages, and excessive secretion in the bronchi and bronchioles. There were areas of patchy acute inflammatory cell infiltration of the trachea, bronchi, and bronchioles.

In skin-irritation tests, Ballantyne and Swanston found that guinea pigs were more sensitive than rats with regard to erythema, but less so for edema. Rabbits were more affected in both categories.

Ballantyne et al.[3] examined the ocular effects of CN on rabbits. Results of tests with CN at 1-10% in polyethylene glycol (PEG) showed that severity and duration of effects were concentration-related. Lacrimation, chemosis, and iritis were more severe and persistent when the vehicle for CN was corn oil and PEG than when it was trichloroethane (TCE) or trioctylphosphate (TOF). Blepharitis was most severe when CN was dissolved in corn oil. CN dissolved in corn oil or PEG caused a greater degree of keratitis than that dissolved in TCE or TOF.

Gaskins et al.[8] investigated the acute toxicity of CN given by stomach tube in various solvents in male and female rats. Moderate to severe gastroenteritis was observed, often followed by death

TABLE 4-20

Summary of Lesions Found in Monkeys Exposed to CN[a]

	No. Monkeys Showing Lesions														
	Ct 3,025 mg·min/m^3 (1,219 mg/m^3, 2.5 min)					Ct 9,251 mg·min/m^3 (1,682 mg/m^3, 5.5 min)					Ct 21,699 mg·min/m^3 (2,411 mg/m^3, 9.0 min)				
Lesion	12 h	24 h	72 h	1 wk	30 d	12 h	24 h	72 h	1 wk	30 d	12 h	24 h[b]	72 h	1 wk	30 d
Pulmonary edema	—	—	1/2	—	—	1/2	2/2	—	2/2	—	2/2	3/3	2/2	1/2	—
Pulmonary congestion	—	—	1/2	1/2	—	1/2	2/2	2/2	1/2	—	—	3/3	—	—	—
Pulmonary hemorrhage	—	—	1/2	—	—	—	—	—	—	—	—	3/3	—	—	—
Cardiac hemorrhage	—	—	—	—	—	—	—	—	—	—	1/2	1/3	—	—	—
Bronchorrhea	2/2	1/2	1/2	—	—	1/2	2/2	—	1/2	—	—	—	—	—	—
Bronchitis	—	2/2	1/2	—	—	2/2	2/2	—	1/2	—	2/2	—	1/2	—	1/1
Pneumonia	—	—	—	—	—	—	2/2	—	—	2/2	—	—	2/2	1/2	1/1
Emphysema	2/2	1/2	2/2	2/2	—	1/2	2/2	2/2	1/2	2/2	1/2	3/3	2/2	2/2	—
Atelectasis	—	—	—	2/2	—	1/2	—	1/2	1/2	2/2	—	—	—	1/2	—
Froth in trachea and bronchi	2/2	1/2	1/2	—	—	1/2	2/2	2/2	1/2	—	1/2	1/3	1/2	1/2	—
Edema of larynx	—	—	—	—	—	—	—	—	—	—	1/2	1/3	—	—	—
Aspiration	2/2	—	—	—	—	—	—	—	1/2	—	1/2	—	—	—	1/1
Hemorrhagic gastroenteritis	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—
Corneal ulcer	—	—	—	—	—	—	—	—	—	—	—	—	2/2	—	—
Tracheitis	—	—	1/2	—	—	—	—	—	1/2	—	2/2	—	2/2	—	—
Interstitial inflammatory cell infiltration	—	—	—	—	—	—	—	—	2/2	—	—	—	—	—	—
No lesions	—	—	—	—	2/2	—	—	—	—	—	—	—	—	—	—

[a] Data from Striker et al.[36]

[b] Three monkeys died spontaneously, leaving only one in this dosage group to be killed at 30 d.

TABLE 4-21 LD50 Values of CN Administered Orally, Intraperitoneally, and Intravenously in Rats, Rabbits, Mice, and Guinea Pigsa

Route	Species	Dosage, mg/kg	LD_{50} (5% confidence limits), mg/kg
Intravenous	Rabbit (male)	25-100	31 (25-35)
	Rabbit (female)	24-48	30 (16-39)
	Mouse (male)	40-113	81 (66-109)
	Rat (female)	25-63	41 (36-49)
Intraperitoneal	Rat (male)	30-50	36 (26-47)
	Guinea pig (female)	14-40	17 (5-22)
Oral	Rat (male)	100-400	127 (113-144)
	Rabbit (female)	78-200	118 (69-143)

[a] Data from Ballantyne and Swanston.[4]

TABLE 4-22 Mortality in Rabbits, Mice, and Guinea Pigs after Inhalation of CNa

Species	Duration of Exposure, min	Average Concentration, mg/m^3	Ct, $mg \cdot min/m^3$	No. Exposed	14-Day Deaths
Rabbit (male)	15	417	6,255	20	11
	15	593	8,895	20	6
	20	559	11,180	20	10
	25	651	16,275	20	16
	30	742	22,260	15	14
	37	749	27,713	20	19
Rabbit (female)	20	465	9,300	8	3
	15	638	9,570	10	0
	15	665	9,975	10	2
	15	720	10,800	10	4
	30	423	12,690	10	7
	30	652	19,560	5	5
	30	742	22,260	5	5
	60	551	33,060	10	10
Mouse (female)	15	600	9,000	50	7
	15	640	9,600	50	6
	30	592	17,760	52	11
	30	652	19,560	22	5
	37	719	26,603	51	20
Guinea pig (female)	15	243	3,645	20	0
	20	465	9,300	10	0
	30	490	14,700	20	16
	30	667	20,010	20	18
	30	764	22,920	20	20
	60	627	37,620	20	20

[a] Data from Ballantyne and Swanston.[4]

(Table 4-23). They tested rabbits for eye and skin irritation. A 1% w/v solution of CN in TCE caused a reddening of the eyes for 48 h. Washing of the eyes 10 min or less after treatment almost eliminated chemosis. Because the aim of these experiments was to estimate the harmful effects of commercial formulations (Mace, etc.), 25 formulations were tested. Only one, which contained 4.3% w/v of CN, produced permanent eye injury in rabbits. Others, containing 0.04-2%, produced no permanent eye or skin injury. Testing for skin irritancy was performed by applying 0.5 ml of various commercial CN-containing preparations on either intact or abraded rabbit skin, with patches covering the areas for 24 h. This usually produced a necrotic eschar 5-6 d after treatment. Washing of the skin did not prevent further injury, even if done 10 min after application. Skin recovery took 3-5 wk.

McNamara et al.[25] tested corn oil dilutions of CN in topical applications of 0.05 ml to rabbit eyes and skin. The results were as follows:

Eyes:	0.5 mg--no effect
	1.0 mg--transitory conjunctivitis
	5.0 mg--corneal opacity
Skin:	5.0 mg--erythema and necrosis

Rothberg[33] studied sensitization to CN in guinea pigs. Using the Landsteiner technique, he applied acetone solutions of CN both topically and by intradermal injection. Twelve induction doses were given to eight animals by each route. After a 2-wk rest period, challenge was made topically at a fresh skin area. There were seven positive reactions in each group of eight animals. Sensitization to CN also occurs in humans.[24,29]

Gwynn and Salaman[11] tested a number of compounds, including CN, as possible promoters of skin carcinogenesis when 9,10-dimethyl-1,2-benzanthracene (DMBA) was used as an initiator. Treatment consisted of 0.3 ml of a 0.1% or 0.15% acetone solution of DMBA on the clipped backs of mice, followed after a delay of 21 d by application of CN at a maximal tolerated concentration once or twice a week for 12-15 wk. There was a significant increase in tumors 22 wk after the start of secondary treatment.

Although CN may be a promoter, the absence of data from thorough carcinogenicity tests or from short-term mutagenicity tests makes it impossible to reach a conclusion regarding its carcinogenicity.

The National Toxicology Program performed a subchronic study of CN to generate data on the maximally tolerated dose (MTD) of this agent preparatory to launching a full-scale chronic-toxicity and carcinogenicity bioassay.[38] The test was conducted in Fischer 344

TABLE 4-23 Acute Oral Toxicity of CN and CS in Ratsa

		Mean LD_{50} (95% Confidence limits), mg/kg[b]		
Solvent	Concentration of CN or CS, % w/v	CS	CN	LD_{50} Ratio, CS:CN
TCE	4	102 (78-134)	260 (210-322)	2.5
Carboxymethylcellulose sodium, 0.5% w/v	2[c]	71 (59-86)	358 (219-440)	5.0
Propylene glycol in water, 65% v/v	1	82(73-90)	178 (144-221)	2.2
Corn oil	2[c]	224 (184-273)	264 (234-298)	1.2
Dimethyl sulfoxide	4	52 (47-80)	318 (250-400)	6.1
TOF	2	258[d]	358[d]	1.4

[a] Data from Gaskins et al.[8]
[b] Deaths occurred within 48 h of dosing, regardless of solvent. Gross abnormalities consisted of moderate to severe gastroenteritis.
[c] Suspensions.
[d] Estimated.

male and female rats and in $B6C3F_1$ hybrid male and female mice. Six groups of 20 rats and mice divided equally between sexes were exposed to a CN aerosol by inhalation at concentrations of 0 (control), 0.25, 0.5, 1.0, 2.0, and 4.0 mg/m^3 for 6 h/d, 5 d/wk for 13 wk, for a total of 65 exposures of 6 h each. In rats, reduced weight gains were seen in males at 4.0 mg/m^3 and in females at 0.25, 0.5, 1.0, and 4.0 mg/m^3. In mice, weight gain appeared normal at all concentrations, except at the last weighing. There appeared to be a slight increase in liver:body weight ratio in mice and rats at most concentrations. However, there were no gross clinical signs in rats or in mice, except those related to irritation of the eye involving opacity. Microscopically, no lesions were observed, compared with controls. On the

basis of these results, it was recommended that the chronic study be conducted at 1.0 and 2.0 mg/m^3 for male and female mice and rats.

TOXICOLOGY IN HUMAN STUDIES

Immediate Effects

Exposure to CN causes an immediate burning sensation or stinging in the eyes, nose, throat, and exposed skin. Lacrimation, salivation, rhinorrhea, and dyspnea or a constricting sensation in the chest follow. The lacrimatory action persists for about 20 min after exposure, but conjunctivitis and blepharospasm may last for 24 h. CN is more toxic than CS or CR. High concentrations of CN may result in chemical injury to the eye, with corneal and conjunctival edema, loss of corneal epithelium, and chemosis.[2,20]

Acute injuries of the eyes, primarily from effects of blast and missiles, may occur from tear-gas weapons, such as pen guns. The immediate effects of these injuries include swelling and edema of the lids, with penetration of skin, conjunctiva, cornea, sclera, or globe by gunpowder and CN; conjunctival ischemia and chemosis; corneal edema, erosion, inflammation, or ulceration; and focal hemorrhage.[13,20]

Injuries in Confined Spaces

The use of CN in open spaces, as in riot control, causes copious lacrimation, rhinorrhea, and a burning sensation in exposed skin. These effects pass quickly in fresh air. A report of Thorburn[37], however, showed that results of exposure in confined spaces can be severe. In the incident described by Thorburn, 44 prison cells, each with an occupant, were sprayed with a CN aerosol, some more than once. The exhaust system did not remove the CN; some lingered in the cells as long as 4 d. After the spraying, inmates took showers while still clothed, and clothing was not changed. Twenty-eight men required medical attention; eight were hospitalized. Besides dyspnea, conjunctivitis, vomiting, allergic reactions, fainting, and pharyngitis, four men had facial burns and three developed blisters around the ankles, apparently from drainage of CN- contaminated water in clothing. One man had first- and second-degree burns over 25% of his body, and 10 men not admitted to the hospital suffered first- and second-degree skin burns. There was no serious eye injury.

Many of the serious effects on these prisoners were caused or exacerbated by a delay in treatment. It is likely that the initial spraying would have produced serious casualties even if they had not been kept in wet clothes impregnated with CN. The immediate effects

of CN observed after an incident in confined spaces include lacrimation, conjunctivitis, conjunctival edema, sore throat and pharyngeal erythema, dyspnea, cough, and skin burns.[37] CN may cause death in confined spaces from which escape is not possible. If death occurs from high concentrations of CN, the postmortem examination may reveal pulmonary edema and congestion, intra-alveolar hemorrhage, necrosis of respiratory mucosa and formation of pseudomembranes, and bronchopneumonia.[6,9,25,35]

Wind-Tunnel Aerosol Tests in Man

Punte et al.[31] tested volunteers in a wind tunnel at an air-speed of 5 mph to establish CN tolerance time--the length of time a subject could remain in the CN-containing airstream. The tolerance time varied with the subject. CN aerosols were generated from acetone solutions and had a mass median diameter of about 0.6 μm. Such particles can reach and remain in the deep region of the respiratory tract. The immediate effects of such exposures were tingling of the nose and rhinorrhea, burning of throat and eyes, lacrimation, and blurred vision. Some subjects suffered dyspnea. Mild conjunctivitis was observed, but this and the other symptoms passed rapidly when the subject left the wind tunnel.

Effectiveness, Lethality, and Tolerance Time

The ICt_{50} and LCt_{50} of CN are 20-213 and 7,000-14,000 mg·min/m^3, respectively.[25] As an aerosol, it has a TC_{50} for the eye of 0.3 mg/m^3 and an IC_{50} of 20-50 mg/m^3.[2] In field conditions, lacrimation occurs at 10 mg/m^3. The human LCt is 8,500-25,000 mg·min/m^3.[2] The tentative maximal safe inhaled Ct for humans is 350 mg·min/m^3.[31]

The estimated ICt_{50} of CN for man is 80 mg·min/m^3, on the basis of a 1-min exposure.[25] This value is not valid for all conditions; the authors cited incapacitating concentrations of 20 and 40 mg/m^3 for 1 min under other conditions.

Estimates of the LD_{50} for humans have been based on animal data, such as shown in Table 4-23. From these and other data, official estimates of LD_{50} and safety factors have been made. A few deaths from CN poisoning have been reported, as discussed later.

TEAR-GAS WEAPONS

Forensic Aspects

The most popular weapon for personal protection against assaults and robberies over the last 20 yr is the tear-gas pen gun, a simple, inexpensive, inconspicuous device resembling a pen that carries a cartridge loaded with Mace. The load is a 1:1 mixture of CN and silicic anhydride; the propellant is smokeless powder. The cartridge is sealed with a foam rubber wad and acrylic coating.[34] The numerous injuries, especially eye injuries, inflicted by these cartridges have prompted the development of a spray with a charge of CN in 1,1,1-trichloro-2,2,2-trifluoroethane and petroleum ethers or other vehicles.[29]

Experimental Studies

Stahl et al.[34] tested the effects of closeup and contact shots. When a standard cartridge (with 0.26 g of CN) was fired 12 in. from the axilla of a dog, the wad caused abrasions, edema, and erythema, but did not penetrate the skin. A shot fired in contact with the skin caused a penetrating wound of the skin and axilla. Exploration of the wound 30 d later disclosed an encapsulated wad and necrosis of the axillary tissues, but no major injury to the neurovascular tissues. Another test was made by firing a magnum cartridge (with 0.7 g of CN) 6 in. from the flank of a nonanesthetized dog. This caused a nonpenetrating wound with ecchymosis of 2 cm^2. A second magnum cartridge fired in contact with the chest wall caused a large wound and almost immediate death. Muscles and pericardium were perforated, and a rib was broken.

Adams et al.[1] tested the effects of tear-gas cartridges fired 4 in. from the exposed sciatic nerves of six rabbits. Animals were killed for examination at intervals up to 32 d. Loss of nerve function was noted at 3-4 d. The odor of CN in the wound was noted for up to 6 d. In animals examined at 15 and 32 d, heavy scar tissue surrounded the nerves and nearby muscle. A second group of rabbits was treated by exposing the nerves and dusting them with 0.2 g of the CN mixture from a cartridge. Loss of nerve function occurred, as in the first group. All animals showed the beginning of axon breakdown on the seventh day. Adams et al. concluded that CN is a toxin when in contact with nerves; thus, particles of it driven into tissue by the force of a closeup shot can inflict serious injury.

Accidental Injuries

Adams et al.[1] reported three hand injuries that resulted from accidental discharges of tear-gas guns at close range. Surgery was required in all three to alleviate pain and in two to remove wadding and other foreign material. All three patients suffered continuing pain and some loss of sensation, apparently from the toxic action of CN on nerves.

Levine and Stahl[21] examined files of the Armed Forces Institute of Pathology and found records of 13 men who had lost eyes because of accidental or intentional discharges of tear-gas weapons close to the face. They emphasized the multiple nature of such wounds from the agent, wadding, and other debris. In old cartridges, the charge of CN may be clumped and thus increase entry of CN into tissue.

Many other reports of eye injuries have been published. Hoffman[13] recommended outlawing all tear-gas weapons. Oksala and Salminen[27] reported six cases, Hoepping[12] reported 20 cases of keratitis and other ocular injuries from pen guns, and Laibson and Oconor[16] treated five cases of eye injury. Oaks et al.[26] urged that police officers be instructed to handle tear-gas guns properly and, when prisoners are in pain after gassing, to get medical attention promptly. One patient shot in the face at close range with a tear-gas gun was imprisoned without medical treatment for 44 h; his sight took a month to recover.

SENSITIZATION IN HUMANS

CN is a skin sensitizer. Rothberg's demonstration of this in guinea pigs has ample confirmation in clinical reports of sensitization in man.[33] Penneys et al.[29] reported treating two patients for allergic contact dermatitis, each having had two or more exposures to the agent. In the course of treatment, one patient was given a patch test with Mace (a formulation containing CN), the other a patch test with CN. A patch test with Mace was applied to one investigator's skin, and one with CN to the skin of another. All four test subjects had positive reactions. Penneys et al. applied 0.009% CN (0.01 the concentration found in Mace) to 30 nonexposed volunteers. Eight of these subjects were then given patch tests with 0.25 ml of 0.9% CN for 24 h. Five of the eight developed allergic contact sensitivity to the agent. They were challenged with 0.009% CN 2 and 3 wk later. Erythema, edema, and vesiculation developed at the test area. Using these subjects, Penneys et al. demonstrated cross sensitization to 1,1-dichloroacetophenone, but not to 1-bromo-acetophenone, p-chloroacetophenone, or acetophenone. Maibach and Marzulli[23] showed that CN is a potent contact sensitizer with a capacity for producing cross-reactions with CS.

Penneys et al.[29] tested the speed of action of CN on skin. They applied Mace to the forearms of three sensitized subjects in five spots. The Mace was then washed off with soap and water at 30 s or 1, 2, 3, or 4 min. There were no reactions to a 30-s exposure, one at 1 min, two at 2 min, and three at 4 min. In a riot, when CN is used, no rioters would be expected to remove the agent quickly by washing, and any who had a previous exposure to Mace or CN might suffer dermatitis.

CN may cause primary irritant dermatitis or allergic contact dermatitis by delayed hypersensitivity. After sensitization, acute exposure to CN causes itching, erythema, edema, vesiculation, purpura, and necrosis.[28] Jolly and Carpenter[14] reported that an accidental discharge of a pen gun resulted in erythema and edema 24 h later; the patient had been exposed to CN 5 yr earlier. Queen and Stander[32] reported severe reactions to CN 17 yr after a first exposure to the agent.

LONG-TERM EFFECTS

Acute eye burns caused by discharge of tear-gas weapons at close range have been reported. In these instances, the charge and seal, as well as the CN, penetrated the tissues, thereby causing mechanical damage.[13] However, gross contamination of the eyes with CN can also result in severe and permanent corneal injury.[10] CN is thought to act on the eye by two distinct mechanisms. In one, it exerts a lacrimatory, reversible, and essentially noninjurious effect on corneal nerve endings at low concentrations; in the other, it causes an injurious, denaturing reaction on the nerve endings (reversible only with difficulty), probably with other components of cornea and conjunctiva affected at high concentrations.[10] CN induces contact dermatitis,[28] but it is not known whether this leads to persistent skin or respiratory problems as reported in animals for toluene diisocyanate.[15]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

Between 1958 and 1972, 99 human subjects underwent experimental exposures to CN at Edgewood Arsenal. Sixty-nine subjects had aerosol exposures in a chamber that they entered masked; they removed the masks after the agent concentration had equilibrated. Thirty subjects had direct skin applications of CN.

Exposure data are available on 68 of the 99 subjects: 16 had one to five aerosol exposures in 1958, 44 had one to three aerosol exposures in 1965, and eight had one dermal application in 1968. No exposure data are available on the other 31 subjects: nine had one

aerosol exposure and performed simulated battlefield functions in 1966, five had one dermal application in 1967, and 17 had one dermal application in 1972.

The only dosage information on the sixteen 1958 aerosol-exposure subjects is exposure time, which was recorded on seven of the subjects and ranged from 0.32 to 3.63 min. The 44 subjects who underwent aerosol exposures in 1965 experienced Ct's of 6-315 mg·min/m^3 and exposure time of 0.15-3 min. The eight dermal-exposure subjects in 1968 had 0.01-0.025 ml of CN applied to their bare or clothed arms.

The effects on the aerosol-exposure subjects were transient, generally resolving within minutes of removal of the agent. There also seemed to be tolerance in experienced subjects, often increased by closing the eyes. Predominant effects of aerosol exposure were ocular: lacrimation, blepharospasm, conjunctivitis, and, rarely, palpebral edema. Respiratory effects of aerosol exposure were nasopharyngeal irritation, rhinorrhea, and, rarely, dyspnea. Skin irritation was prominent on shaved areas. Other rare effects of aerosol exposure were headaches and dizziness. No laboratory analyses were recorded for aerosol exposure.

Only one of the eight 1968 dermal-exposure subjects had erythema at the exposure site, which lasted for 7 h. The CN was applied to his skin. Five of the 1968 dermal-exposure subjects had normal results of laboratory analyses--including urinalysis, complete blood count, blood urea nitrogen, alkaline phosphatase, and serum glutamic oxalotransferase--7 d after exposure.

In summary, among the 68 subjects from Edgewood on whom there are data, there were probably no permanent ocular or pulmonary injuries. These were short, low-dose exposures, and effects on the eyes and respiratory system were transient; symptomatic recovery was complete within minutes. Information on the dermal effects of CN exposure is minimal. Sensitization to CN is likely, causing allergic contact dermatitis and possible systemic allergic reactions (e.g., pulmonary fibrosis) on re-exposure, although there is no evidence that this occurred among the Edgewood subjects.

DISCUSSION

It has been suggested that reactions of CN with sulfhydryl groups may be involved in the toxic effects of CN.[2,7,11] Reaction with nucleophilic centers (e.g., sulfhydryl groups) at the binding or catalytic sites of enzymes may lead to enzyme inhibition.

Castro[5] investigated the inhibitory actions of several alkylating agents on cholinesterase (ChE) and found that CN was "an instantaneous and noncompetitive inhibitor." The inhibition of ChE was reversible by dilution or dialysis. Castro concluded that inhibition of ChE by CN does not involve the sulfhydryl group, but possibly the histidine or methionine groups of the enzyme. He suggested that the rapid and reversible ChE inhibition by CN might explain the rapid onset of lacrimation and the correspondingly quick recovery when the subject moves to clean air.

SUMMARY

- CN is moderately toxic (more so than CS), but nonlethal, except at high dosage. There appear to be no lasting effects on eyes or skin of Edgewood subjects after single or multiple aerosol exposures.
- CN was found to be active as a promoter in one study. Adequate tests have not been conducted, however, to permit conclusions about the carcinogenicity of CN itself. Similarly, the available information permits no conclusions with regard to mutagenicity. Studies now being conducted by the National Toxicology Program should provide more information on these subjects.
- CN is a strong sensitizing agent, often producing allergic contact dermatitis after a single exposure. Edgewood subjects exposed to aerosolized CN might now be skin-sensitized and at greater risk of hypersensitivity to inhaled CN, if effects on CN parallel those of toluene diisocyanate on animals in this regard.
- The use of CN-loaded (Mace-loaded) pen guns and pistols presents severe hazards, often not caused by the agent itself, because of improper handling and accidental discharge.

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DIBENZ[B,F][1,4]OXAZEPINE

CHARACTERISTICS

Dibenz[b,f][1,4]oxazepine (CR) is a pale yellow solid, with a molecular weight of 195.2 and a melting point of 72.5°C (Table 4-1). It is slightly soluble in water (3.5×10^{-4} mol/L at 20°C), but freely soluble in most organic solvents.[2]

Bandman and Savateyev[8] and Choie and Landis[10] published recent reviews on CR.

CYTOTOXICITY

Lee and Webber[14] studied the toxicity of CR in HeLa cells in culture. In vivo experiments had disclosed only negligible effects on tissue cells, except for the sensory elements of the nervous system. Lee and Webber believed that suitable cell cultures might react to compounds of moderate toxicity, thus constituting sensitive tests that would indicate which cellular elements were attacked by compounds like CR.

CR was added to cell cultures at various concentrations. The agent was washed out, cultures were incubated for 7 d, and effects of CR were judged in terms of changes in the morphology of the cells. After exposure at 4×10^{-5} mol/L for 3.5 h, there was no change in cell morphology. After exposure at 1.4×10^{-4} mol/L for 1.75 h, there was cytoplasmic shrinkage and increased staining of cell membranes. Cells with aggregations of heavily stained blebs, usually surrounding the nucleus, were about 20% of the population. After exposure at 1.4×10^{-4} mol/L for 3.5 h, the same alterations were seen, but in greater numbers. In the growth inhibition test, ranging tests yielded a GID_{50} (inhibition of growth for 50% of the population) of 10^{-4} mol/L for CR. A second series of tests with a narrow range of concentrations gave a final value of 8.7×10^{-5} mol/L. The authors speculated that results with lacrimators are based on interference with sulfhydryl-containing enzymes.

Upshall[21] exposed pregnant rats and rabbits to CR at several doses and by several routes of administration to test for teratogenic or embryolethal effects. Inhalation exposure times were 5-7 min for rats and rabbits. Other conditions were as follows: Rats and rabbits were exposed to aerosolized CR at Ct's 10,000 and 1,000 on days 6-15 or 6-18 of pregnancy, respectively. Other rats were exposed to CR by intragastric administration at 2, 20, and 100 mg/kg on days 6, 8, 10, 12, and 14 of pregnancy. Other rats were exposed at 400 mg/kg CR intragastrically on day 7, 10, or 13 of pregnancy. Rabbits were exposed to CR by intragastric administration at 0.2, 2, and 20 mg/kg on days 6, 8, 20, 12, 14, 16, and 18 of pregnancy or intravenously at 14.1, 15.8, or 17.8 mg/kg on selected days of pregnancy. Recorded results included number of litters, number of live fetuses, litter size and weight, placental weight, increase in placental weight, and number of abnormal litters. High inhalation and intragastric doses of CR were not shown to be teratogenic, but the selected exposure concentrations did not reach maternally toxic values. Intravenous administration to rabbits at a dose close to the maternal LD_{50} caused fetal deaths. The author concluded that CR is

neither teratogenic nor embryolethal to rats and rabbits when given by inhalation or intragastric intubation. He did not offer a satisfactory resolution of the rabbit-injection studies.

TOXICITY IN ANIMALS

Acute Effects

CR is a potent peripheral sensory irritant of low toxicity by the usual routes of administration.[3] It appears safer than CS, which replaced CN and DM in turn as riot-control agent because of greater effectiveness and lower toxicity. Table 4-20 shows comparative toxicities of these compounds in several species.[2]

Ballantyne[3] studied the toxicity of CR in several species of laboratory animals (Table 4-24). CR was more toxic to rabbits and rats than to mice by single-dose intravenous injections. There were no sex-related differences for rats and mice. Signs of poisoning developed in seconds. Survivors appeared normal in 1 h. Animals that died did so in 4-10 min, with congestion of alveolar capillaries and liver sinusoids on autopsy examination. Survivors, sacrificed for autopsy 15 d later, exhibited no abnormalities. Toxic signs appeared in 2-5 min after single-dose intraperitoneal injection, with deaths 1-4 d after injection. Survivors recovered in about 24 h. Animals that died showed congestion of alveolar capillaries, some hemorrhage, and congestion of liver, kidneys, and small intestine. Survivors, examined 14 d later, showed no abnormalities. Survivors of orally administered CR showed ataxia and weakness for 1-2 d, then appeared normal. Animals that died did so in 1-6 d, with congestion of gastric and intestinal mucosa, liver, and lungs and renal tubular necrosis. Survivors were normal when sacrificed for necropsy.Table 4-25, Table 4-26, and Table 4-27 summarize results of inhalation experiments. Few deaths occurred, even at the highest exposures. Rats, rabbits, guinea pigs, and mice were exposed to smokes from grenades, both with and without CR. CR contributed very little to the toxic effects of grenade smokes. The toxicities of CR, CS, and CN are compared in Table 4-28.

Owens et al.[17] and Biskup et al.[9] studied the effects of 1% solutions of CR in propylene glycol, dipropylene glycol, diethylene glycol monomethyl ether, and propylene glycol/water solutions. These solutions were tested in guinea pigs, rats, rabbits, dogs, and monkeys. Doses were administered to the eyes (0.2 ml) and skin (1.0 ml) and by intratracheal insufflation (0.5 ml). No test revealed residual damage. The intent of these studies was to assess the effects of the solvents as carriers of CR.

TABLE 4-24 Acute Oral and Parenteral Lethal Toxicity of Solutions of CR in Various Small Mammals[a]

Route	Species	Sex	LD50 (95% Confidence Limits), mg/kg
Intravenous	Mouse	Male	130 (101-167)
	Mouse	Female	112 (103-121)
	Rat	Male	68 (60-77)
	Rat	Female	68 (61-76)
	Rabbit	Female	47 (42-68)
Intraperitoneal	Rat	Male	817 (747-1,007)
	Rat	Female	766 (719-818)
	Guinea pig	Female	463 (337-607)
Oral	Mouse	Female	4,000
	Rat	Male	7,500 (6,400-12,100)
	Rat	Female	5,900 (5,100-8,500)
	Rabbit	Female	1,760 (1,350- 2,400)
	Guinea pig	Female	629 (555-712)
Percutaneous	Rabbit	Female	450 (in corn oil)
	Rabbit	Female	400 (in petrolatum paste)
	Rabbit	Female	1,500 (in DMSO)

[a] Data from Ballantyne[3]

TABLE 4-25 Exposure of Rats to CR Aerosols[a]

Duration of Exposure, min	CR Concentration, mg/m^3			
	Average	Range	Mean Ct, mg·min/m^3	14-Day Mortality, no. deaths/no. exposed
15	870	780-965	13,050	0/5
30	960	900-1,140	28,800	0/5
60	970	860-1,050	58,200	0/5
60	1,370	1,220-1,570	82,200	0/5
70	1,360	1,070-1,500	95,200	0/5
84	1,230	910-1,740	103,320	0/5
80	1,470	1,120-1,680	117,600	0/5
120	1,650	1,200-2,060	198,000	0/5
123	2,030	1,770-2,200	249,690	0/5
180	2,380	2,300-2,520	428,400	0/5

[a] Data from Ballantyne[3]

TABLE 4-26 Exposure of Rabbits, Guinea Pigs, and Mice to CR Aerosolsa

Duration of Exposure, min	CR Concentration, mg/m^3			14-Day Mortality, no. deaths/no. exposed		
	Average	Range	Mean Ct, mg·min/m^3	Rabbits	Guinea pigs	Mice
14	670	480-1,210	9,380	0/20	0/20	0/40
120	490	350-620	58,800	0/5	0/5	0/20
120	570	550-610	68,400	0/10	0/10	0/20
193	690	530-880	133,170	0/10	1/10	0/20
113	1,500	1,290-1,930	169,500	9/20	5/20	2/40

[a] Data from Ballantyne[3]

Duration of Exposure, min	CR Concentration, mg/m^3			14-Day Mortality, no. deaths/no. exposed
	Average	Range	Mean Ct, mg·min/m^3	
60	710	610-800	42,600	0/10
220	1,060	1,000-1,180	233,200	0/10
200	1,850	1,310-2,030	370,000	1/10
180	2,450	2,350-2,680	441,000	2/10

[a] Data from Ballantyne[3]

TABLE 4-28 Comparative Single-Dose Lethal Toxicity of CN, CS, and CRa

		LD_{50} (95% Confidence Limits)[b]		
Route	Species (Sex)	CR	CS	CN
Inhalation (smoke)	Rat	139,000 (119,000-157,000)	68,000 (60,000-77,000)	23,000 (20,000-27,300)
	Rabbit	160,000 (134,000-188,000)	63,000 (50,000-80,000)	15,800 (8,700-28,600)
	Guinea pig	169,000 (139,000-201,000)	35,000 (25,000-45,000)	15,400 (11,300-21,100)
	Mouse	203,000 (180,000-229,000)	76,000 (61,000-119,000)	
Inhalation (aerosol)	Rat	428,400	88,460 (77,400-98,500)	3,700-18,800[c]
	Rabbit	169,500	54,100 (42,630-70,400)	5,840-11,480[c]
	Guinea pig	169,500	50,010 (42,750-60,220)	3,500-13,140[c]
	Mouse	169,500	67,200 (59,200-78,450)	18,200-73,500[c]
Intravenous	Mouse (male)	130 (101-167)	48 (41-63)	81 (66-109)
	Mouse (female)	112 (103-121)	--	--
	Rat (male)	68 (60-77)	--	--
	Rat (female)	68 (61-76)	28 (25-30)	40 (34-49)
	Rabbit (female)	47 (42-68)	27 (25-30)	29 (16-39)
Intraperitoneal	Rat (male)	817 (747-1,007)	48 (43-54)	38 (31-54)
	Rat (female)	766 (719-818)	--	--
	Guinea pig (female)	463 (337-607)	73 (62-78)	17 (8-21)
Oral	Mouse (female)	4,000	--	--
	Rat (male)	7,500 (6,400-12,100)	1,366 (1,184-1,780)	126 (113-144)
	Rat (female)	5,900 (5,100-8,500)	1,284 (1133-1530)	--
	Rabbit (female)	1,760 (1,350-2,400)	142 (61-236)	118 (93-143)
	Guinea pig (female)	629 (555-712)	212 (190-243)	157 (122-229)

[a] Data from Ballantyne[3]
[b] In mg·min/m^3 for smokes and aerosols; in mg/kg for intravenous, intraperitoneal, and oral routes.
[c] Range of values from several sources.

Ocular Effects

Rengstorff et al.[19,20] supplemented the ocular exposures of Owens et al.[17] by testing the effects of a 5% solution of CR in propylene glycol in the rabbit eye. Owens et al. had instilled 0.2 ml of 1% solutions of CR in propylene glycol, dipropylene glycol and diethylene glycol, monomethyl ether in single doses and on 5 successive days. Neither rabbits nor monkeys suffered more than mild and transitory eye effects.

Rengstorff et al.[20] applied 0.025 ml of a 5% solution of CR 5 d/wk for 4 wk. The animals were kept under observation for 60 d. Instillation of the daily doses was followed by a brief period of blepharospasm (about 15 min), after which the eyes appeared to be normal. During the test period, superficial and slit-lamp examination did not reveal any injury to the eyes. At the end of the experimental period, the eyes were examined by light and electron microscopy. No abnormalities were found.

Ballantyne et al.[6] conducted extensive investigations of the effects of CR on the eyes of rabbits. Similar tests were applied with CN.

Eye effects were as follows:

- Lacrimation: very mild at 1 and 2% for 1 h, mild at 5 and 10% for 2-3 d.
- Blepharitis: mild at 2% for 24 h, up to 2 d at 5 and 10%.
- Chemosis: mild to moderate at 5 and 10% for 2 d.
- Hyperemia: mild at 1 and 2% for 48 h, moderate at 5 and 10% for up to 3 d.
- Iritis: mild in 2 of 10 rabbits at 5%, in 3 of 10 at 10%.
- Keratitis: mild at 10% for 21 d, at 5% for 3 d.
- Effects of solvents: less severe than with CN for all solvents.
- Solid CR: 5 mg dropped into the eye caused minor lacrimation, blepharitis, and chemosis, clearing in 1 h.
- CR aerosols: 30-min exposures at 10,200 mg·min/m^3 and 17,130 mg·min/m^3 resulted in only mild lacrimation and conjunctival injection, clearing in 1 h.

- Corneal thickness: increase in thickness was dose-dependent, and cornea returned to normal in 2 d after CR at up to 2%, in 13 d after CR at 10%.
- Intraocular tension: CR at 0.5-5% caused increases of 6-40%, which subsided in 1 h.

Ballantyne et al. concluded that these experiments demonstrated a much greater degree of safety for CR and less (almost nonexistent) damage to the eye than CN. Colgrave et al.[11] examined the fine structure of exposed rat lungs by optical and electron microscopy after exposure to CR aerosols at high concentrations, as follows:

Concentration, g/m^3	Time, min	$Ct,x10^3$, $mg \cdot min/m^3$
1.15	68	78
1.22	115.5	141
1.02	158	161

No animals died from these exposures. Examinations of the lung tissues showed mild congestion, some capillary damage in the form of endothelial ballooning, and some endothelial swelling and thickening. These effects were judged reversible.

Lung Effects

Pattle et al.[18] exposed 10 rats to a CR aerosol, generated pyrotechnically, at 115,000 $mg \cdot min/m^3$ for 1 h. The lung surfactant and organelles from which it appears to be derived showed no changes up to 15 d after exposure, when examined by light and electron microscopy.

Metabolism and Physiology

Balfour,[1] using tritium-labeled CR to investigate uptake and metabolism, found that both intact cornea of the guinea pig and corneal homogenates take up CR readily and metabolize it to a lactam derivative. The metabolic process appeared to take place in the corneal cells. Homogenization of the corneas supported this idea: after homogenization, the metabolic activity was found in supernatant fluid. The author speculated that the lactam derivative of CR is not involved in the process of irritation, but that methyl derivatives are involved.

Lundy and McKay[15] studied the effects of intravenous CR on cardiovascular activity in anesthetized cats. Dose-dependent stimula

tion of heart rate and blood pressure of short duration was seen, with increased arterial catecholamine content. Blood pressure increased by about 5% after a dose of CR at 25 mg/kg and about 60% after 200 mg/kg. The brevity of the increase was attributed to rapid metabolism of CR. Pretreatment with phentolamine completely blocked the increase in blood pressure. Pretreatment with propranolol almost completely blocked the tachycardia. Cats pretreated with 6-hydroxydopamine did not show cardiovascular stimulation. Bilateral adrenalectomy did not alter the cardiovascular response to CR. The pressor effect of CR was attributed to release of norepinephrine from adrenergic nerve endings.

Additional studies of Lundy and McKay[16] suggested that the CR-induced increases in heart rate and blood pressure in the cat are mediated by the sympathetic nervous system.

Lundy and McKay were aware of the report of Ballantyne et al.[5] describing the cardiovascular changes (decrease in heart rate and increase in blood pressure) that follow "splash contamination" of the human face (no details given). The results of their experiments on the cat heart suggested to Lundy and McKay that the cardiovascular effects described by Ballantyne et al. could be explained by the absorption of enough CR to produce a systemic effect on the heart via the sympathetic nervous system. The second paper of Lundy and McKay, discussing the splash effect, appeared in 1977. However, in the 1976 report of Ballantyne et al.,[5] the possibility of systemic effects of absorption was rejected, because the authors concluded that, even with a whole-body "drench," not nearly enough CR could be absorbed fast enough to cause the immediate increase in blood pressure observed in their experiments. They did not refer to work of Lundy and McKay, which appeared around the same time.

Leadbeater and Maidment[13] studied the absorption of CR given to rats as an aerosol, by gavage, or by intravenous injection. Absorption from the aerosol was measured by scintillation counting of the ^{3}H-labeled CR. Both ^{3}H and ^{14}C labels were used for other experiments. Absorption and metabolism were rapid. Radioactivity derived from the CR aerosols was detected in the blood within 15 s of the beginning of exposure. The plasma half-life of CR given intravenously was about 5 min; the half-life of the CR absorbed through the lungs was about the same. CR and its metabolites were found in the blood within 10 min after intragastric administration. The immediate metabolic products were not identified, but glucuronides were identifiable later. Leadbeater and Maidment, like Lundy and McKay, believed that the rapid absorption of CR may imply a systemic involvement in its physiologic effects.

TOXICITY IN HUMANS

Ocular Effects

Ballantyne and Swanston,[7] applying procedures developed with CS, measured threshold concentrations of CR in saline solution for producing blepharospasm: in the rabbit, 7.9 x 10^{-5} M; in the guinea pig, 3.5 x 10^{-5} M; and in man, 8.6 x 10^{-7} M. Threshold concentrations for sensation were 4.9 x 10^{-7} M in the human eye and 2.1 x 10^{-6} M in the human tongue. The authors cited data from which a CR threshold concentration of 4 x 10^{-3} mg/m^3 of air was calculated. The threshold concentration of solutions in the eye is 4.9 x 10^{-7} M, or 4.1 x 10^{-2} mg/L of solution. The corresponding value for aerosols is 4 x 10^{-6} mg/L of air, so the human eye is much more sensitive to CR aerosols than to solutions. From the data in Table 4-29, it can be seen that, for the rabbit and guinea pig, CS is more potent than CR at the threshold concentration. For the human eye and tongue, however, CR is more potent. Caution must be used in extrapolating data from animals to humans. The authors calculated a safety factor of several thousand between the highest concentration used in these threshold-concentration tests and the 5% (0.256 M) concentration that is the lowest likely to produce just-detectable damage to the eye.

Cutaneous Effects

Weigand and Mershon[22] tested 39 subjects for skin reactions to CR, using 1-cm^2 patches soaked with CR-propylene glycol solutions. Each patch was wetted with 0.1 ml of 0.01, 0.05, 0.10, 0.25, 0.50, or 1.0% CR. Exposure times were 5 and 30 min, and tests were made at 18.3 and 25.6°C. The concentration of CR did not affect the time of onset of sensation; higher temperature decreased onset times. Subjects differed widely, both in time of onset and termination of irritation and in reported intensity of sensation. Intensity was not related to concentration of CR, exposure time, or temperature. The degree of erythema was variable, but it disappeared in 2-4 h. All CR concentrations were judged to be relatively harmless.

Holland[12] tested skin reactions to CR by putting measured amounts of CR powder on the skin sealed under 4-cm-diameter watch

glasses. Some were dry, others wetted with 2 drops of saline solution. After a 1-h exposure, the CR was removed and the skin was washed. Results are presented in Table 4-30. Erythema appeared in 10 min and faded out by 30 min after removal of the CR. No swelling or vesication developed, and no residual skin changes were seen. In comparing the results with those of similar tests of CS and CN, Holland commented that all reactions to CR are transient and mild, whereas CS causes longer-lasting erythema and CN causes blistering.

Ballantyne et al.[4] have given a general description of the effects on humans of dilute solutions of CR in a water-polyethylene glycol mixture as a whole-body spray. Their report was intended mainly as a compendium of symptoms with recommended procedures for treatment, if needed. A detailed report on the effects of these "drenches" was published by Ballantyne et al.[5] They examined the effects of CR and CS when subjects were given either showers or sprays from a hose, as might be experienced by rioters if a water cannon were used. CR solutions for the drenches were made up with 3.3% (v/v) dipropylene glycol monomethyl ether as a cosolvent. Control tests were run with water alone and water plus dipropylene glycol monomethyl ether. The control drenches, like other cold showers, caused a mild shock and a transient increase in blood pressure.

CR-containing drenches were tested at 0.001% and 0.0025% for both individuals and groups. Within a minute after these drenches, the subjects felt an intense stinging of the eyes, with blepharospasm. Then the facial skin began to sting; this increased to a strong burning sensation. Lacrimation, rhinorrhea, and salivation also occurred. In another minute, the stinging and burning spread to the neck, shoulders, back, and genitalia. Other parts of the body were less strongly affected. After 15-20 min, sensation deceased to a mild tingling. A mild erythema of the skin, lasting 1-2 h, developed.

Physiologic Studies

All the subjects in the studies of Ballantyne et al.[4] were monitored for changes in blood pressure. CR drenches caused an immediate increase in blood pressure. A mean increase in systolic pressure of 45 mm Hg was observed after drenches with 0.001% CR, and a mean of 59 mm Hg after drenches with 0.0025% CR. Corresponding increases in diastolic pressure were 23 and 29 mm Hg. Increases above control values were highly significant, but the mean peak increases for the two concentrations did not differ significantly. Increases in blood pressure lasted about as long as the sensory irritation. Concomitant with the increase in blood pressure, Ballantyne et al. observed nonuniform changes in heart rate. The first report[4] stated that "heart rate in the majority of subjects

TABLE 4-29 Comparison of Threshold Irritant Response of Eyes of Guinea Pig and Rabbit and Eyes and Tongue of Man to CR Solutionsa

			Threshold Concentration (95% Confidence Limits		
Species	Organ	Response	CR	CS	Potency Ratio, CR:CS
Guinea pig	Eye	Blepharospasm	3.5 (2.8- 4.3) x 10^{-5} M	2.2 (1.9- 2.4) x 10^{-5} M	0.63:1
Rabbit	Eye	Blepharospasm	7.9 (5.1-12.5) x 10^{-5} M	5.9 (3.8-10) x 10^{-5} M	0.75:1
Man	Eye	Blepharospasm	8.6 (6.8-12.5) x 10^{-7} M	3.2 (2.1- 6.1) x 10^{-6} M	3.7:1
Man	Eye	Sensation	4.9 (3.8- 6.5) x 10^{-7} M	7.3 (4.2-11.2) x 10^{-7} M	1.5:1
Man	Tongue	Sensation	2.1 (0.7- 3.1) x 10^{-6} M	6.8 (5.0-10.6) x 10^{-6} M	3.2:1
Predicted minimal incapacitating concentration (by log-probit plot)			3.3 x 10^{-6} M	2.7 x 10^{-5} M	8.2:1

[a] Data from Ballantyne and Swanston.[7]

Amount of CR, mg	Reactions			
	Dry		Wet	
	Irritation	Erythema	Irritation	Erythema
0.5			+4/4	+4/4
1.0			+4/4	+4/4
2.0	−0/2	+2/2	+6/6	+6/6
5.0	+3/6	+3/6	+6/6	+6/6
10.0	+3/6	+3/6	+6/6	+6/6
15.0	+1/2	+2/2	++2/2	++2/2
20.0	+2/2	+2/2	+++2/2	+2/2
25.0	+1/6	+1/6	+++5/6	++4/6

[a] Data from Holland.[12]

[b] −, negative; +, mild; ++, moderate; +++, severe.

usually falls." The second report[5] noted that "pulse rates increased in all subjects but with marked individual variations." "Five subjects out of the 13 had a relative bradycardia during the first few minutes after drenching, after which pulse rates increased." Ballantyne et al. appear to have considered the changes in blood pressure as much more important than the rate changes. Exercise after the drenches did not affect the increase in systolic blood pressure, but the diastolic pressure decreased. The authors believed that the increase in blood pressure was brought about by the irritant effect of the agent, and not by a cold pressor effect or by absorption of CR to produce a pharmacologic action. Because exercise after the drench did not add to the increase in blood pressure, it was concluded that the CR drench presented no more of a hazard than exercise alone.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

From 1963 to 1972, 97 subjects underwent experimental exposures to CR at Edgewood: 33 had aerosol exposures in a chamber, and 64 had cutaneous exposures.

In 1963, four subjects had aerosol exposures of 4.8-34 mg·min/m^3 (duration, 17.5 s to 1 min). In 1967, 29 subjects had aerosol exposures of 0.01-1.7 mg·min/m^3 (duration, 1 s to 6.75 min); one had two exposures.

In 1969, 24 subjects had open patch tests with 0.1 or 0.25-1.0% CR applied to their foreheads, faces, necks, and hands for 5 or 30 min, and 20 had closed patch tests with 0.01, 0.1, or 0.25-1.0% CR applied to their foreheads for 5 or 30 min.

In 1972, 11 subjects wearing protective goggles in the wind tunnel had CR sprayed on their faces from 3 or 12 ft for 10 s. The exposed areas were then washed. Concentrations of the CR sprays are not available. Also in 1972, nine subjects had cutaneous exposures to CR and CN on the same day; other data on these subjects are not available.

Several CR subjects exposed in the aerosol chamber commented that CR was milder than "tear gas." The effects of exposure to CR aerosol were transient and predominantly respiratory and ocular. All subjects with aerosol exposures had upper respiratory tract irritation with choking. Many subjects had dyspnea; some were tachypneic. Ocular irritation from aerosol exposure was common, often causing blinking, closing of the eyes, and lacrimation and interfering with the subject's ability to carry out simulated battlefield functions.

The effects of CR in the open and closed patch applications were stinging and erythema at the exposure sites, which resolved by 24 h

after exposure. Applying the CR under a Telfa patch did not worsen the effects.

The effects of skin sprays in the wind tunnel included skin irritation at the exposure site, sometimes accompanied by erythema; lacrimation and conjunctivitis; upper respiratory tract irritation; and, rarely, numbness at the exposure site. The effects generally began after the exposure and worsened initially. Two wind-tunnel subjects said the effects of CR were similar to or worse than those of CS.

Many of the aerosol and wind-tunnel subjects had laboratory analyses 7 d after exposure. There were no abnormalities in the results that had not been present in pre-exposure analyses.

Given the available data on short-term exposures of Edgewood subjects to CR, as well as literature data, it is not possible to predict whether long-term health effects will result from the CR exposures.

SUMMARY

CR has been studied as a possible replacement for CS. CR has a lower mammalian toxicity than either CN or CS. Its effects are in general the same as those of CS, but they could be achieved with substantially lower quantities of agent.

Not only is the acute toxicity of CR extremely low, with an estimated human LCt_{50} over 100,000 mg·min/m^3, but the overt signs of exposure are even more transitory than those of CS. Eye irritation passes in 15-30 min, and skin irritation in 15-20 min. Erythema, which develops only on contaminated skin, passes in about an hour and does not lead to vesication or to contact sensitization. The abrupt increase in blood pressure, which has been observed after whole-body drenches of CR in solution, subsides rapidly. Although available results show no long-term health effects of exposure to CR, there are no available data on the mutagenicity and carcinogenicity of this compound, and the data on teratology are limited.

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DIPHENYLAMINOCHLORARSINE

CHARACTERISTICS

Diphenylaminochlorarsine (DM) is a canary-yellow crystalline solid (see Table 4-1). It is insoluble in water, but soluble in organic solvents; it melts at 195°C and boils at 410°C.[5] It was first prepared by Weiland in Germany (1915) and independently (1918) by Adams in the United States. During and after World War I, it was known as Adamsite.[1] Sim[10] described its odor as that of burning fireworks. DM can be disseminated as an aerosol, either from pyrotechnic mixtures in grenades or bombs, sprayed as a solution, or dispersed as a dust. It has been described as an irritant smoke, a sternutator, and a "sneeze gas."

TOXICITY IN ANIMALS

No reports were found that provide information on whether DM is mutagenic, carcinogenic, or teratogenic.

Owens et al.[5] conducted extensive investigations of the incapacitating effects of DM in humans and its toxicity in several species of laboratory animals. McNamara et al.[4] summarized this work in 1969. The information given here is based on both these reports, which were the outcome of a request from the President's Science Advisory Committee. It was apparently believed that previous research on DM, done mainly soon after World War I, was insufficient and unsatisfactory because of the variability in the methods used and in agent purity. Furthermore, because all the early work had been done with DM dispersed in laboratory tests, it was decided to test the results of dispersion from munitions.

The immediate responses of various species of animals to DM are as follows:

- Mice, rats, and guinea pigs: The animals become hyperactive at once. In a few minutes, lacrimation and salivation begin. Hyper-activity gives way to lethargy, with labored breathing. This state may persist for 1-2 h after removal from the exposure chamber. Survivors return to normal in about 2 wk.
- Rabbits: The animals exhibit ocular and nasal irritation, hyperactivity, dyspnea, and convulsions.
- Dogs: Hyperactivity, jumping, and barking are the first signs; they are followed by salivation, retching, and vomiting. The dogs become atactic and, after removal from the chamber, hypoactive. Gagging and vomiting persist for 24 h. Little food or water is consumed for a week, after which survivors return to normal.

- Monkeys: During exposure, salivation, rhinorrhea, dyspnea, vomiting, and ataxia are seen. After exposure, lethargy, coughing, and vomiting persist for 24-48 h.
- Goats: During exposure, the animals are hyperactive, shaking their heads, rearing up, frothing at the mouth, then becoming atactic. Survivors are hypoactive and gag and vomit for a week.
- Swine: During exposure, the animals have dyspnea, salivation, frothing at the mouth, and ataxia. Survivors have dyspnea, loss of weight, and dehydration.

McNamara et al.[4] reported LCt_{50}s in seven species of animals exposed to DM dispersed in 10% acetone solutions. The combined LCt_{50} was 12,305 mg·min/m^3 (range, 10,283-14,726) (Table 4-31).

Owens et al.[5] exposed monkeys, dogs, and guinea pigs on 10 consecutive days to DM aerosols from a No. 113 grenade. The mean daily exposures were at about the LCt_5 in one set of animals and at the LCt_{20} to LCt_{25} in another set. In both sets, the accumulated uptake should have killed all the animals if given in a single exposure. The low concentration killed five of eight monkeys. At the high concentration, slightly more monkeys and guinea pigs and fewer dogs than would have been expected died from a single exposure. There was little indication of cumulative toxicity. Exposures and mortality are in Table 4-32.

Punte et al.[7] summarized their observations on mice, rats, and guinea pigs and stated that animals killed (or dying) after exposure to DM aerosol at Ct's greater than 500 mg·min/m^3 showed hyperemia of the trachea, pulmonary congestion, edema, and pneumonia. At less than 500 mg·min/m^3, no pathologic changes were observed.

Striker et al.[12] conducted a study of the effects of DM dispersed from No. 113 grenades. Thirty Macaca mulatta monkeys in groups of 10 were exposed as follows:

Average DM Concentration, mg/m^3	Duration of Exposure, min	Ct, mg·min/m^3
855	3	2,565
1,708	5	8,540
2,615	11	28,765

The animals were killed and examined at 12 and 24 h and 3, 7, and 30 d. At the low exposure, one animal examined 12 h after exposure had superficial tracheitis, edema of the tracheal and bronchial mucosa, and beginning bronchorrhea. All others were free of lesions related

TABLE 4-31 Acute Inhalation Toxicity of DM Disseminated from a 10% Acetone Solution[a]

Species	Bliss-Calculated LCt_{50}, mg·min/m^3	Concentration, mg/m^3	Duration of Exposure, min	Mortality Fraction	Time to Death, h[b]
Monkey	40,000	296	135	6/6	28, 43, 149, 190(2),* 248
	25,085	214	117	6/6	43, 47, 67, 148, 235, 307
	20,800	219	95	4/6	42, 65, 238, 286
	16,720	209	80	3/6	192, 278, 350
	12,555	279	46	0/6	--
	5,040	297	20	0/6	--
Dog	16,720	209	110	6/6	10, 16, 17, 35 (3)
	12,555	279	45	4/6	18, 20, 42, 116
	9,060	206	44	5/6	63, 86, 278, 136, 356
	5,940	297	20	1/6	305
	2,960	212	14	0/6	--
Goat	41,600	210	198	6/6	4, 16(2), 72, 77, 113
	30,000	227	132	6/6	22(2), 71, 95, 240, 552
	19,640	216	91	4/6	18, 90, 198, ?
	9,800	233	42	3/6	20(2), 239
	5,062	230	22	0/6	--
Swine	61,000	223	273	3/6	5.5, 20, 167
	41,600	210	198	2/6	4, 335
	10,000	227	132	2/6	47(2)
	19,640	216	91	1/6	42
	9,900	206	48	0/6	--
Rat	61,000	223	273	20/20	4, 8, 20(4), 47 (5), 71, 95(2), 118(2), 124, 147(2), 168
	40,000	296	135	20/20	1(2), 47(2), 120(10), 190 (4), 216(2)
	25,085	214	117	18/20	29, 110(12), 134, 158, 211 (3)
	19,640	216	91	14/20	68(3), 140(3), 146, 148, 166 (6)
	16,720	209	80	1/20	11
	12,555	279	45	1/20	21
	5,940	297	20	0/20	--
Guinea pig	16,720	209	80	17/20	11(6), 17, 35 (7), 42, 64, 96
	12,555	279	45	19/20	19(14), 26(2), 528(2), 552
	5,940	297	20	11/20	16(8), 21(2), 40
	2,960	212	14	8/20	14, 16, 38(5), 70
	1,100	220	5	1/20	230
Rabbit	40,000	296	135	6/6	2.25(6)
	34,560	300	115	6/6	2(5), 24
	29,140	107	95	6/6	2(4), 2.5, 24
	20,900	279	75	6/6	1.5, 2, 24(3), 48
	11,070	246	45	4/6	1.2, 2, 24, 48
	8,050	268	30	4/6	24(2), 48, 72
	4,290	286	15	5/6	24, 72(2), 216, 240

[a] Data from McNamara et al.[4]
[b] Numbers in parentheses are numbers of deaths at stated time, if other than 1.

TABLE 4-32 Mortality in Animals Exposed for 20 Consecutive Days at at Dosages Below LCt50a

Species	Mortality after Single Exposure, %	Observed Mortality Fraction
Group 1--Average daily exposure, 11,609 mg·min/m^3:		
Monkey	3-13	5/8
Dog	1-6	1/8
Guinea pig	1.2-6	6/20
Group 2--Average daily exposure, 17,302 mg·min/m^3		
Monkey	16-46	8/8
Dog	8-28	2/8
Guinea pig	8-26	18/20

[a] Data from Owens, et al.[5]

to the exposure. At the middle exposure, all superficial signs of exposure disappeared in 24 h. Two monkeys examined at 12 h showed bronchorrhea, focal pulmonary edema, and pulmonary congestion. At 24 h, edema and congestion were more pronounced. One monkey had membranous tracheitis and focal areas of hemorrhage into the parenchyma of the lung. At 72 h, edema and congestion had largely cleared. At 7 d, one animal appeared healthy, and the other had areas of emphysema and atelectasis. After 30 d, one monkey showed emphysema and atelectasis, and the other had extensive, early pneumonia. At the high exposure, eight monkeys died within 24 h. One animal killed for examination at 24 h showed early bronchopnemonia, pulmonary edema, emphysema, ulceration of the tracheobronchial tree, and some visceral congestion. The last monkey died at 29 d with the same array of lesions. No abnormalities in serologic or hematologic values were found in any of these animals. In summary, the most important health problem resulting from exposure to DM was pulmonary edema. This seemed to reach a peak at 24 h. If the animals survived beyond that time, they had little residual damage.

Striker et al.[11] considered that, at high exposures, animals may have held their breath. They may also have diluted or washed out some of the agent by coughing, gagging, etc. Lower exposures were studied in which seven groups of five monkeys were

Concentration, mg/m^3	Duration of Exposure, min	Ct, mg·min/m^3
291	2	582
291	10	2,910
272	20	5,440
330	40	13,200
99	2	198
108	12	1,296
77	60	4,620

exposed to DM generated from an M6A1 grenade as follows:

At 582 mg·min/m^3, the first three pairs of monkeys showed only slight pulmonary congestion; at 1 wk and 30 d, congestion and edema were greater. At 2,910 mg·min/m^3, focal pulmonary edema and bronchorrhea were present at 12 h; the edema cleared by 24 h, but bronchorrhea and bronchitis persisted to 30 d. At 5,440 and 13,200 mg·min/m^3, lesions were similar, but increasingly severe. When the concentration of DM was reduced to about 100 mg/m^3, the external signs of intoxication were mild. At 198 mg·min/m^3, edema, congestion, and bronchorrhea were evident when the lungs were examined; at 72 h, the edema and congestion had cleared, and the bronchorrhea was likewise clear by 1 wk; these lesions persisted at 30 d. At 1,296 mg·min/m^3, the lesions were somewhat intensified, persisting up to 30 d. Surprisingly, in animals exposed to 4,620 mg·min/m^3, signs of labored breathing and edema were marked, but edema, congestion, and bronchorrhea, present up to 72 h, were not found at autopsy at 7 and 30 d. These results seem to bear out the original hypothesis of the authors that exposures to DM at fairly low Ct but for long periods produce relatively more severe effects than short exposures at high Ct.

Roberts et al.[8] examined the effects of DM on the gastrointestinal tract as a possible factor in poisoning. Using both intravenous and oral lethal doses in dogs, they monitored central venous pressure, right ventricular pressure, cortical electric activity, alveolar CO_2, respiratory rate, heart rate, electrocardiogram, and gastric activity. Both amplitude and rate of gastric activity were greatly increased by the introduction of DM, lasting for 15-20 min before decreasing to normal. Pretreatment with trimethobenzamide HCl, which prevents vomiting in dogs given peripherally or centrally acting emetics, did not counteract the DM-induced gastric activity, but chlorpromazine was effective, probably because of its atropinelike action. Blocking the nervous supply to the stomach did not cancel the DM effect. The authors concluded that DM affects the stomach directly and that the primary cause of death is its effect on the lungs, as seen in the experiments of Striker et al.

Owens et al.[5] tested the effects of DM suspensions in corn oil on rabbit eyes. DM was instilled in the eye in doses of 0.1, 0.2, 0.5, 1.0, and 5.0 mg. At 0.1 mg, there was no effect. Mild conjunctivitis was seen at 0.2 mg; at 0.5 mg, mild blepharitis was also seen. Corneal opacity persisting over the 14-d observation period resulted from 1.0 and 5.0 mg. Corn oil suspensions of DM (100 mg/ml) were applied to the clipped backs of rabbits in groups of six in doses of 1, 10, 50, 75, and 100 mg. At 10 mg and higher, necrosis of the skin was observed.

Rothberg[9] tested DM for skin sensitization in guinea pigs; the findings were negative.

TOXICITY IN HUMANS

Ballantyne[1] described the effects of human inhalation exposures to DM as beginning with acute pain in the nose and sinuses. Pain in the throat and chest follow, with sneezing and violent coughing. Next there is eye pain, lacrimation, blepharospasm, rhinorrhea, salivation, nausea, and vomiting. Recovery is usually complete 1-2 h after exposure. The onset of symptoms is, however, delayed for several minutes, in contrast with the onset of symptoms after exposure to CS and CN, which have very short latent periods--this permits the absorption of much more DM before a warning is perceived.

Owens et al.[51] estimated that threshold doses of DM for irritation of the throat, irritation of the lower respiratory tract, and initiation of the cough reflex are 0.38, 0.5, and 0.75 mg/m^3, respectively.

Incapacitating or riot-control agents are used to put persons out of action. DM is less effective in this regard than CS and CN. McNamara et al.[4] and Owens et al.[5] conjectured that there may be greater differences among persons in their susceptibility to DM than in their susceptibility to other agents. Although Owens et al. claimed that thousands of people were exposed to DM (no details given), few reports involved controlled tests.

Recent tests of the ICt_{50} for humans were probably more reliable than tests soon after World War I, but information is limited. McNamara et al.[4] summarized the results of two tests[3,6] by saying that "human exposures . . . indicated that men could tolerate concentrations of 22 to 92 mg/m^3 for 1 min or more"; "a concentration range of 22 to 220 mg/m^3 would appear to be intolerable to 50% of a population for 1 min"; and "these values are applicable to experimental situations."

In 1959, toxicity data for mice, guinea pigs, and dogs were combined to give an estimated LCt_{50} for humans of 14,000 mg·min/m^3 for a single exposure. More recent estimates for three means of dispersion,[4] based on seven animal species combined, are as follows:

Form of Dispersion	ICt_{50}, mg·min/m^3	ICt_{50}, mg·min/m^3	Safety Factor
Laboratory dispersions	11,000	22 or 220	50-500
M6A1 grenade	44,000	22 or 220	200-2,000
Commercial grenade	35,000	22 or 220	160-1,600

McNamara et al.[4] reported the accidental death of a man exposed for an uncertain time (5-30 min) to DM at a concentration estimated at 1,130-2,260 mg/m^3.

No investigations of long-term effects of DM on humans or animals appear to have been undertaken.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

Sixty-seven human subjects underwent experimental exposure to DM: 29 in 1958 and 38 in 1966-1968, of whom exposure dataare available on 23 and 31, respectively. Subjects were exposed in an aerosol chamber; they wore masks when they entered, and the masks were often removed after some intervals in the chamber.

The subjects tested in 1958 underwent one to five exposures on 1 or 2 d. Ct's, available for at least one exposure of 14 of the 1958 subjects, ranged from 7.1 to 100 mg·min/m^3; exposure times, available for eight subjects, ranged from 1 min to 4 min 28 s. Subjects tested in 1966-1968 underwent only one exposure each; Ct's and exposure times, available for 31 subjects, were 7.1-236 mg·min/m^3 and 45 s to 10 min, respectively.

Some subjects were transiently sickened by their exposures. Effects lasted up to several hours after removal from the agent. Predominant symptoms were related to respiratory tract irritation: burning sensations of the respiratory passages, choking sensations, dysphonia, dyspnea, coughing, and sneezing. Nausea was common. Other, less frequent effects were retching, anorexia, headache, dizziness, lacrimation, salivation, and urinary frequency. Objective signs of DM exposure were infrequent and included rhinorrhea, wheezing, conjunctivitis, and diaphoresis.

Laboratory results from 7 d after exposure are available for 31 of the 1966-1968 subjects. No abnormalities in routine

clinical blood differential counts and urine analyses not seen before exposure were seen 7 d after.

Although DM has greater acute toxicity to the respiratory tract than CS and CN, Edgewood subjects appeared to recover shortly after exposure. Given the available information on DM and the short low-dose exposures, it is impossible to predict whether Edgewood subjects exposed to DM will suffer any long-term effects of the exposure.

SUMMARY

DM is a moderately toxic riot-control agent that produces symptoms of slightly delayed onset and a relatively long recovery period. Because they cause symptoms of more rapid onset and recovery and are less toxic, CS and CN may be said to be more effective riot-control agents and to make DM obsolete as a riot-control agent.

Castro[2] found DM and CS to be active inhibitors of cholinesterase and suggested that this characteristic might explain their lacrimatory effect. Roberts et al.[8] demonstrated a direct effect of DM on gastric activity and, like Striker et al.,[11,12] found solid evidence that its lethal effects are of respiratory origin.

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BROMBENZYL CYANIDE

CHARACTERISTICS

Brombenzyl cyanide (CA)--also called α-bromobenzyl cyanide, α-bromotolunitrile, bromobenzylnitrile, and α-bromophenylacetonitrile--was the most powerful lacrimator used in World War I (see Table 4-1). It was introduced by the French army and adopted as the standard U.S. lacrimator in 1918. It was temporarily abandoned because of its reactions with metal and instability in storage.[3,10] Interest in it revived when a need arose for a chemical that was more persistent than CS and CN in enclosed areas, such as rooms and earthen tunnels.[1] In this connection, vapors of CA from ground contamination are lacrimatory for 15-30 d.[6]

CA is a yellow-white solid, with a molecular weight of 195.97, a melting point of 25°C, a boiling point of 225°C[10] and the odor of sour fruit. Sim described its activity as immediate in onset and causing eye and respiratory irritation. Rapid recovery (2-10 min) from its effects is produced by moving to fresh air. It is low in skin irritancy.93

Witten[10] reported that tolerance to CA develops more easily than tolerance to CS. In an atmosphere containing CA at 0.44 mg/m^3, subjects lacrimate freely in 30 s. While still in the CA atmosphere, they stop lacrimating in 7-15 min. Exposure at a Ct of 5 mg·min/m^3 is incapacitating,[7] but Oberst et al. reported that men exposed in a chamber tolerated 30 mg/m^3 for 10 min.[5]

A comparison of some other characteristics of CA, CN, and chloropicrin (PS) follows:[6]

Substance	Volatility at 20°C, mg/m^3	Threshold Lacrimatory Concentration, mg/m^3
CA	130	0.15
CN	106	10.30
PS	165,000	2.00

TOXICITY IN ANIMALS

Oberst et al. [3] questioned the reliability of LCt_{50}s for CA in early reports. They then tested eight species of animals at "high" concentrations (105-168 mg/m^3 for 12-120 min) in an exposure chamber, using CA of 90% purity. Table 4-33 shows the concen trations, exposure times, mortality fractions, and times to death, and Table 4-34 summarizes the LCt_{50}s.

TABLE 4-33 Mortality of Animals Exposed to CA Vapor at High Concentrations (10-day Observation)a

Species	Ct, mg·min/m^3	Concentration, mg/m^3	Duration of Exposure, min	Mortality Fraction	Time to Death, h[b]
Mouse	6,120	136	45	1/10	168
	8,400	140	60	5/10	144(5)
	10,500	140	75	10/10	16.5, 40.5, 64.9 (3), 88.5(3), 136.5, 216
Rat	5,850	130	45	0/8	--
	6,120	136	45	0/6	--
8,040	134	60	0/7	--	
8,400	140	60	0/6	--	
10,500	140	75	0/6	--	
11,880	132	90	1/6	96	
15,960	133	120	4/7	24(2) 120(2)	
17,850	119	150	2/8	22, 120	
21,420	119	180	5/8	17(5)	
Guinea pig	5,850	130	45	1/8	120
	6,120	136	45	1/6	114.5
8,040	134	60	0/7	--	
8,400	140	60	2/5	151(2)	
10,500	140	75	4/6	64, 136(2), 161.5	
11,880	132	90	5/6	1, 40, 88(2), 94	
15,960	133	120	6/7	41.5, 137.5(5)	
17,850	119	150	6/8	18.8, 118.8(4), 138.8	
21,420	119	180	8/8	3(2), 15.8(3), 111.8(3)	
Rabbit	5,850	130	45	1/8	368
	8,040	134	60	4/7	20.6, 53.3, 140.9(2)
	10,500	140	75	4/6	140.9(2)
	10,500	140	75	4/6	20, 22.7, 88, 336
	11,880	132	90	6/6	16, 40, 64, 88(3)
	15,960	133	120	7/7	1, 2, 17.5, 41.5 (2), 45, 65.5
Monkey	8,400	140	60	0/3	--
	12,690	141	90	2/7	72, 144
	17,040	142	120	4/8	24, 72, 77, 78
	22,050	147	150	5/6	36(2), 71, 144, 312
Dog	8,400	140	60	0/2	--
	10,200	136	75	1/4	48
	13,320	148	90	6/8	23(2), 25, 36, 46, 120
	17,340	145	120	5/6	28, 36, 47(2), 168
Pig	1,548	129	12	0/6	--
	1,932	161	12	1/6	16.7
2,340	117	20	0/4	--	
3,150	105	30	1/6	21.9	
4,680	156	30	2/6	16.5(2)	
5,640	141	40	3/6	5.5(2), 20.9	
6,120	136	45	3/3	48, 72(2)	
10,500	140	75	3/3	1.3, 16(2)	
Goat	3,990	133	30	0/6	--
	6,160	154	40	0/6	--
	6,840	152	45	2/6	18.5, 120
	6,943	131	53	0/6	--
	10,080	168	60	5/6	16(2), 18.5, 23.5, 96

a Data from Oberst et a1.[3]
b Numbers in parentheses are numbers of deaths at stated time, if other than 1.

All species except rats and guinea pigs responded to CA vapor rapidly. All had lacrimation and salivation, with various kinds of eye-rubbing, blinking, licking, and vomiting. Goats were the least responsive. Dogs, rabbits, and monkeys seemed most susceptible to eye damage. Conjunctivitis, blepharospasm, and corneal ulcers were common. The rhesus monkey appeared most susceptible to cutaneous irritation; dogs and pigs seemed non-susceptible in this regard. Respiratory damage was common and appeared to be the cause of death in animals that did not survive. The data from all species were combined for an LCt_{50} value of 11,095 mg·min/m^3. This was considered a tentative value for humans.

Oberst et al.[4] also examined the toxicity of CA at lower concentrations. In this series of tests (at 8-29 mg/m^3 for up to 168 h), five species were used. Table 4-35 shows the mortality fractions; relatively few animals died. Dogs were most likely to die, possibly because, being mongrels, they were in poor health to begin with.

Signs of CA irritation were similar to those seen at high concentrations, but less severe. Corneal damage seen in monkeys and rabbits early during exposure disappeared while the animals were still in the test chambers. Survivors showed no apparent after-effects. No estimates of ICt_{50}s or LCt_{50}s were made from these data.

LCt_{50}s for CA disseminated from the M6 oil candle were obtained by Ballard et al.[1] These experiments were performed in generally the same way as those done by Oberst et al., but this method of agent dispersion produced both vapor and aerosol in widely varying proportions; the concentration of CA also varied widely (255-1,707 mg/m^3). The LCt50s for the four species used are therefore not reliable. A comparison of these values with those reported by Oberst et al.[3] suggests that combustion products from the candle may have been partly responsible for the deaths.

	LCt_{50}s, mg·min/m^3	
Species	Ballard et al.	Oberst et al.
Monkey	10,831	16,287
Dog	4,675	12,037
Rat	11,740	18,859
Guinea pig	4,785	10,214

Bezahler et al.[2] performed pathologic examinations on 207 animals of various species that died during or after the previously described exposures. Controls were not available. Lesions were not distinguishable from those produced by common laboratory animal diseases, and an arbitrary decision was made that CA was responsible for death if it occurred within 6 h of exposure. Laryngeal edema was commonly seen, and respiratory lesions were generally the cause of death.

A second arbitrary group was established for animals that died 6-24 h after exposure. Tracheobronchitis and bronchopneumonia appeared to be the causes of death, except that in half the rats and two-thirds of the guinea pigs the lesions were insufficient to explain their deaths. After 24 h, nearly all the animals that eventually died had severe respiratory lesions. In the absence of controls, the investigators were reluctant to say that CA caused the lesions found in survivors.

Tests of guinea pigs for skin sensitization potential with CA yield negative results; CN and CS were positive.[8]

TOXICITY IN HUMANS

Although the human LCt_{50} was arbitrarily set by combining values for eight animal species (Table 4-34), neither ICt_{50} for humans nor long-term effects in humans or animals have been reported.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

In 1966, 13 human subjects underwent experimental exposures to CA at Edgewood. The subjects wore masks when they entered an aerosol chamber and removed the masks after equilibration of the chamber CA concentration.

Each subject had only one CA exposure. The exposure Ct's ranged from 0.9 to 204 mg·min/m^3. Exposure times, available for 11 subjects, ranged from 50 s to 10 min.

Effects of CA exposure were transient. Ocular irritation, often accompanied by conjunctivitis, was predominant. Upper respiratory tract irritation with rhinorrhea also occurred.

Routine blood differential counts and urinalyses analyses obtained 7 d after CA exposure are available on 12 subjects. One subject had minimal leukocytosis (WBC, 12,800) that was not seen in his pre-exposure laboratory analyses.

Exposure was for a short term and at a low dose, however, given the absence of data on chronic effects it is not possible to estimate long term health effects of the CA exposure.

SUMMARY

CA, the most powerful lacrimator used in World War I, was discarded in favor of CN because of storage stability problems resulting from its reactivity with steel and iron containers. A resurgence of interest during the 1960s led to its use to clear persons out of tunnels in Vietnam. The LCt_{50} for humans has been estimated at 11,095 $mg \cdot min/m^3$ on the basis of animal data. CA is not a skin irritant or skin sensitizer in animals.

TABLE 4-34 CA Vapor LCt50s for Eight Animal Speciesa

Species	LCt_{50},[b] mg·min/m^3	95% Confidence Limits
Mouse	7,968	7,100 - 8,942
Rat	18,859	15,151 -23,474
Guinea pig	10,214	8,580 -12,160
Rabbit	8,021	6,714 - 9,582
Monkey	16,287	13,132 -20,201
Dog	12,037	9,161 -15,815
Pig	4,852	3,580 - 6,576
Goat	8,401	7,043 -10,020
All species	11,095	9,661 -12,741

Monkeys--Monkeys usually were blinking and rubbing their eyes within 1 min. They kept their eyes partially closed most of the time. As early as 26 min, most monkeys appeared to be distressed; they were salivating and breathing through their mouths. In 80 min, some were gasping and making choking sounds.

Dogs--Immediately after the dogs were placed in the chamber, they began to blink and lick their mouths. Occasionally, choking was observed at 30 s. They were salivating within 2.5 min. Rhinorrhea, partially closed eyes, and vigorous scratching at the window to escape usually were noted in 5 min. By 28 min, salivation was profuse, and headshaking, retching, and corneal opacity were present. Vomiting and phonation occurred in 1 h, dyspnea and coughing in 2 h.

Pigs--Usually, pigs were active, salivating, blinking, and shaking their heads in 30 s. Difficult breathing (mouth breathing) was observed in 15 min. At approximately 18 min, they fought, squealed, and either closed or partially closed their eyes. Occasionally, vomiting was observed in 30 min.

[a] Data from Oberst et al.[3]

[b] Concentration, 105-168 mg/m^3.

TABLE 4-35 Mortality of Animals Exposed to CA at Low Concentrationsa

Run No.	Avg. Concentration, mg/m^3	Duration of Exposure, h	Avg. Ct,[b] $mg \cdot min/m^3$	Mortality Fraction							
				Mouse	Rat	Guinea Pig	Rabbit	Monkey	Dog	Guinea Pig	Goat
27	10.1 (6.9 - 13.1)	12	7,272	0/10	0/6	0/6	0/6	2/6	5/6	0/6	0/6
36	8.1 (7.1 - 9.5)	8	3,888	--	--	--	--	--	0/6	--	--
36	8.5 (7.7 - 10.6)	12	6,120	--	--	--	--	--	2/3	--	--
46	8.4 (7.1 - 10.6)	18	9,072	0/9	0/6	0/6	1/6	0/6	1/3	0/6	1/4
41	11.7 (9.7 - 13.9)	9	6,318	--	--	--	--	--	2/6	--	--
41	11.3 (9.7 - 13.9)	12	8,136	--	--	--	--	--	0/3	--	--
43	15.4(12.9 - 18.0)	24	22,230	4/10	0/6	0/60	0/6	0/6	5/6	0/6	0/6
44	12.7 (7.7 - 20.6)	24[c]	18,288	--	--	--	--	--	1/1	--	--
44	12.6 (7.7 - 18.2)	34	25,826	--	--	--	--	--	2/6	--	--
44	14.0 (7.7 - 20.6)	40	33,686	0/10	0/6	0/6	1/6	0/6	--	0/6	0/6
45	12.7 (9.0 - 18.9)	44.5	40,320	--	--	--	--	--	2/2[d]	--	--
45	13.6 (9.0 - 18.9)	54.5	45,690	--	--	--	--	--	1/1[d]	--	--
45	17.3 (9.0 - 18.9)	90	83,331	--	--	--	--	--	1/1[d]	--	--
45	11.0 (9.0 - 18.9)	100	86,538	--	--	--	--	--	1/1[d]	--	--
45[e]	12.3 (4.9 - 18.9)	168	124,108	0/10	0/6	1/6	1/6	0/6	0/1	0/6	0/6

[a] Data from Oberst et al.[4]
[b] Cumulated each time concentration of a sample was determined.
[c] Estimated.
[d] Dogs died during exposure.
[e] Except for dogs, two animals of each species were necropsied immediately after exposure, two animals after 1 wk, and two animals after 4 wk (one rabbit and one guinea pig at 4 wk).

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CHLOROPICRIN

CHARACTERISTICS

Chloropicrin (PS)--trichloronitromethane, CCl_3NO_2, Green Cross, Klop--is a colorless, volatile, slightly oily liquid with an intense odor (see Table 4-1). It is very slightly soluble in water, soluble in ether, and miscible with benzene, absolute alcohol, and carbon disulfide.[10,11] It boils at about 112°C.

Used extensively as a harassing agent in World War I, PS is a lacrimator and a lung irritant with a tendency to cause nausea and vomiting. In acute toxicity, it is intermediate between chlorine and phosgene;[10] unlike phosgene, it has no latent period. During Oorld War I, it was known in Germany as Green Cross, because artilOery shells were so marked,[2] and Klop, because it was made at the Klopper Werke at Breloh in the Lüneberg Heath. Büscher[2] described PS as a fairly volatile liquid, resisting decomposition by water; it was often combined in a ratio of 35:65 with diphosgene as a charge for shells. Sim[9] said that it was no longer of interest as a military chemical.

PS is used as an insecticide and sterilizing agent, mainly for cereal grain in storage or in ships' holds. As a soil fumigant, it is used to control nematodes, microorganisms, and weed seeds. Treated soil must be left unplanted for about 2 wk, because PS damages seeds. It is also a warning agent in fumigants, such as methyl bromide, because of its strong odor.[15,16] At 1 ppm in air, it causes lacrimation and smarting pain.[3]

TOXICITY IN ANIMALS

Toxicity data on PS are scanty. Short-term studies have been extremely limited, and long-term studies are lacking.[15,16]

The 1980 Registry of Toxic Effects of Chemical Substances[6] listed the following animal-toxicity data:

- Oral: rat LD_{50}, 250 mg/kg.
- Intraperitoneal: mouse LD_{50}, 25 mg/kg.
- Inhalation: mouse LC_{50}, 1,600 mg/m^3 for 10 min.
- Inhalation: cat LC_{Lo}, 800 mg/m^3 for 20 min.
- Inhalation: rabbit LC_{Lo}, 800 mg/m^3 for 20 min.
- Inhalation: guinea pig LC_{Lo}, 800 mg/m^3 for 20 min.

Silver et al.[8] reported a median lethal concentration of 1,500 mg/m^3, by inhalation, for 10-min exposure of mice. Because the 11 separate runs of 20 mice varied widely in their LC values 10 d after exposure, Silver et al. noted that physiologic variations were caused by differences in age, nutrition, etc.

The United States Testing Co. performed tests to measure the toxicity of PS administered by inhalation, by mouth, and cutaneously.[4] Conditions and results were as follows:

- Inhalation toxicity: The LC_{50} was the concentration that would kill 50% of the population within 14 d when administered by continuous inhalation (over 90% of particles under 10 um) for 1 h. Five male and five female Sherman adult rats were used at each dose. LC_{50}: 25.5 ppm.
- Oral toxicity: Animals used were as above. The LD_{50}0was the dose that would kill 50% of the animals within 14 d when given orally by intubation. LD_{50}: 37.5 mg/kg.
- Skin absorption: The LD_{50} was the dose that would kill 50% of the test animals within 14 d after continuous contact with the skin of rabbits for 24 h. No data on numbers of animals were given. LD_{50}: 100.0 mg/kg.
- Skin corrosion: The dose sought was the dose that would cause visible destruction or irreversible alteration in skin tissue, such as ulceration or necrosis, after 4 h of contact with the skin of rabbits. Six albino rabbits with clipped backs were used for each dose. Corrosive dose: 0.5 ml (the only dose listed).

TOXICITY IN HUMANS

PS may enter the body through the skin, respiratory tract, or gastrointestinal tract. It has a pungent odor and its effects are evident several hours after exposure. The acute effects of PS include burning eye discomfort, lacrimation, headache, photophobia, burning sensation in the nose and throat, coughing, nausea and vomiting with colicky abdominal pain, diarrhea, and occasionally pulmonary edema.[5] The concentration necessary to cause fatal pulmonary edema is 2.4 g/m^3, and methemoglobinemia has been associated with PS intoxication/[15].

Although PS has lost status as a chemical-warfare agent, its toxic potential makes it an industrial and agricultural hazard. The Occupational Health Guideline[14] described it as a severe

irritant of the eyes, skin, and respiratory tract. Exposure of humans at 119 ppm for 30 min results in pulmonary edema and death. The Guideline gave an extreme tolerance time of 1 min at 15 ppm, but added that a few seconds at 4 ppm is disabling. Concentrations of 0.3-0.37 ppm result in painful eye irritation in 3-30 s. The permissible concentration in the workplace is 0.1 ppm (0.7 mg/m^3), averaged over an 8-h day.[14] The Guideline stated that, even though PS is not a skin sensitizer, persons exposed to it once show reduced tolerance to other toxic effects.

A Center for Disease Control publication[12] described the effects of a PS-containing mixture of methyl bromide used to fumigate a 15-acre field near suburban housing. Although the field was covered with plastic sheeting after fumigation, 35 (26%) of 133 nearby residents developed eye irritation, nausea, coughing, vomiting, or other typical symptoms of exposure to PS a few hours later.

A similar public-health problem was reported from Japan by Okada et al.[7] PS was used as a fumigant for leaf tobacco. Large numbers of farm workers reported symptoms. Of 760 workers and nearby residents surveyed, five required medical attention and 72% reported one or more symptoms. The five workers, evaluated more extensively, experienced lacrimation, vertigo, headache, fatigue, and orthostatic hypotension for several hours after exposure to PS. The other persons surveyed had lacrimation, headache, coughing, nausea, and anorexia for 1-3 d after exposure; and 30 had symptoms for over 1 mo after exposure. Age and symptoms were positively correlated.

MUTAGENICITY

Like many other irritants, PS has not been tested thoroughly for mutagenicity. However, in one early study, Auerbach[1] tested its capacity to induce sex-linked recessive lethal mutations in the fruit fly, Drosophila melanogaster. In all broods of flies derived from PS-treated males, the mutation frequencies were consistent with those of untreated laboratory stocks. A total of 4,454 chromosomes were tested, and the mutation frequency was 0.2%. In contrast, flies treated with mustard gas had a frequency of 5.2%.

The sex-linked recessive-lethal test in Drosophila continues to be highly regarded in modern genetic toxicology. In current mutagenicity testing, however, it is generally recommended that tests be conducted in more than one experimental organism and that more than one genetic end point be studied.

Because we have found no evidence in the scientific literature that PS has been studied in other mutagenicity tests, some uncertainty remains in categorizing it as nonmutagenic.

CARCINOGENICITY

The National Cancer Institute[13] studied the carcinogenicity of PS. Osborne Mendel rats and B6C3F1 mice were given PS in corn oil by gavage. Rats of both sexes were initially given 46 and 23 mg/kg per day, but the dosage for males was increased to 56 and 28 mg/kg per day at week 5, because males appeared to tolerate the agent better than females. Some early deaths caused periodic suspensions of the dosing, so the overall pattern was intermittent. Dosing was terminated at 78 wk, but observation continued for an additional 32 wk. Mice were given 50 and 25 mg/kg per day. At week 14, the dosage was increased to 70 and 35 mg/kg per day for a total of 78 wk. Controls were treated with corn oil. Animals were necropsied, and slides prepared from 29 different tissues. Survival curves for rats and mice are shown in Figure 4-4 and Figure 4-5.

No statistically significant differences in tumor incidence were found in rats or mice. It was concluded that, because of high early mortality, too few rats lived long enough to develop late-appearing tumors.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

From 1955 to 1971, 136 subjects underwent one or two experimental exposures to PS in equipment tests. The subjects remained in a test chamber for up to 4 h with gas masks in place and sometimes simulated battlefield functions.

Exposure records are available on 46 subjects exposed to PS vapor in 1955 and 12 subjects exposed in 1967. No dosages are listed. Data are not available on 17 subjects exposed to PS in 1955, 23 in 1956, nine in 1960, 11 in 1965, nine in 1967, three in 1969, and six in 1971.

Apparently, there was minimal gas-mask leakage during the experiments involving the 58 subjects on whom there are records, and the PS had no acute effects on these subjects. No laboratory analyses are recorded.

Some experimental information on PS indicates a possibility of skin absorption. During the Edgewood experiments, effective masks seemed to prevent acute inhalation toxicity, but subjects exposed to PS vapor for 4 h might have absorbed small amounts through their skin.

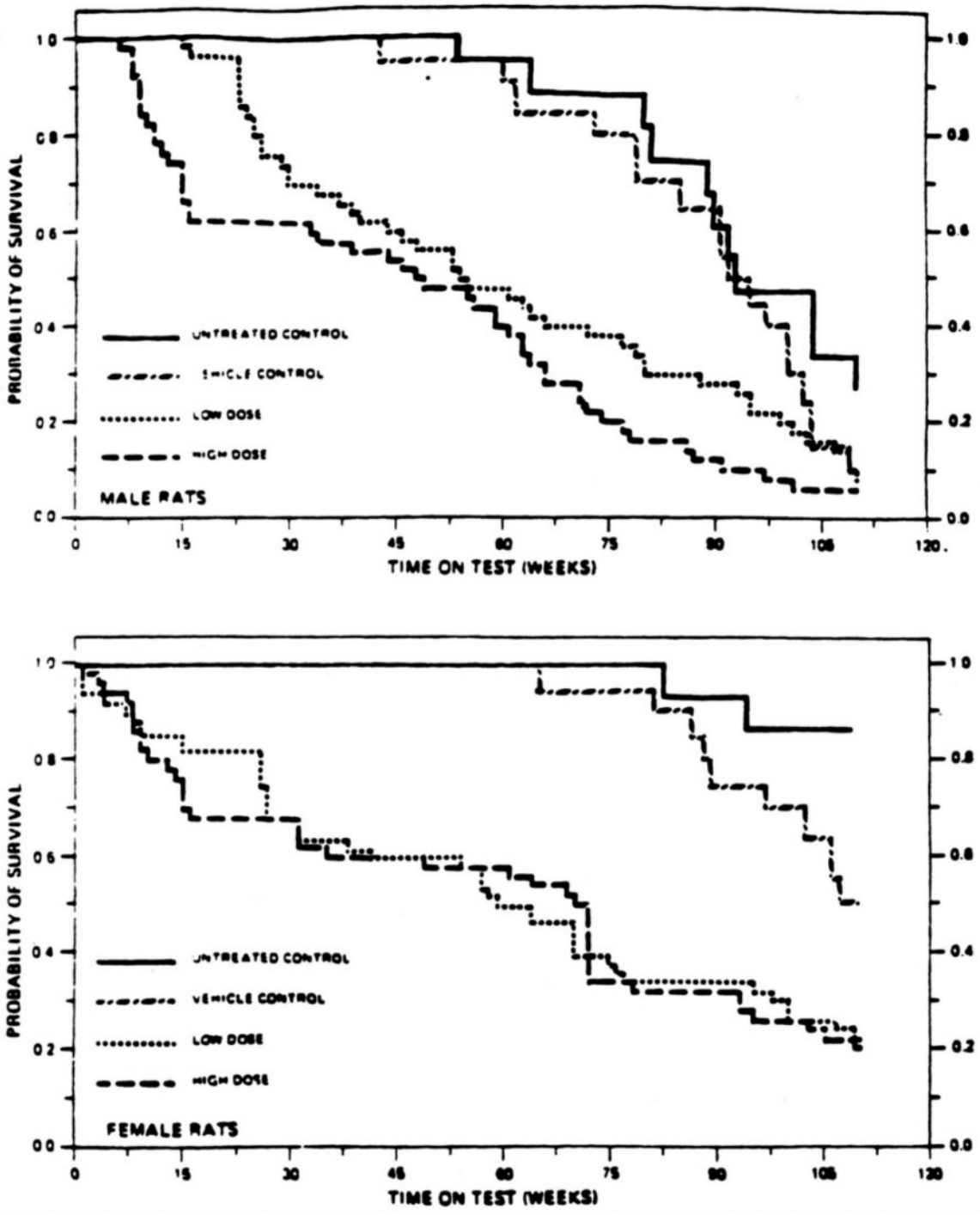

FIGURE 4-4 Survival of rats exposed to PS. Data from U.S. National Cancer Institute.[13]

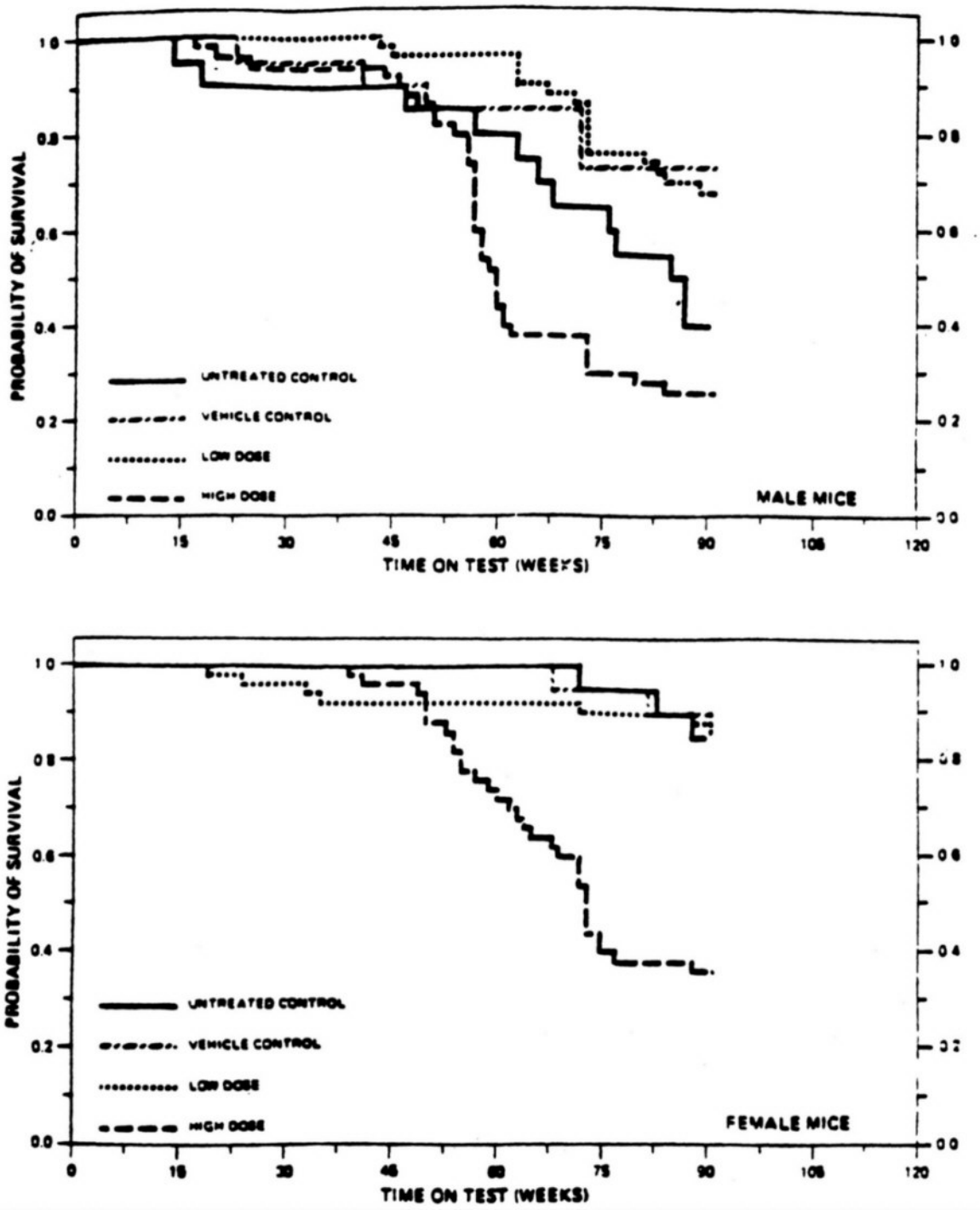

FIGURE 4-5 Survival of mice exposed to PS. Data from U.S. National Cancer Institute.[13]

PS is acutely quite toxic to humans, but little is known of its long-term health effects.

DISCUSSION

Despite widespread use as an insecticide, nematocide, and intermediate in chemical processes, the toxic potential of PS has not been fully evaluated. Absence of lethal effects may well be explained by its powerful odor, which alerts workers to its presence in contaminated areas. Animal-toxicity data suffer from variations in the methods used. One long-term study of carcinogenicity in rats was unsatisfactory because of high early mortality.

SUMMARY

PS is acutely toxic, with a variety of effects on animals. PS has not been tested thoroughly for mutagenicity. A test for sex-linked recessive lethal mutations in Drosophila was negative. However, because we have found no evidence that PS has been studied in other mutagenicity tests, uncertainty remains in categorizing it as nonmutagenic. A single carcinogenicity test in rats and mice yielded no evidence of carcinogenicity, but the test was flawed by the occurrence of deaths among experimental animals.

Because of its widespread use in agriculture, transportation, and industrial chemistry, further studies of its chronic toxicity and long-term effects are needed.

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NONANOYL MORPHOLIDE

CHARACTERISTICS

Nonanoyl morpholide--pelargonic morpholide or EA 1778--is a fatty acid amide with a molecular weight of 227 and a boiling point of 120-130°C at 0.5 mm Hg (see Table 4-1). Classed as a pungent, because of its pungent odor, it is chemically related to alkaloids found in black pepper.[1,3,4] Its synthesis has been described by Rice et al.[3]

TOXICITY IN ANIMALS

The available data on this compound are sparse. Two reports were obtained, one on animal, the other on human exposures.

Punte et al.[1] compared the toxicities of CN, DM, and nonanoyl morpholide in rabbits, rats, mice, and guinea pigs. Results are given below.

- Intravenous toxicity: The acute LD_{50} in rabbits is about 21 mg/kg, making it comparable with CN and somewhat less toxic than DM by this route (Table 4-36).
- Ocular toxicity: Instillation of 0.5 or 1.0 mg into the rabbit conjunctival sac produced mild to moderate conjunctivitis that lasted a week. If the eyes were washed out with water a few minutes after dosing, the conjunctivitis lasted 24 h.
- Inhalation toxicity: Aerosol exposure (mass median diameter, 1.8 μm) produced similar toxic signs in rats, mice, and guinea pigs. A brief period of hyperactivity was followed by salivation and lacrimation. Lethargy and labored breathing began after 5-15 min and lasted 1-2 h. Other toxic signs subsided within minutes of removal to fresh air. Table 4-37 presents data on inhalation toxicity, and Table 4-38 compares nonanoyl morpholide with CN and DM for lethal effects.

The animals that died or were sacrificed for autopsy examination after exposure to nonanoyl morpholide showed hemorrhagic edema of the lungs. Their intestines showed hemorrhages, dilation, and degeneration of the villi and loss of epithelium in some areas.

Results on animals were used to estimate a maximal safe inhalation Ct of 500 mg·min/m^3 for human exposures.

TABLE 4-36 Intravenous Toxicity of CN, DM, and Nonanoyl Morpholide in Rabbitsa

Compound	No. Tested	LD_{50}, mg/kg
CN	20	20
DM	32	6
Nonanoyl morpholide	18	21

[a] Data from Punte et al.[1]

TABLE 4-37 Computed Inhalation LCt50s of CN, DM, and Nonanoyl Morpholide in Various Animal Speciesa

	Rats		Mice		Guinea Pigs	
Compound	No. Tested	LCt_{50},[b] mg·min m^3	No. Tested	LCt_{50},[b] mg·min/m^3	No. Tested	LCt_{50},[b] mg·min/m^3
CN	80	3.7	80	78.6	70	3.6
DM	24	3.7	42	22.4	56	7.9
Nonanoyl morpholide	60	58	60	130[c]	60	19.0

[a] Data from Punte et al.[1]
[b] Multiply values by 1,000 to obtain LCt_{50}.
[c] Estimated by extension of dosage-mortality curve.

TABLE 4-38 Computed Inhalation LD50s for CN, DM, and Nonanoyl Morpholidea

	LD_{50} mg/kg[b]		
Compound	Rats	Mice	Guinea Pigs
CN	14.1	58.8	1.1
DM	14.1	17.9	2.4
Nonanoyl morpholide	23.2	104	5.7

[a] Data from Punte et al.[1]
[b] Based on minute volume, retention, and body weight.

TOXICITY IN HUMANS

Using results on animals as a basis, Punte et al.[2] exposed healthy subjects to nonanoyl morpholide at several concentrations. Response times are shown in Figure 4-6. Table 4-39 compares ECt_{50}s of nonanoyl morpoholide, DM, and CN. The human responses to exposure sometimes provoked comments that compared nonanoyl morpholide with pepper. The symptoms after 3-min exposure at a Ct of 20-40 mg·min/m^3 were lacrimation, coughing, and a burning sensation of the nose, throat, and eyes. Nausea sometimes followed, but only if the subject had recently eaten a large meal. Symptoms were relieved in 10-15 min by exposure to fresh air.[4]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

In 1958, 32 subjects underwent experimental exposure to nonanoyl morpholide in an aerosol chamber at Edgewood. At least some subjects wore masks during exposure. Exposure data are available on 30 subjects. Subjects had one to four exposures on 1 or 2 d. Exposure durations, available for 15 subjects, ranged from 10 s to 8 min. No Ct's are avilable.

The effects of nonanoyl morpholide exposure on the subjects were generally transient. The principal effects were due to respiratory tract irritation and included rhinorrhea, cough, substernal pain, and dyspnea. Nausea was also common, and vomiting occurred if the subject had eaten before the test. Headache sometimes occurred 1 h after exposure; in one subject, headache lasted for a week. Lacrimation, sometimes associated with conjunctivitis, occurred less frequently than other effects. No laboratory analyses are available.

DISCUSSION

Although they are not extensive, the data of Punte et al.[1,2] suggest that nonanoyl morpholide is less toxic than CN or DM. Nonanoyl morpholide appears more irritating than CN or DM, but may have less persistent effects. No definitive information is available on the possibility of long-term effects of exposure to nonanoyl morpholide. However, given the available information on nonanoyl morpholide and the short-term low-dose exposures of Edgewood subjects to it, the Committee believes that long-term health effects on the subjects are unlikely.

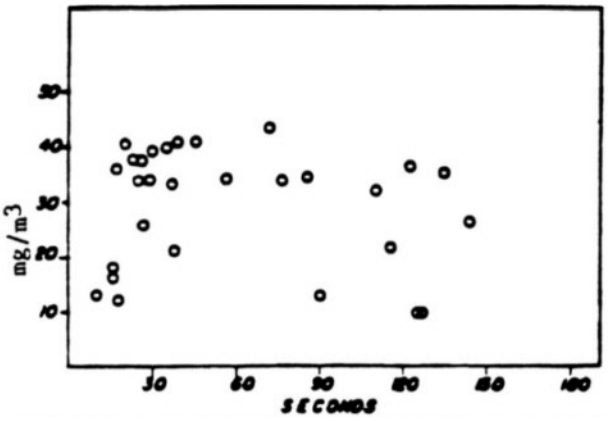

FIGURE 4-6 Response (tolerance time) of 26 subjects exposed to nonanoyl morpholide for 180 s. Reprinted with permission from Punte et al.[2]

TABLE 4-39 Estimated Airborne Ct Needed to Produce Response (Acute Irritation) in 50% of Subjects for Various Exposure Times to CN, DM, and Nonanoyl Morpholidea

Compound	1-Min ECt_{50}, mg·min/m^3	2-Min ECt_{50}, mg·min/m^3	3-Min ECt_{50}, mg·min/m^3
CN	213[b]	119	93
DM	[c]	38[b]	19
Nonanoyl morpholoide	39	29	21

[a] Data from Punte et al.[2]
[b] Estimated on basis of limited data.
[c] Too few responses to make estimate.

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1-METHOXY-1,3,5-CYCLOHEPTATRIENE

CHARACTERISTICS

Riot-control agents--such as CS, CN, and CR--are solids. Edgewood scientists[9] felt that dissemination and decontamination would be simpler with a volatile liquid agent. Research led to the production and testing of 1-methoxy-1,3,5-cycloheptatriene (CHT, EA 4923), a liquid substance of high volatility (Table 4-40) with physiologic effects typical of riot-control agents (e.g., lacrimation, skin irritation, and mucous membrane irritation).

CHT, known as tropilidene in England,[2] is pale yellow, with a molecular weight of 92, a boiling point of 117°C at 749 mm Hg, and a freezing point of -79.5°C. It is unstable when exposed to air or light. Early Edgewood samples were difficult to produce in a pure state, ranging from 78 to 89%, with EA 4922 (a relatively inert isomer) forming most of the remainder.

CHT is less irritating than CS and CR to humans. It is roughly as toxic as CR and less toxic than CS by inhalation in animals, and it has the capacity to penetrate skin or rubber.[9]

TOXICITY IN ANIMALS

Brown et al.[2] evaluated the safe handling of CHT, using only four males and four females per test. They used 96% pure CHT. In gavage studies, the LD_{50} of CHT in rats and mice was 57 and 171 mg/kg, respectively. Clonic convulsions suggested CNS involvement as a mechanism of toxic effects. Undiluted CHT applied to clipped back skin of rats under an impermeable bandage for 24 h produced an LD_{50} of 442-884 mg/kg. When 1 ml of CHT was applied to a 2 x 2-in. patch that was then placed on the clipped back skin of rabbits under an impermeable cover, and this procedure was repeated daily for 3 d for 6 h at a time, severe damage was caused, with necrosis and ulceration. Application of 1 ml of CHT to the clipped, uncovered backs of two rabbits and 10 guinea pigs daily for 23 d, produced gross skin irritation, with spongiosis, acanthosis, and necrosis. Attempts to sensitize guinea pigs yielded negative results. Undiluted CHT applied to rabbit eyes caused severe conjunctivitis that cleared in 24 h; there was swelling of lids, but no corneal involvement.

Early studies at Edgewood were conducted on dogs.[9] Severe neurologic damage was observed in one animal dosed with degraded agent (a dark mixture with a nonvolatile component). This observation led to further studies, including the use of CHT stabilized with antioxidants. Percutaneous toxicity was tested by applying CHT to the backs of clipped dogs and covering the area with a Saran plastic sheet held in place with a cloth jacket. Jackets were removed after 24 h, and the dogs' skins were then decontaminated. Results (Table 4-41) showed that ataxia, tremors, and death can be produced by these procedures. Other tests involved topical applications of CHT at 0.5 and 1.0 ml/kg (86.7 mol% pure) on two sets of dogs; stabilized and non-stabilized material was used. Eight dogs (two per test) were tethered outdoors and exposed uncovered for 4 h at 75°F. A second group of eight dogs was similarly tested at 100°F. No dogs died, but one showed transitory mild tremors after application of the stabilized agent at 1.0 ml/kg. The percutaneous LD_{50} in dogs (occluded skin) was estimated at about 500 mg/kg. Results were similar for stabilized and nonstabilized CHT. Ocular instillation of 5 1 (2 d), 10 1 (1 d) and 50 1 (4 d) of freshly distilled, about 90% pure agent caused mild erythema, inflammation, and iritis in rabbits, which cleared in 4-6 d. Other samples and degraded fractions given in single instillations of 10-50 1 produced varied but increased eye injury, including permanent corneal opacity, and in some cases injury to skin.[9]

The combined effects of inhaled and topically applied CHT were tested on eight dogs on which stabilized and nonstabilized CHT was applied at 1.0 and 0.5 ml/kg.[4] The dogs were placed in an unventilated 441-ft^3 room whose atmosphere was saturated with CHT and exposed twice, once for 1.5 h and a second time for 6 h. Effects of

1.5-h exposures were long-lasting, but not lethal. Deaths occurred from the 6-h exposures, however (Table 4-42). The effects of inhaled CHT were studied in dogs by allowing CHT equivalent to 0.5 and 1.0 ml/kg to evaporate from absorbent cotton in a closed cubicle. No ill effects were seen after exposure for 1.5 h. The effectiveness of decontamination procedures with bleach or soap solution was tested (Table 4-43).

McNamara et al.[7] used several species of animals and several routes of administration. Various samples of CHT were used, both controlled (relatively pure and stabilized) and uncontrolled (containing degradation products). They stated that toxic signs associated with controlled and uncontrolled samples were often the same. The intravenous LD_{50}s of neat agent (uncontrolled samples) were 99, 88, and about 20 mg/kg in the mouse, rabbit, and dog, respectively. The percutaneous (covered-patch) LD_{50}s in rabbits varied from 480 to 1,000 mg/kg for different samples. The acute inhalation LCt_{50}s (controlled samples) were 184,000, 176,000, and 63,000 in the rat, guinea pig, and dog, respectively. Death usually occurred within 24 h of exposure to lethal doses. Neuromuscular weakness persisted for several months in some dogs after the cutaneous (covered) application of CHT at 500 mg/kg or intravenous administration at 10 mg/kg. Persistent weakness was seen in rabbits after cutaneous exposure. Some samples of CHT produced corneal lesions in rabbits. Necropsies showed no lesions after CHT was given intraperitoneally, intratracheally, ocularly, or cutaneously. Pulmonary lesions were found after intravenous administration to dogs and after inhalation by dogs, rats, and guinea pigs. In surviving dogs and guinea pigs, but not always in rats, the lesions were reversible.

Biskup et al.,[1] also at Edgewood, compared the parenteral toxicities of six agents, including CHT. LD_{50}s in the mouse, rat, and rabbit are shown in Table 4-44.

Toxicity studies were conducted at Stanford Research Institute (SRI) with CHT under contracts with Edgewood. The first part of the SRI work was concerned with the pulmonary and neurologic lesions caused by CHT in dogs and monkeys. Monkeys tolerated high concentrations of CHT for short times and low concentrations for periods up to 100 min without adverse, irreversible effects. Ct's of 985-105,100 mg·min/m^3 were tested. No significant changes in blood pressure, heart rate, or respiration rate were observed. A comparison with CS indicated that CS can produce lung lesions at a Ct of 47,000 mg·min/m^3 in dogs and 20,000 mg·min/m^3 in monkeys. These are much lower than Ct's of CHT that produced no significant lesions. Neurologic lesions were produced by intravenous administration of CHT in dogs, but not in monkeys. This confirms the early results at Edgewood and suggests that dogs may be peculiarly susceptible to this type of treatment.[4] ^{14}C-labeled CHT was used to

study distribution and excretion. No indication of retention in any organ was observed.[4] A summary of percutaneous toxicity of CHT in dogs with occluded dressings is in Table 4-45.[3] In a later study, the inhalation of CHT in goats was studied at SRI. Eighteen goats were tested: six were controls, six were given a low dose (Ct, 3,000 mg·min/m^3), and six were given a high dose (30,000 mg·min/m^3). Three of the six goats in the high-dose test died before the exposure ended, so useful data were not obtained. All exposures, however, lowered the white-cell count and the hematocrit. No change in blood-gas contents was seen. No significant changes in biochemical or pathologic factors were found.[10]

TOXICITY IN HUMANS

During the chemical synthetic work that led to the development of CHT, several men were exposed to its vapors. The resulting lacrimation and irritation of the mucous membranes passed quickly with no after-effects. The compound was therefore judged to be a potent irritant, but relatively harmless.[9] Because of these observations, human volunteers and animals were tested simultaneously at Edgewood.

Fourteen men were exposed to various concentrations of CHT to establish an ICt_{50}. Nine of these men withstood exposures of 28-64 mg·min/m^3. These tests were terminated before an ICt_{50} could be established, because some animal tests suggested that the compound might be toxic at higher concentration.[7] Eventually, it was found that CHT had been degraded by exposure to air and light. Purified and stabilized agent was then prepared for further toxicity testing, but no further human tests were conducted at Edgewood. Accidental exposures reported by SRI involved ocular, cutaneous, and inhalation routes. Irritant effects developed at once and disappeared 15-30 min after removal of the agent. No after-effects were reported.[3]

MUTAGENICITY

CHT has not been tested thoroughly for mutagenicity. On the basis of unpublished studies conducted by Edgewood, it has been suggested that CHT is nonmutagenic in the Salmonella reverse-mutation test,[11] the micronucleus test in mice,[11] and a dominant-lethal test in rats.[6] Limitations in the available data base, however, make it impossible to reach clear conclusions regarding the genotoxicity of CHT.

The Salmonella reverse-mutation test reported for CHT does not seem to have been an adequate negative test. In strains TA1538 and TA100 with metabolic activation, the range of dosages was too

limited, and there was no suitable positive control for strain TA1537. Moreover, the data on strain TA98 could suggest a positive response, in that there was a fourfold increase in numbers of revertants per plate. If this increase were reproducible, one could conclude that CHT is positive, rather than negative. The available information on the size of the experiments, their reproducibility, and the variability of the data is also too limited to support the general conclusion that CHT is nonmutagenic in bacteria.

It was reported that CHT was nonmutagenic in a test for dominant lethal mutations in rats.[11] Treatment was by inhalation at concentrations of 100 and 4,000 μl/m^3; treatment involved single 20-min exposures or five 20-min exposures on consecutive days. Not having reviewed the dominant-lethal test data, we can neither accept nor refute the claim that CHT was negative in this test. However, a negative result in dominant-lethal tests is not generally regarded as strong evidence of lack of genotoxicity.[8]

CHT was tested for its capacity to cause an increase in the frequency of micronuclei in polychromatic erythrocytes in mouse bone marrow. Micronucleus formation is an indicator of chromosomal damage, and CHT had no apparent effect in the test. It should be noted, however, that the procedures used may not have conformed to current standards for a sensitive micronucleus test. For example, Heddle et al.[5] recommended examining at least 500 polychromatic erythrocytes from each of at least eight animals for every dosage and time for which results were analyzed; they also recommended that sampling be extended to at least 72 h after treatment and that the highest possible dosages (e.g., 80% of the 7-d LD_{50}) be used. If the test is conducted by a less sensitive procedure, some mutagens may not be detected. In the tests conducted at Edgewood, the highest dosage was about 25% of the LD_{50}. Three mice were treated per group by single intraperitoneal injection, and they were killed to determine results after only 24 h.

In our view, it is premature to reach a conclusion regarding the genetic toxicity of CHT. Although the mutagenicity tests conducted at Edgewood provided no evidence that CHT is mutagenic, they were not adequate to support the general conclusion that it is nonmutagenic. One can therefore make no statement about the possibility that CHT could pose a mutagenic hazard for man. For a thorough discussion of bases for determining the likelihood that a given chemical is mutagenic in humans, see the recent report of the National Research Council Committee on Chemical Environmental Mutagens.[8]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

In late 1969 and early 1970, 16 subjects each underwent one experimental exposure to CHT in an aerosol chamber at Edgewood. The duration of exposure among the 16 subjects ranged from 30 s to 5 min. Ct's ranged from 15.4 to 64 mg·min/m^3.

The effects of exposure on the subjects were transient, with complete resolution by 15 min after leaving the chamber. The predominant effects were lacrimatory, causing incapacitation due to eye closure and blurred vision lasting several minutes after exposure. Dermal irritation and rhinorrhea occurred. One subject had "chest congestion."

Laboratory analyses were performed 9 d after exposure to CHT. Two subjects had slight increases in SGOT after exposure (31.5 and 44.5 IU), representing slightly less than a doubling of their preexposure control values. SGOT was normal in both 1 mo later. One subject had a slight persistent increase in alkaline phosphatase 9 and 15 d after exposure (13.7, 14.3 KA), representing an increase to approximately 1.5 times his pre-exposure control value. Other tests of liver function performed on the subject with increased alkaline phosphatase 21 d after exposure had normal results. One subject had a decrease in hemoglobin content, from 16.8 g before exposure to as low as 13 g after exposure. A decrease in hematocrit from 48% to 44% accompanied the decrease in hemoglobin, and reticulocytes were 0.8% after exposure. Results of hemoglobin analyses from 9 to 20 d after exposure ranged from 13 to 14.2 g in the subject whose hemoglobin decreased, but his white-cell count was normal. These subjects were exposed to no other chemicals at Edgewood before the postexposure laboratory analysis. The slight abnormalities in SGOT after exposure to CHT might have represented idiosyncratic hepatic reactions to the chemical. Complete recovery is likely. The increase in alkaline phosphatase and the decrease in hemoglobin after CHT exposure are difficult to relate to the exposure.

Given the available information on CHT and on the Edgewood subjects exposed to the chemical, long-term health effects of the exposure on the subjects are difficult to predict.

SUMMARY

CHT appears to be a powerful lacrimator and irritant with a high safety factor and a short recovery time. It is highly volatile and dissipates rapidly in open air or in ventilated enclosures.

CHT appears safer than CR or CN. Neuromuscular damage in one dog from degraded CHT led to further studies, which demonstrated severe

eye and skin damage from some of the degraded products, including neurologic signs, but no permanent damage in surviving animals.

Data are insufficient to support a conclusion regarding the mutagenicity or carcinogenicity of CHT.

TABLE 4-40 Comparative Data on CS, CR, and CHTa

Characteristic	CS	CR	CHT
Volatility at 20°C, mg/m^3	0.36	0.63	8,484[b]
ICt_{50} for man, mg·min/m^3	6.9	0.15	ca.50
LCt_{50}, mg·min/m^3:			
Guinea pig	36,439	169,000	176,000
Dog	29,748	57,171	63,322
Percutaneous LD_{50} for dogs, mg/kg	No data available	No data available	708-1,000[c]

[a] Data from Simmons et al.[9]
[b] Volatility measured at 25°C.
[c] Animals held in room approaching saturation for 6 h.

TABLE 4-41 Effects of CHT Administered Cutaneously in Nonrestrained, Clipped Beagles Decontaminated at 24 hours with Bleach and Watera

Sample Composition,[b] mol%	Condition	Dosage ml/kg	Results
"Control"-- fraction 5 (88.2% EA 4923, 9.4% EA 4922, by NMR)	Occluded	0.125	2/2 normal at 24 h
		0.25	2/2 normal at 24 h
		0.50	1/2 dead at 2-3 d; 1/2 sacrificed at 5 d
Nonvolatile residue from 31AS in DMSO	Occluded	0.25	2/2 normal at 24 h
		0.50	2/2 normal at 24 h
		1.0	2/2 atactic at 2-3 d, but normal at 5 d
31AS sample (77.6% EA 4923, 11.7% EA 4922)	Occluded	0.125	2/2 normal at 24 h
		0.25	1/2 normal at 24 h; 1/2 unable to stand, convulsing at 24 h, and sacrificed at 2 d
		0.50	1/2 dead at 30-46 h; 1/2 with salivation, tremors, postural instability, but normal at 13 d
"Control"-- fraction 9 (85.1% EA 4923, 10.3% EA 4922), stabilized with 1% antioxidant	Occluded	0.25	1/2 dead at 2-3 d; 1/2 with tremors, emesis, postural instability, but normal at 7 d
		0.50	1/2 dead at 4-5 d; 1/2 with tremors, weakness, emesis, postural instability, but normal at 7 d
		1.0	2/2 dead at 1-3 d
"Control"-- fraction 8 (89.4% EA 4923, 8.4% EA 4922)	Nonoccluded	0.25	2/2 tremors, but normal at 2 d
		0.50	2/2 tremors, but normal at 2 d
		1.0	1/2 tremors, salivation, weakness, but normal at 5 d

[a] Data from Simmons et al.[9]
[b] EA 4923 = CHT. EA 4922 = relatively inert isomer of CHT.

TABLE 4-42 Toxicity of CHT Administered to Skin without Occlusion in Nonrestrained Beagles Held in Saturated Environmenta

Time in Environment	Dose ml/kg	Mortality fraction	Survivor Recovery Time,[b] d
1.5 h:			
Nonstabilized	0.5	0/2	6, 6
	1.0	0/2	N.E., 28
Stabilized	0.5	0/2	N.E., 6
	1.0	0/2	6, 20
6 h:			
Nonstabilized	0.5	1/4	6, 7, 23
	1.0	3/4	5
Stabilized	0.5	0/4	N.E., 2, 7, 13
	1.0	2/4	4, 19

[a] Data from Simmons et al.[9]
[b] N.E.=neuromuscular effect such as tremors, weakness or ataxia noted.

TABLE 4-43 Effect of Decontamination with Bleach and Water or with Soap Solution and Water at Various Intervala after Skin Application of CHT at 1.0 ml/kg to Nonrestrained Beaglesa

Sample	Decontaminant	Decontamination Time, min	Results
"Control"-- fraction 4 (87.5 mol%)	Bleach followed by water	5	1/1 no toxic signs noted
		30	1/1 slight tremors at 3-22 h, but normal at 4 d
		60	1/1 dead at 30-72 h
"Control"-- fraction 4	Soap solution followed by water	5	1/1 no toxic signs noted
		30	1/1 slight tremors, but normal at 4 d
		60	1/1 dead at 23 d
"Control"-- fraction 4	Bleach followed by water	45	1/1 tremors, weakness at 1-19 d, but normal at 19 d
		60	1/1 tremors, weakness, postural instability at 3-25 d, but normal at 28 d
"Control"-- fraction 4	Soap solution followed by water	45	1/1 found drowned in water pan on day 4
		60	1/1 tremors, weakness, postural instability at 3-25 d, but normal at 25 d
"Control"-- fraction 9, stabilized with 1% antioxidant	Bleach followed by water	75	1/1 dead at 22 d
"Control"-- fraction 8	Soap solution followed by water	75	1/1 tremors, weakness at 2 h-11 d, but normal at 11 d

[a] Data fom Simmons et al.[9]

TABLE 4-44 Parenteral Toxicity of CHT in Rat, Mouse, and Rabbit

Species	Route[b]	LD_{50}, mg/kg
Mouse	Oral	478 (412-555)
Mouse	Intraperitoneal	13 (12-15)
Rat	Oral	282 (246-324)
Rat	Intraperitoneal	21 (16-27)
Rat	Intravenous	13 (11-15)
Rabbit	Intravenous	8 (7-9)

[a] Data from Biskup et al.[1]
[b] Administered in polyethylene glycol 200.

TABLE 4-45 Toxicity of CHT Administered Cutaneously to Dogsa

Amount Applied,[b] ml/kg	Time, h	Acute Signs	Acute Mortality
1.0 (O)	1	1/8 (neurologic)	0/8
0.5 (O)	16 or 24	Not recorded	8/8
0.5 (O)	2	No toxic signs[c]	--
0.5 (O)	4-6	Toxic signs[c]	--
0.5 (O)	1 every 4 d for 4 doses	1/3 (neurologic)	0/3
0.25 (O)	1 every 4 d for 4 doses	0/3	--
0.125 (O)	1 every 4 d for 4 doses	0/3	--
0.5 (N)	24	0/4	0/4

[a] Data from Dilley et al.[3]
[b] O = occluded, N = nonoccluded.
[c] Number of animals not stated.

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EFFECTS OF HUMAN EXPOSURES TO 123 IRRITANT CHEMICALS AT EDGEWOOD IN TWO-MAN TESTS

RL SOP No. 70-3, dated June 1, 1967, describes methods used at Edgewood for searching for and selecting toxic chemicals. Some of the details in connection with exposure of human volunteers to experimental irritant chemicals are described. Human volunteers were exposed to compounds after review of animal screening data and approval by committees based on a conclusion that the experimental chemicals were safe for human use. Generally, two volunteers were exposed to each substance. Subjects were exposed in a wind tunnel at an air-speed of 5 mph and were asked to resist leaving the test atmosphere (up to 1 min) until exposure was unbearable.

During 1962-1972, 123 irritant chemicals were tested. Further details on the chemicals are available from the NRC repository of Edgewood data. The substances were classified as irritants on the basis of the preliminary animal studies. Except where noted below, exposures were for 1 min or less in an aerosol chamber; each subject was exposed to a chemical only once.

AGENTS THAT CAUSED SLIGHT OR NO EFFECTS

Of the 123 irritant chemicals, 64 caused slight or no effects on the exposed subjects. Two subjects were exposed to each of the 64 chemicals, except CS 40806, to which only one subject was exposed.

The following 64 irritant chemicals caused slight or no effects:

301021	CS39241	CS40850	CS43166
CS1086	CS39242	CS41462	CS43168
CS4659	CS39666	CS41468	CS43169
CS15442	CS39715	CS41592	CS43945
CS20409	CS40320	CS41623	CS43974
CS23653	CS40325	CS41725	CS43988
CS27474	CS40332	CS42055	CS43989
CS29780	CS40679	CS42057	CS44854
CS30800	CS40683	CS42213	CS45514
CS36650	CS40686	CS42216	CS45659
CS36667	CS40781	CS42740	CS46345
CS36722	CS40785	CS42824	CS47137
CS37149	CS40804	CS43000	CS47148
CS37200	CS40805	CS43001	CS47563
CS38355	CS40806	CS43013	CS48418
CS38731	CS40841	CS43014	CS61804

CS38731 might have been contaminated with dioxin. Despite a lack of complete toxicity data, it seems unlikely that the short, single exposures to the 64 irritant chemicals that caused slight or no acute effects on the exposed subjects will cause long-term health effects.

LACRIMATORY AGENTS

The predominant effects of 42 of the 123 irritant chemicals were ocular, including eye irritation, eye closing, lacrimation, and conjunctivitis.

Of the 42 lacrimatory agents, 34 caused very mild effects--generally eye irritation, sometimes associated with dermal and upper respiratory passage irritation. The 34 mild lacrimatory agents were tested on two subjects each, except 119135 (four subjects), CS46398 (one subject), EA 2542 (17 subjects), EA 3365 (17 subjects), EA 4922 (six subjects), and CS815799 (ylidineamine, 21 subjects). The mild lacrimatory agents include:

118055	CS30749	CS41377	EA 2305
119135	CS30785	CS41722	EA 2329
302049	CS30799	CS43331	EA 2413
CS2127	CS31979	CS43981	EA 2433
CS5146	CS37270	CS46398	EA 2542
CS5616	CS38756	CS48861	EA 3176
CS18692	CS40331	EA 2284	EA 3365
CS30747	CS40849	EA 2302	EA 4922
CS30748			CS815799

CS38756 and CS40849 might have been contaminated with dioxin.

Additional details are available on EA 2542 and CS815799. In 1963, two subjects underwent one exposure each to EA 2542 at Ct's of 39 and 57 mg·min/m^3. No effects were described. In 1969, EA 2542 was tested further on 15 subjects, who had one exposure each. Ct's ranged from 29.8 to 65.8 mg·min/m^3, but no exposure durations are available. The subjects experienced irritation of eyes, nose, throat, and periorbital sites. Results of postexposure laboratory analyses were normal.

In 1972, 21 subjects underwent exposure to CS815799. Seven subjects had aerosol exposures at a Ct of 50 mg·min/m^3; one was exposed twice. CS815799 exposure caused mild ocular and respiratory irritation. Laboratory analyses 7 d after exposure revealed increased SGOT (43.8 and 42.7 IU) in two subjects, both of whom had normal pre-exposure SGOT. One of these two subjects also had 6-10 white cells in urinary sediment--a finding not seen in preexposure urinalysis.

Fourteen CS815799 subjects were exposed cutaneously with 1% solution, which caused mild irritation. One subject was exposed twice.

Given the absence of followup data, it is not possible to predict whether short exposures to the 34 mild lacrimatory agents will have long-term health effects among the exposed subjects. The increase in SGOT in CS815799-exposed subjects might have represented hepatic reactions to the chemical, but recovery was probably complete.

Eight of the 42 lacrimatory agents caused more severe effects than the other 34, namely more prolonged incapacitation in association with lacrimation and eye closing: 118539, 123175, 126312, CS encapsulated in gelatin, CS36579, FOGOILCS, EA 2366, and CS-DM mixtures.

The CS-DM mixtures were tested on 88 subjects. The CS:DM ratio in the mixture was 1:10. Thirty-eight CS-DM exposures occurred in 1958, and half the 1958 subjects had two exposures each. Fifty of the CS-DM exposures occurred in 1966. Ct's were 0.5-40 mg·min/m^3. Exposure durations, when recorded, were 16 s to 2 min. The principal effects were ocular irritation, lacrimation, and conjunctivitis, sometimes associated with upper respiratory tract irritation and cough. One subject required analgesic therapy after exposure because of severe discomfort. Results of laboratory analyses 7 d after exposure, were available for all the 1966 CS-DM subjects, as well as some of the earlier subjects. One subject had an increase in SGOT (to 60 IU) in postexposure laboratory analysis, but his pre-exposure SGOT had also been slightly high (33.6 IU).

In 1963, two subjects underwent short, single exposures to 118539, which caused lacrimation and severe conjunctivitis, salivation, nasal irritation, chest constriction, and cough. In 1965, eight subjects underwent single exposures to 123175, which caused lacrimation and eye closing for several minutes after cessation of exposure. In 1966, 12 subjects underwent single exposures to CS36579, which caused eye pain, profuse lacrimation, eye closing and incapacitation that lasted several minutes after cessation of exposure, nasal irritation, and dyspnea. In 1963, two subjects underwent short, single exposures to EA 2366, which caused lacrimation and conjunctivitis, respiratory tract irritation and dyspnea, and nausea.

Given the available information on subjects exposed to 118539, 123175, 126312, CS36579, and EA 2366 and their short, low-dose exposures, one cannot predict long-term health effects of these agents. The discomfort associated with the exposures was marked, but exposures were short and recovery appeared complete.

RESPIRATORY IRRITANTS

Seventeen of the 123 irritant chemicals caused predominantly respiratory effects, which were generally mild and transient. Ten caused nasal and throat irritation, including cough. These upper respiratory irritants were tested on two subjects each, except CS5635, CS42818, and EA 2129, which were tested on four subjects each. Other upper respiratory irritants were CS24302, CS41458, CS42822, CS42984, CS43010, CS43109, and EA 3437.

Seven respiratory irritants caused primarily a sensation of chest constriction and dyspnea. EA 2097 (CS14632) was tested on 19 subjects, 15 of whom had two exposures each, and EA 2214 was tested on four subjects. Other lower respiratory irritants were tested on two subjects each: 118609, 119400, CS36273, CS42985, and CS43329.

Given the lack of followup information on the primary respiratory irritant chemicals, it is not possible to predict whether they will have long-term health effects. Because these were short, low-dose exposures in which the acute effects were generally mild, with complete recovery, the Committee believes that long-term health effects are unlikely.

CONCLUSIONS

The Committee analyzed published studies describing the in vivo and in vitro properties of the agents used and reviewed short-term data collected by the U.S. Army on volunteers. The ability to provide definitive answers to the questions raised by the charge to the Committee was limited by the absence of long-term followup studies of the soldiers and by the sparseness of chronic studies of these compounds in animals or in humans after industrial exposure.

In general, the Committee found insufficient evidence to evaluate these chemicals, except mustard gas. Mustard gas is an experimental mutagen and human carcinogen at high doses. Data on the other irritants are insufficient to evaluate their mutagenicity, carcinogenicity, or other long-term effects. Tests of all these chemicals involved few exposures and low doses.

MUSTARD GAS (H)

Mustard gas is highly reactive and has vesicant and systemic toxic effects. It is an alkylating agent that is mutagenic in various laboratory test systems, including mammalian germ cells, but data are inadequate to predict the extent of its genetic risk in humans. Mustard gas is also carcinogenic in experimental animals

and humans. Other possible long-term effects of mustard gas are related to its systemic toxicity; specifically, it can cause blindness, permanent scarring of the skin (which may lead to skin tumors), and chronic bronchitis. Reported instances of long-term injury such as carcinogenesis in workers in a Japanese mustard production plant, were associated with exposure at high, long-term dosages. Information is insufficient to project risks associated with smaller exposures to mustard gas; however, serious long-term adverse effects in the small number of soldiers who received one or a few low-dose exposures at Edgewood seem unlikely (except for some cases of permanent scarring). Some of those exposed at Edgewood suffered skin injuries that took several weeks to resolve. However, in view of the small number of persons tested (about 150 healthy men) and the very low dosages involved, it is unlikely that a statistically significant increase in the risk of cancer or other chronic disease can be detected in those exposed to mustard gas at Edgewood. When exposed, the Edgewood subjects were wearing gasmasks and impregnated clothing--an ensemble being tested for efficacy against toxic contamination.

o-CHLOROBENZYLIDENE MALONONITRILE (CS)

Results of experimental studies in microorganisms and short-term experiments in laboratory animals suggest that long-term medical abnormalities in soldiers exposed to CS are unlikely. Acute tissue changes produced in animals and humans seem reversible and not likely to become chronic in the absence of recurrent exposures. Followup information on the long-term state of health of exposed soldiers is not yet available, but no reports have indicated that Edgewood subjects have experienced any long-term sequelae.

CHLOROACETOPHENONE (CN)

CN, a moderately toxic irritant, has immediate effects on the eyes, skin, and respiratory tract. CN is a strong skin-sensitizing agent, but is rarely lethal. The Committee found no evidence of lasting ocular or respiratory effects in 99 volunteers exposed experimentally at Edgewood between 1958 and 1972 when subjects were evaluated 2 wk after cutaneous administration or inhalation of aerosol. Allergic contact dermatitis or hypersensitivity in these volunteers on re-exposure to CN is possible. There has been no systematic study of the possible mutagenic and neoplasm-promoting effects of CN with current scientific methods.

DIBENZ[b,f][1,4]OXAZEPINE (CR)

CR, a mild lacrimatory irritant, manifests less acute toxicity than CN and CS. At low doses, it causes transient effects. There are a few studies on long-term health effects, including potential mutagenicity and teratogenicity. The available data are insufficient to predict long-term health effects. The small number of exposures and the small number of subjects exposed to CR at low doses at Edgewood make the occurrence of demonstrable effects in these subjects unlikely.

CHLOROPICRIN (PS)

PS is acutely toxic and has a variety of sensory effects in animals. It has not been evaluated thoroughly for mutagenicity or carcinogenicity. Like those exposed to mustard gas, the subjects exposed to PS were wearing gasmasks, and small numbers of soldiers were exposed to small doses. PS is unlikely to have produced detectable long-term health effects in volunteers exposed at Edgewood.

BROMBENZYL CYANIDE (CA), DIPHENYLAMINOCHLORARSINE (DM), and 1-METHOXY-1,3,5-CYCLOHEPTATRIENE (CHT)

CA, DM, and CHT are unlikely to have produced measurable long-term health effects in volunteers exposed at Edgewood. But there are no specific toxicologic data on the mutagenicity and carcinogenicity of these compounds. CHT is less toxic than CN or DM when administered acutely.

NONANOYL MORPHOLIDE

The Committee does not expect long-term health effects in volunteers tested with nonanoyl morpholide at the dosages used at Edgewood. As with CA, DM, and CHT, specific toxicologic data regarding its potential in this regard are not available.

123 IRRITANT CHEMICALS

A total of 123 irritant chemicals were generally tested on only two subjects each. There are no data on their mutagenicity, carcinogenicity, or other long-term health effects. However, because the exposures were small, detectable adverse effects seem unlikely.

APPENDIX A

Part 1. Reproduced from Volume I, Anticholinesterases and Anticholinergics

HISTORY OF THE EDGEWOOD TESTING PROGRAM

INTRODUCTION

Human experimentation appears to have been an integral part of the history of the U.S. Army chemical warfare (CW) research efforts until its suspension in 1975. On June 28, 1918, the President directed the establishment of the Chemical Warfare Service (CWS). Four years later, in October 1922, the CWS created a Medical Research Division to conduct research directed at providing a defense against chemical agents. No matter how exhaustively an agent was tested in animals, it was felt that its efficacy in humans also had to be studied.

In early 1941, the threat of war increased the urgency of the development of protection against CW agents and, consequently, engendered a need for a larger source of volunteers. Formal authority to recruit and use volunteer subjects in CW experiments was initiated in 1942. The Secretary of War was asked to rule on the permissibility of using enlisted men for testing agents of the mustard-gas type. In July 1943, the CWS was assigned responsibility for all medical research related to CW. This extension of the CWS mission included toxicological research and the study of hazards to the health of personnel in the CWS.

The issue of the use of human volunteers was considered by the Armed Forces Medical Policy Council during the early 1950's. The Council concluded that essential data could not be obtained unless human volunteers were used, and the use of humans in medical research was authorized. By 1954, the Chemical Corps (formerly CWS) had established a framework within which to conduct human experimentation, but it lacked an adequate pool of volunteers. In 1955, it was decided that the most practical source of volunteers would be enlisted men stationed at Army installations in the vicinity of Edgewood Arsenal. It was emphasized that voluntary consent of each human subject was absolutely essential. It was also stated that, in all experiments involving volunteer subjects, the subjects would be thoroughly informed of all procedures and of what might be expected as a result of each test. Furthermore, each volunteer would be free to determine whether he desired to participate in a given experiment. In October 1959, approval was granted for the conduct of research on volunteers to investigate defense against incapacitating CW agents.

The search for incapacitating agents intensified when the Kennedy administration took office. The involvement with incapacitating agents represented a departure from an earlier period, begun in 1946, when interest in highly toxic (acute) anticholinesterase chemicals resulted from their development in Germany during World War II. The basic purpose of a military incapacitating agent is to produce temporary ineffectiveness without permanent injury or death. Incapacitating agents (anticholinergic chemicals) and highly toxic (acute) anticholinesterase chemicals produce functional and structural effects on the nervous system which cause rapid or delayed effects on an individual's performance and behavior.

PROCEDURES USED AT THE EDGEWOOD CHEMICAL TESTING PROGRAM

A fairly extensive discussion of the procedures used is provided in the Inspector General's report, Use of Volunteers in Chemical Agent Research, prepared by Colonel James R. Taylor and Major William H. Johnson and dated March 1976 (listed in Appendixes C,D).

RECRUITMENT OF VOLUNTEERS

Recruiting teams (initially administrative officers, but later often including military physicians from the Edgewood laboratory) visited Army installations where a briefing, usually with a film and handouts, was presented to a large number of enlisted men. Generally 10 to 20 percent of the audience expressed interest and these men were asked to complete a personal history, which included medical and psychologic items and the Minnesota Multiphasic Personality Inventory (MMPI). It was not unusual for 400-600 men to request assignment in the course of a tour of seven to ten installations. Of these, no more than 100 were selected and eventually assigned for a 1- to 2-month period of temporary duty at Edgewood Arsenal.

The “incentives” for volunteering consisted of a small monetary allowance (approximately $1.50 a day for temporary duty), the assignment of only light duties while at Edgewood, and almost every weekend free. Some volunteers were genuinely interested in the scientific and experimental aspects; however, if curiosity or the desire to “test one's self” seemed too strong, the applicant was usually not accepted.

As a group, the volunteers were above average in physical and mental qualifications, with a mean IQ near 110, good behavior records, deviations of the population mean on all scales.

GUIDELINES FOLLOWED IN THE PROTECTION OF SUBJECTS

The Nuremburg and Helsinki guidelines were regarded by the investigators and their supervisors as appropriate constraints in studies performed on volunteers, although this was not clearly articulated in official memoranda until the mid 1960s. The provision of accurate, informative explanations of what was planned and what might be expected was regarded as essential to the continuance of the program. Written consents, witnessed by medical staff members, were required from the outset and became more elaborate with time. However, minutes of hearings conducted by the U.S. Senate Subcommittee on Health and Subcommittee on Administrative Practice and Procedure, September 10-12, 1975, stated that the consent information was inadequate by current standards.

INVESTIGATORS

When BZ studies were begun in 1960, the need for a psychiatrist with biologic training and interest was recognized and one was assigned to the program in January 1961. Physicians trained in internal medicine, anesthesiology, cardiology, surgery, dermatology, ophthalmology, neurology, and other specialities were assigned as the program proceeded. Many were research-oriented and have since gained excellent reputations in academic medicine at leading universities.

SELECTION OF DOSES FOR HUMAN TESTS

Subthreshold doses based on estimates from animal potency studies were used in the first few subjects. For example, the earliest exposures to BZ, one of the anticholinergic test compounds, were at doses between 0.1 and 0.5 μg/kg, which was less than one-tenth the incapacitating dose (ID) ultimately established at approximately 5.5 μg/kg. The intravenous route was preferred initially, but other routes of administration were also used. Inhalation studies were sometimes undertaken after a compound had been thoroughly studied by one of these parenteral routes. Oral and percutaneous studies were performed when effectiveness via these routes was of interest.

As the program developed, it became customary to test agents at dose increments of 40 percent, once the approximate effects of the lower doses were known. Placebos were used in some studies, but the cost with respect to subject confinement time, staff workload, and delay in achieving estimates of potency made this impractical except in special casses (e.g., evaluation of antagonists). Instead, low and high doses were assigned in a randomized manner by someone not involved in an experiment. Placebo responses were minimal. Signs of drug effects at all but the lowest doses were significant and made the value of placebo or “no treatment” inconsequential.

RANGE OF DOSES

Rarely did the intramuscular or intravenous doses exceed 1.5 times the incapacitating dose. Inhalation doses were higher, but potencies were lower by this route (usually about 60 percent of that by the intravenous or intramuscular route). Compared with doses described in the scientific literature on atropine coma therapy [18,19,20,21,22 and 23] or scopolamine therapy,[19] the BZ doses to which volunteers were exposed appear modest. As much as 20 times the ID_{50} of atropine and 30-40 times the ID_{50} of scopolamine have been administered in the past by clinicians--often to older and less robust patients. Many patients received multiple exposures of this magnitude over a period of days or weeks. These therapeutic procedures, reported several decades ago in refereed journals, actually stressed and advocated the benefits of such treatment, despite occasional deaths (most of which appear to have been caused by hyperthermia).

SAFETY MARGIN

The safety margin of a drug is defined as the ratio of the lethal dose (LD) to the effective dose (ED). Sometimes, ratio of the LD_{50} to ED_{50} is used, although a more conservative approach favors the use of the ratio of LD_1 to ED_{99} (standard margin of safety). In the case of incapacitating agents, much reliance is placed on extrapolation from animal experimentation, and estimation of the LD_1 is generally unreliable.

Many other extrapolation techniques have been used in manipulation of animal lethality data in an effort to generate a reasonable human estimate. By taking a conservative approach with data on deaths at low doses, one can derive estimates for man that are modest and in keeping with clinical judgement. Such methods depend on procedures developed and applied in toxicology.

APPENDIX A

Part 2. Clinical Research Department, SOP No. 5, August 12, 1968 (Revised)

VOLUNTEER SCREENING AND SELECTION

The purpose of this SOP is to provide guidelines for the psychological/ psychiatric selection of volunteers. There are several standard forms used for this purpose and each will be discussed.

1. Screening Data form (medical history). A "yes" answer on any item without a recommendation of a medical officer for acceptance will reject the individual.
2. When the GT score is available a very low score (below 90 or 80) will reject the individual.
3. MMPI. These are "rules of thumb." Lacking a scientific basis for choosing, these represent advice rather than dogma, but should be followed if possible.

A. Clinical Scales (Hs, D, Hy, Pd, Mf, Pa, Sc, Ma and Si)

1. Overall profile. Reject if any five of the above scales are over 65.
2. Mark profiles borderline and carefully examine family history for indication of psychological problems if--

a. L and K both exceed F by at least 15 scale points.
b. F exceeds both L and K by at least 15 scale points.

3. Pd, Pa, Sc Pattern (psychoticism)

a. Reject if any two of these three are among the two highest scores on the clinical scales.
b. If Pa or Sc is above 80, and mark as border-line if either exceeds 70.
c. Reject if Pa and Sc are both above Hs, D, and Hy.

4. Pd, Mf, Ma Pattern (Sociopathic Deviate; “Acting Out”)

a. Reject if Pd and Ma are both above 65, and there is a history of “acting out.”
b. Reject if Pd is above 70, and there is a history of “acting out.”
c. Reject of Mf is above 80 in combination with Pd, Pa, or Sc above 65.

5. Hs, D, Hy, Pt, Si Pattern (neuroticism)

a. Reconsider overall picture, history, etc., if any four of these are above 70.
b. Reconsider overall picture, history, etc., if any two of these are above 80.
c. Reconsider overall picture, history, etc., if Pt is above 80.

The most common exception to these rules is the active, ambitious, college graduate with Pd and Ma above 65, but no history of acting out. In all but the most extreme cases it is well to obtain corroborating evidence from the Family History.

4. Family and Developmental History. The Family History (SMUEA Form 6-85) contains information about a wide range of the potential volunteer's activities, as well as tapping various levels of consciousness. For routine screening certain items are useful.

1. Trouble in school, with the civilian police, or Article 15s. A pattern of this sort is indicative of an “acting out” type of person.
2. Interviews with a psychiatrist for anything other than routing screening, e.g., peace corps selection, etc.
3. A history of fighting after heavy drinking, especially with a bad temper.
4. Blatant and diffuse expressions of hostility on the Picture Frustration Test pp 15-16.

Other items: slow rate of promotion, lack of clear cut goals, excessive depreciation of self value, and generally bizarre answers

on the Sentence Completion may be clues but must be interpreted to relation to other information available.

The screening on the basis of the MMPI is usually done by the psychologist or psychiatrist. The Family Histories are read by the Medical Officers in Psychopharmacology with each officer reading his share. During this phase of the screening only those histories that have survived the preceding steps are read. The purpose of this screening is to pick those volunteers chosen to come to Edgewood for any type of test.

After all this material has been read and the volunteers rated, a list is furnished the administrative office of about 80 first choice names and 80 alternates. These names are usually given to the administrative office by the tenth of the month which precedes the month they are to report to Edgewood.

When the volunteers arrive at Edgewood they are interviewed by the officers in the Psychopharmacology Branch.

At the time of the screening interview, on the basis of the interview, history, questionnaire (sentence completion and Picture Frustration tests) and MMPI scores a rating will be applied to each candidate to separate out the following groups and an entry will be made upon the Physical Examination sheet opposite the heading "Psychiatric" characterizing the candidates qualification for drug testing, as follows:

Rating	Qualification (on PE form)
A	OK for psychochemical testing
B	Low-dose psychochemicals only
C	No psychochemicals
D	Equipment only

The ratings are to be defined as follows:

A. No apparent or overt psychologic problems and no tendency to somatize or act-out intra-psychic tension. Many assets, few liabilities. Flexible. Good ego strength. Age - appropriate maturity and responsibility. A clear sense of identity. Such conflicts as are evidenced are few in number, situational, and usually conscious. Normal MMPI and Family History.

An exceptionally well adjusted candidate who impresses the interviewer by his flexibility and ease in handling anxiety and hostile or aggressive impluses should be rated A+. These men will be used for such psychochemical tests as are considered to be of greater than usual stress.

B. Adequate, flexible, good ego strength. Gets along fairly well. An appropriately mature and responsible person. History is good but may have one or more negative items such as: a very minor offense for which an Article 15 is given or a civilian arrest for a minor, non-occurring matter (symptomatic of immaturity). Minor personality distortions which do not interfere with optimum functioning. These men are not optimal candidates for psychochemical testing but the interviewer expects they would suffer at most negligible psychic trauma from experience with the effects of psychochemicals.

C. Any tendency to psychosomatic reactions or aggressive physical acting-out should drop a candidate at least to this group. These men are good cooperative subjects who, however, are not candidates for psychochemical tests but may be used for other drug tests. They may be somewhat dull or non-verbal, have obvious neurotic traits, immaturity, rigidity or other apparent liabilities, but with good reality assessment and no borderline or psychotic tendencies at present or at any time in past history does not include bizarre circumstances or severe and continued traumatization.

D. These men fall into the lower end of a scale of group whose characterization agrees roughly with those rated as C. Some definite emotional pathology is tolerated in this group as well as some bizarre or unusual responses on the questionnaire tests and border-line or aberrent scores on some MMPI scales. Numerous but minor offenses (2 or 3 Article 15). These men may be used for equipment testing and at the discretion of the responsible medical officer may be used for local drug testing but should not be subjected to any systematic drug. Hysterical, or schizoid personalities and any but minor tendencies towards somatization should drop a candidate to this group. Some men who arrive at Edgewood with diagnosible physical or emotional disorder may be allowed to participate in the program but with D rating and their participation in any particular test must be OK'd by the responsible physician.

Such men who are untrustworthy, sociopathic, grossly disturbed or pathologic or have criminal history or a history of recurrent, severe or recent psychotic episodes should not be selected as volunteers and if they arrive at Edgewood, should be returned to their home station. This decision should ordinarily be made during the initial screening upon the basis of severe distortion of MMPI scores or very bizarre or unappropriate items on the history or questionnaire tests.

Under no circumstances should this SOP be construed to supplant or replace the judgement of the medical officers in the selection procedures, who may deviate from these guidelines at their discretion. Deviation from the SOP may also be done in a systematic way

if adherence would interfere with the accomplishment of a particular investigation, as, for example, a study of the effects of psychochemicals upon depressed subjects. But the conditions of such an experiment would demand an unusual attention to the safety and well-being of the volunteers selected.

APPENDIX B

DIGEST REPORT OXIMES

by

J. Henry Wills

INTRODUCTION

The group of oximes that has been administered to human volunteers by or under the auspices of the Biomedical Laboratory of the Edgewood Arsenal at Aberdeen Proving Ground includes several salts of N-methylpyridinium-2-formyl oxime, N,N'-trimethylene-bis-(4-formylpyridinium oxime)bisbromide and -bischloride, N,N'-methyleneoxymethylene-bis(4-formylpyridinium oxime)bischloride, and the ketoxime diacetylmonoxime. Two of the widely used salts of the monopyridinium oxime are the chloride (pralidoxime chloride), referred to here as I, and the methane sulfonate (contrathion), referred to as II (or P2S). The bispyridinium bisoximes above are known, respectively, as trimedoxime bromide (III), trimedoxime chloride, and obidoxime chloride (IV). The ketoxime is known as DAM (V). The designations by Roman numerals are used hereafter for these oximes.

Other salts of N-methylpyridinium-2-formyl oxime are identified by the abbreviation 2-PAM followed by the common symbols for elemental anions (such as 2-PAM I for the iodide) or the names of organic anions (such as 2-PAM tartrate). Other salts of N,N'-trimethylene-bis-(4-formylpyridinium oxime) are identified by appending the designation for the anion to the abbreviation TMB-4.

These and other oximes were developed to be reactivators of cholinesterase that had been inhibited by organophosphorus anticholinesterase compounds;[1,4] they were considered initially and briefly to be complete antagonists of the toxic actions of these substances. This idea had to be abandoned when 2-PAM I was found[5,6] to be much more effective as an adjunct to atropine than as a sole therapeutic agent in antagonizing intoxication by organophosphorus anticholinesterase agents. Furthermore, 2-PAM I and I antagonized particularly the blockage of nicotinic cholinergic neuromuscular transmission at the motor endplate on skeletal muscle--an effect that can be reproduced to some extent with d-tubocurarine and other curaremimetic agents.

The efficacy of oximes as adjuncts to atropine in treating intoxication by anticholinesterase agents depends on both the agent

and the oxime. For instance, I is potent as an adjunct to atropine in treating experimental animals intoxicated by sarin or VX, but is only mildly effective as an adjunct to atropine in treating intoxication by tabun[6,8] and almost completely ineffective in treating intoxication by soman,[9] whereas III is moderately effective as an adjunct to atropine in treating experimental intoxication by tabun. None of the oximes considered here is outstandingly effective as an adjunct to atropine in treating intoxication of laboratory animals by soman.[9,13]

The failure of these oximes to antagonize the alterations in normal function induced by soman has been attributed[14] to hydrolytic dealkylation of the phosphorus atom in the phosphonyl residue attached to the active center of cholinesterase; that results in an alteration in the electronic field around the phosphorus atom that renders oximate ions unable to sever the bond between the phosphorus atom and the serine residue in the active center of the enzyme. The aging reaction, identified first with DFP,[15] has been found to proceed particularly rapidly in the phosphonyl residue from soman on inhibited cholinesterase.[13,16] A large dose of I was found[17] to stop the aging process in experimental animals, but reactivated only a part of the cholinesterase that had been inhibited. The amount of I required for this purpose was so large (104 mg/kg intravenously) as to carry a high hazard of toxic action. Furthermore, there is some uncertainty about the importance of aging in the response of an organism to soman. For example, the repetitive depolarization of skeletal muscle fibers after a single indirect stimulus that follows a dose of soman was stopped by TMB-4 Cl_2 without detectable reactivation of cholinesterase in the vicinity of the motor endplate.[18] Similar results have been obtained with d-tubocurarine,[19] gallamine,[19] and piperidyl methylandrostanediol.[20] Crone[21] reported that d-tubocurarine chloride in vitro at 10^{-4} M completely prevented for 6 h the aging of red-cell acetylcholinesterase that was inhibited by sarin and that gallamine triethiodide at the same concentration markedly slowed the aging of similarly inhibited cholinesterase. Because the response of skeletal muscle after a dose of soman was affected by a dose of d-tubocurarine that would have yielded a concentration in the blood of no more than 0.45 x 10^{-6} M, it is difficult to believe that Crone's effect can explain fully the in vivo action of d-tubocurarine in antagonizing the neuromuscular blocking action of soman.

In 17 rats, twitch contraction of the anterior tibialis muscle in response to single indirect stimuli continued until a mean dose of soman had been given that was more than 2,100 times the minimal dose that altered the magnitude of the twitch.[22] Eventual failure of the twitch response was seen to be always connected with marked slowing of the heart. This observation raises the possibility that the effect of soman on the twitch response of skeletal muscle depends on

a deficient supply of blood to the muscle, rather than on an effect on neuromuscular transmission. However, a similar indirect action seems not to explain the effect of soman on the response of skeletal muscle to repetitive indirect stimulation. Skeletal muscles become unable to maintain a tetanic response to repetitive indirect stimulation at doses of soman below those that affect significantly cardiovascular function. The indication that TMB-4 Cl_2 can antagonize the effect without inducing reactivation of cholinesterase at the neuromuscular junction[18] illustrates the importance of knowing what actions other than reactivation of cholinesterase may reside in the molecules of not only III but also the other oximes with which this review is concerned.

LETHALITY OF SINGLE DOSES IN EXPERIMENTAL ANIMALS

Several compilations of the toxicities of oximes have been published.[23,24,25,26,27,28 and 29] Additional information on the toxicities of the oximes under consideration is available from several sources, including Namba,[30] Lindsey et al.,[31] Wills,[32] and Crook and Cresthull.[33] Table 1 summarizes the available information on the single-dose lethalities of the oximes by giving typical values, without attribution to specific investigators. In comparing lethal doses of the various oximes, assuming that the oximate radical is the biologically active portion of the molecule, knowledge of the relative amounts of this radical in the various compounds is important. Table 2 gives values for this measure; the last column of the table gives values for the relative lethal activities of the compounds derived from the data in Table 1. It is obvious that there is no measurement in Table 1 on which all eight oximes can be compared, so that the relative lethality values in Table 2 should not be taken too seriously; they may be approximately correct in order. Lethality after oral administration was omitted from the consideration of relative lethality because it obviously differed qualitatively from lethality by other routes of administration; e.g., all five of the oximes given to mice orally were less toxic than 2-PAM I, although all were more lethal than 2-PAM I by any other route of administration. When the two rankings in Table 2 are compared by means of Kendall's rank correlation coefficient, there is only a 90% chance that the ranks in the two measurements are significantly correlated Thus, the relative amounts of the oximate radical in the molecules of the different oximes may not explain completely their comparative lethalities.

Seven of 14 dogs given single intravenous doses at 187 mg/kg of 2-PAM I died.[33] Vomiting, weakness, tremor, salivation, loss of reflexes, and convulsion were the most common signs of intoxication in these animals. Seven of 16 dogs given single intravenous doses of III at 57.5 mg/kg by the same investigators died. Weakness, convulsion, tremor, salivation, loss of reflexes, and vomiting were the

most common effects. In general, the signs of toxicity appeared in approximately the following order after each oxime: staggering weakness, collapse, relaxation of muscles, urination, defecation, tremor, convulsion, gasping, salivation, loss of reaction to touch, sound, or pricking with a needle, loss of eye reflexes, apnea, cyanosis, and death. The principal difference in effects between 2-PAM I and III was that muscular weakness was a less immediate response after 2-PAM I than after III.

Cholinolytic drugs have been found[34,35] to increase the lethalities of II, III, and a 1:1 mixture of II and III in mice given intramuscular injections. The changes in the LD_{50}s of II and III induced by a constant dose of atropine were 17.9% and 17.8%, respectively, despite the fact that the LD_{50}s of the oximes alone differed by a factor greater than 2. Parpanit and several mixtures of cholinolytic drugs had effects qualitatively similar to those of atropine. Duke and deCandole[36] reported that intramuscular injection into rabbits of equal doses (30 mg/kg) of I, II, and III resulted in peak plasma concentrations of the oximes about 9 min after the injections, the peak concentration of III being considerably greater than those of I and II. These investigators reported also that, whereas the plasma concentrations of I and III after intravenous injections decreased more slowly than that of II, the plasma concentration of all three after intramuscular injections decreased at about the same rate. Inasmuch as I and II had similar peak blood concentrations (lower than that of III) after intravenous injections, I and II seem to have somewhat larger volumes of distribution in the body of the rabbit than III.

In the rat, absorption of III from a single-loop intestinal preparation during 1 h was found to be only about 13% of that of 2-PAM I.[37] The rate of absorption of I was somewhat lower than that of the iodide; II was absorbed at nearly the same rate as the iodide.[38] Three hours after the oximes were put into intestinal loops, slightly more than one-third as much of III had been absorbed as of 2-PAM I.

Brown[39] found that intracisternal injection of II, after injection of sarin by the same route, was ineffective in overcoming respiratory paralysis and vasomotor stimulation resulting from sarin, but that an intravenous dose of atropine was effective. Edery[40] extended this sort of study with several organophosphorus compounds, atropine, I, III, and V. He found that intraventricular atropine and, to a minor extent, oximes were able to antagonize the effects of intraventricularly injected ethyl pyrophosphate. Intravenous injection of V at 25 mg/kg 1-2 min after intraventricular injection of ethyl pyrophosphate did not modify the effects induced by the organophosphorus compound. Intravenous injection of III at 20 mg/kg or, especially, of 2-PAM I at 50 mg/kg had definite antagonistic effects

TABLE 1 Representative LD50 Values of Eight Oximes Administered to Seven Animal Species

		LD_{50}, mg/kg							
Species	Route[a]	2-PAM I	2-PAM Cl (I)	P2S (II)	2-PAM lactate	TMB-4 Br_2 (III)	TMB-4 Cl_2	Obidoxime (IV)	DAM (V)
Mouse	IV	133	155	--	122	44	--	130	--
	IP	210	140	--	--	60	110	139	68
	IM	230	180	231	--	80	130	160	--
	SC	257	222	165	--	83	--	183	--
	PO	1,650	2,590	3,700	1,920	2,000	--	3,390	--
Rat	IV	147	96	109	--	89	104	140	--
	IP	300	199	--	--	165	--	195	--
	IM	--	150	218	--	137	--	189	--
	SC	--	--	332	--	--	--	--	--
	PO	--	--	7,000	--	--	--	4,000	--
Guinea pig	IM	--	168	305	--	--	--	79	--
Rabbit	IV	--	94	133	--	--	44	83	--
	IM	--	--	245	--	--	--	--	--
Cat	IV	--	--	--	--	--	--	100	--
	IM	--	--	--	--	117	--	188	--
Dog	IV	190	--	--	--	60	--	70	--
Monkey	IM	--	--	356	--	--	--	--	--

[a] IV, intravenous; IP, intraperitoneal; IM, intramuscular; SC, subcutaneous; PO, by mouth.

TABLE 2 Relative Oximate Content and Relative Lethality of Eight Oximes

Oxime	Relative Oximate Content	Relative Lethality
2-PAM I	1.00	1.00
P2S (II)	1.13	1.27
2-PAM lactate	1.17	1.10
TMB-4 Br_2 (III)	1.18	2.56
Obidoxime (IV)	1.47	1.39
TMB-4 Cl_2.	1.48	1.67
2-PAM Cl (I)	1.53	1.33
DAM (V)	2.61	3.13

on the responses induced by prior intraventricular injection of ethyl pyrophosphate. In interpreting these findings, it is pertinent to point out that the doses of the various oximes used correspond with 0.044 mmol/kg of III, 0.189 mmol/kg of 2-PAM I, and 0.248 mmol/kg of V. On the basis of these values, Edery's conclusion that 2-PAM I was the most active antagonist of ethyl pyrophosphate may be questionable.

These and other findings have raised the question of whether quaternary pyridinium oximes can enter the CNS from the general circulation. Kalser,[41] in a study with rats and cats given intravenous injections of I tagged with ^{14}C in the quaternizing methyl group, found that the cerebrum, the cerebellum, the medulla oblongata, and the spinal cord contained only traces of ^{14}C at times at which the blood contained the label at 19-53 μCi/kg. However, Firemark et al.[42] found that the brain of the rat had a concentration of ^{14}C-2-PAM I about one-tenth that in plasma 10 min after intravenous injection of labeled oxime at 20 mg/kg. In the brain, the cerebral and cerebellar cortices had the highest concentrations of the label; the caudate nucleus, the thalamus, and the hypothalamus contained concentrations a little less than half those in the cortices. Rats pretreated with the anthelmintic organophosphate trichlorfon and killed 10 min after intravenous injection of labeled 2-PAM I had a concentration of the label in their brains about twice that found in normal rats. The brain appears, therefore, to be somewhat permeable by 2-PAM I, but distinctly less so than leg muscle, diaphragm, liver, and kidneys according to Kalser's data, and to have that permeability increased by an organophosphorus anticholinesterase compound.

Freshly prepared solutions of II (150 mg/ml) in water or DMSO were applied to the skins of guinea pigs and rabbits, except that on the head and legs.[43] The II in DMSO entered the blood of the rabbit at a peak rate about 3 times that at which II in water penetrated the skin. No uptake of II from the aqueous solution by the guinea pig was detected. The rate of uptake of II from the DMSO solution by the guinea pig was about two-thirds that by the rabbit. Guinea pigs that had been anointed with the DMSO solution of II were given sarin subcutaneously at 200 ml/kg and immediately thereafter atropine sulfate intramuscularly at 15 mg/kg. Similarly anointed rabbits were given sarin intravenously at 100 μg/kg and then atropine sulfate intramuscualrly at 15 mg/kg. For the guinea pigs, the shortest interval between skin application of II in DMSO and sarin administration at which death occurred was 12 h. For the rabbits, the corresponding time was 5 h. It is apparent, therefore, that DMSO can facilitate the movement through guinea pig and rabbit skin of sufficient II to maintain protective blood concentrations of II for reasonable times.

The LD_{50} of I, injected intravenously, for rabbits was decreased by prior intravenous injections of d-tubocurarine at 0.1 mg/kg or atropine sulfate at 2 mg/kg by 63.4% and 9.3%, respectively.[44] Prior intravenous injection of neostigmine bromide at 0.1 mg/kg increased the LD_{50} by 31.2%. These findings indicate that I exerts its principal action on the neuromuscular junction in skeletal muscles and has only a minor effect on muscarinic cholinergic junctions. This conclusion agrees generally with that from Kalser's work. It is reinforced by reports that I blocks transmission in the isolated rat phrenic nerve-diaphragm preparation;[45] that it antagonizes the stimulant action of acetylcholine on isolated frog muscle;[46] that it decreases the response of the frog rectus abdominis muscle to decamethonium and to carbamylcholine, in addition to the response to acetylcholine, but in large concentrations increases partial blockade of transmission in the rat phrenic nerve-diaphragm preparation induced by prolonged exposure to decamethonium;[47] and that it increases, and in high concentrations decreases, the endplate potential.[48]

Wagley[48] found that V produced a dose-related decrease in the endplate potential of the curarized iliofibularis muscle of the frog at 1-3 x 10^{-2} M. A similar effect of 2-PAM I was found at 1-3 x 10^{-3} M, whereas concentrations below 10^{-3} M produced dose-related increases in the endplate potential. Fleisher et al.[47] found that III, unlike I, had no excitatory action on the isolated frog rectus abdominis, but had a more potent effect than I in inhibiting the response of that muscle to decamethonium, carbamylcholine, and acetylcholine.

Kunkel et al.[49] found that intravenous administration of III at 5 and 10 mg/kg had no effect on the response of the cat gastrocnemius-soleus muscle group to supramaximal indirect stimulation at either slow (0.5/s) or tetanizing frequencies. An intravenous dose of 20 mg/kg produced a marked temporary decrease in the response to a tetanizing frequency without altering the twitch response. An intravenous dose of 40 mg/kg almost abolished for 25 min or more the response to delivery of a tetanizing frequency to the motor nerve and decreased by about 80% the tension developed in the twitch response. That dose of III also blocked partially the response of the heart to stimulation of the vagus nerves, but did not modify the changes in blood pressure induced by bilateral occlusion of the carotid arteries or by intravenous injection of acetylcholine chloride or epinephrine chloride at 3 μg/kg. The effect of III, like that of I, seems to be predominantly on nicotinic cholinergic junctions. The effects of TMB-4 Cl_2 on the response of the cat heart to stimulation of the vagi and on contraction of the nictitating membrane after preganglionic stimulation of the superior cervical ganglion were more marked than those of the same intravenous dose (15 mg/kg) of I.[50]

Dultz et al.[23] infused V intravenously into dogs at 50 mg/kg per minute and I at 30 mg/kg per minute. The mean times to death after infusions of the two oximes were 10 and about 33 min, respectively. These times correspond with relative lethal doses of 100 and 196.5, respectively. These values are not far from those for intraperitoneal injections of the two oximes into mice.[100,206] After V, the heart rate rose initially and then, after about 3 min of infusion, began a slow decrease. The diastolic pressure decreased progressively from the start of infusion, whereas the systolic pressure remained fairly steady during the first 4 min of infusion and then began to decrease sharply. The rate of breathing and the tidal volume were reasonably constant during the first 5 min of infusion; the rate of breathing then increased, after an initial brief decrease, to nearly 3 times its original value by 9.5 min after the start of infusion. During the same period, the tidal volume decreased to about one-tenth its original value. At 10 min after the start of infusion, the animal became apneic, the pulse pressure fell to zero, there was a brief period of arrhythmia associated with an elevation of the J segment of the ECG, and then the dog died.

The most striking changes during the early minutes of infusion of I were increases in systolic and pulse pressures. These were accompanied by an increase in breathing rate without much change in tidal volume or heart rate. After about 28 min of infusion, systolic pressure and heart rate began to decrease. About 3 min later, both systolic and diastolic blood pressures fell precipitously with heart rate and tidal volume. Breathing rate had begun to decrease sharply after about 28.5 min of infusion. Terminally, the T wave of the ECG was increased and prolonged, and the voltage of the QRS complex was markedly reduced. Death followed apnea by only a few minutes. The changes reported by these investigators suggest that V kills by CNS depression, whereas I kills by interfering with repolarization and contraction of cardiac muscle.

Ballantyne et al.[51] gave rabbits intramuscular or intravenous injections of II at the LD_{50}. They found that plasma II maintained at less than 90 μg/ml was not lethal. Concentrations of 90-160 μg/ml were not lethal if they persisted for only a few minutes. If concentrations in that range were maintained for 40-50 min, there might be a sudden increase to above 200 μg/ml. A plasma II concentration above that limit generally led to death. The II concentration in the aqueous humor of the eye increased slowly, but an hour after injection was nearly the same as that in the plasma. Thereafter, the II concentration in the plasma and in the aqueous humor decreased at similar rates.

TOXICITY OF REPEATED DOSES IN EXPERIMENTAL ANIMALS

Rats and rabbits received intramuscular injections of solutions of II in saline 5 d/wk for 11 and 9 wk, respectively.[52] Dogs were

given gelatin capsules of II by mouth 5 d/wk for 17 wk. Control groups of rats and rabbits were given physiologic saline intramuscularly on the same schedules as those which received II. No control dogs were included in the experiment; comparisons were made between the dogs given II and "normal" animals. The daily doses of II injected into rats were 50 and 150 mg/kg; those injected into the rabbits were 50 and 100 mg/kg. Dogs weighing 13-17 kg received daily doses of 1 g, or about 59-77 mg/kg.

The rats given II seemed to be normal both grossly and by microscopic examination of sections of tissues at the end of the study. The rabbits had no abnormality clearly attributable to II other than purulent, indurative myositis at the site of injection in nine of 10 animals. Inasmuch as the solutions injected were not stated to have been sterilized, the myositis is not astonishing. The stomachs of the three dogs all had fibrosed mucous membranes at the cardiac and/or pyloric regions. The plasmas of these dogs had subnormal concentrations of albumin and total protein and low albumin:globulin ratios.

The rats given either of the doses of II grew normally, and indeed somewhat more than the controls. The rabbits, as is not unusual, had coccidial infestations of their livers and intestines, but had no lesions attributable specifically to the oxime. The plasma II concentrations of the dogs shortly before they were killed at the end of the exposure period were around 60-90 µg/ml. One of the three dogs had roundworms (Toxascaris leonina) in its intestines and granulomatous nodules in its kidneys and pancreas due to this infestation.

The same investigators made a comparative study of the toxicities of I and TMB-4 Cl_2 in rabbits and dogs, published as an internal report by FOA 1 (C1024-F100) in April 1963 and as a paper in April 1964.[53] Groups of eight rabbits received intramuscular injections 5 d/wk for 12 wk of I at 65 mg/kg, TMB-4 Cl_2 at 30 mg/kg, or physiologic saline at 0.2 ml/kg. The solutions were sterilized by filtration through a Seitz filter. Groups of four young beagles (9-11 kg) were given capsules containing 1 g of TMB-4 Cl_2 or of I 5 d/wk. For TMB-4 Cl_2, this dose was continued throughout the 15 wk of the study; for I, the daily dose was reduced to 0.75 g after the first 2 wk and kept there until the end of the 15-wk study.

Except for local changes at the site of injection, the rabbits given I intravenously suffered no detectable toxic effect other than a minor loss of weight. Those given TMB-4 Cl_2 began to die during the third week of the study, six of eight rabbits having died by the end of the 15-wk period. The blood of the rabbits given either oxime was seen to clot rapidly, the effect being more marked after TMB-4 Cl_2 at 30 mg/kg than after I at 65 mg/kg and lasting for 4-5 h after an intramuscular injection. The dogs given capsules of TMB-4 Cl_2 had no signs of intoxication by the oxime, whereas those given capsules of I had diminished activity, ataxia, and head drop starting

2-3 h after they were given their capsules. After the daily dose of I was reduced, these signs of intoxication disappeared; they became evident again in three of the four dogs during weeks 10-12 of the study. The signs of renewed intoxication appeared on only a few days in each dog and then disappeared again, although administration of I continued. The curves of gain of body weight by the dogs were unaffected by the oximes, and all were judged to be in a normal state of nutrition at the end of the 15-wk period.

At the sites of injection of oxime in the rabbits, various extents of hemorrhage and of purulent, indurative myositis and muscle necrosis were seen. The rabbits that received physiologic saline and five that received intramuscular injections of 2.15 M sodium chloride on 8 d in a satellite experiment had waxy degeneration of muscle at the site of injection. Five rabbits that received intramuscular injections of 2.15 M I on 8 d also had waxy degeneration of muscle at the site of injection. This was stated to be more extensive than that seen in the rabbits given equimolar sodium chloride. No other lesions in the rabbits that seem to be attributable to the oximes were described.

The dogs given capsules of the oximes were found to have hyperemia in their stomachs and intestines. Four of the eight dogs had erosions of gastric mucosa, evident particularly in the apices of the rugae in the fundus and found in animals given I as well as in those given TMB-4 Cl_2. All the dogs had epithelial defects and proliferation of the connective tissue of the lamina propria. Atrophy of stomach glands was seen sometimes. The brains of both the dogs and the rabbits were described as having peculiar, slightly granular, basophilic structures in the white matter of the brain stem. These could appear as spheres or clouds. The figure purporting to demonstrate these structures does not do so clearly enough to permit a guess as to their nature; they may be nothing more than fixation artifacts.

Inasmuch as myositis was reported in the second paper[53] as well as in the first,[52] this response must be induced by the oximes and not by bacteria. The second paper showed that oral doses of I and TMB-4 Cl_2 induced the same sort of scar formation as II in the gastric mucosa, so that this response may be induced by either the oximino group or the quaternary nitrogen atom. It would be informative in this regard to have the results of an experiment in which capsules of pyridine-2-aldoxime and of N-methylpyridinium chloride were administered in a similar fashion.

The toxicities of repeated intravenous doses of I and II in rabbits and dogs have been estimated.[54] Six rabbits were given I at 50 mg/kg 5 d/wk for 6 wk; four rabbits were given II at the same dose on the same schedule. Three dogs were given I at 25 mg/kg b.i.d. 5

d/wk for 6 wk; three other dogs were treated similarly with II. During the 6 wk of the experiment, 91 observations of signs of toxicity were recorded for the dogs given II and 115 for those given I. In both groups of dogs, the most common observation was hyperventilation. This sign accounted for 60% of the observations recorded for the dogs given I and 78% of those for the dogs given II. The next most common sign for I was ataxia; for II it was vomiting. Collapse was the third most common sign with both oximes, but accounted for less than 6% of all the observed signs of toxicity. Tremor and jerking of the head were equally frequent signs of intoxication in fourth place with I, whereas tremor and ataxia had equal incidences in fourth place among the dogs given II. The only other sign reported was salivation, which accounted for less than 2% of the total of recorded signs. Hyperventilation, mentioned above as the most frequent sign of intoxication, was stated to occur only during injections of the oximes and to stop immediately after the end of the injection.

All the dogs given intravenous injections of an oxime had decreases in their blood concentrations of hemoglobin (mean, −23.2%). Two of the dogs given I had increases in their hematocrits, whereas only one of the dogs given II had this sort of change. All three dogs given I had decreases in their leukocyte counts, whereas two of the dogs given II had increases in their leukocyte counts. Of the four dogs that had decreases in their total leukocyte counts, two had increased percentages of polymorphonuclear leukocytes and two decreased percentages. The two dogs with increased percentages of PMN leukocytes had subnormal percentages of lymphocytes. The two dogs with decreased percentages of PMN leucocytes had increased percentages of lymphocytes.

Two rabbits died after the third dose of I, and two other rabbits from this group died during a weekend after having developed diarrhea that lasted 2-7 d. One rabbit given II died after the fourth injection. Another rabbit in this group developed diarrhea during the fifth week and died during the following weekend.

Three rabbits and three dogs given I and two rabbits and three dogs given II were subjected to necropsy at the end of the experiment. No lesions attributable to the oximes were found. Other rabbits housed in the same areas as those used in this study were reported to have developed diarrhea and in some instances to have died, so that the deaths of rabbits during the experiment may have been unrelated to the experimental procedures. The decreased hemoglobin concentration and the diminished white-cell counts in dogs may have been induced by the oximes, but the larger dose of II given to rabbits by Albanus et al.[52] during a longer period did not result in decreases in hemoglobin concentration or in the red-cell count, although it may have induced a decrease in the white-cell count. Crook et al.[54] concluded that I and II have relatively low

toxicities for dogs and rabbits when they are given intravenously in daily doses of 50 mg/kg on 5 d/wk for 6 wk and that I is more likely to induce ataxia than II, but is less likely to induce vomiting.

A comparative study of the toxicities of I, III, and IV administered to rats by gavage and to dogs in capsules has been reported.[55] Groups of five rats were given daily doses of I, III, or IV at 20 mg/kg through intragastric catheters 5 d/wk for 4 wk. Wheezing was the most frequent observation, even occurring in the control group. Wheezing was observed more frequently among the rats given oximes than among the control animals; it was less frequent in the group given IV than in those given the other two oximes. Chronic inflammation of the lower respiratory tract was found in almost all the rats. Irritation of the eyes occurred in a few rats of each group, including the control group. Hematologic measurements, organ weights, body weights, food consumption, and gross and microscopic surveillance of organs and tissues at necropsy revealed no oxime-related changes.

The control dogs (given empty capsules on the same schedule on which other dogs received oxime-containing capsules) and most of those given oximes seemed to be in good health throughout the study. The exceptions were one dog in the group given I that exhibited fasciculations and tremors 10 min after receiving its first capsule, one dog in the group given III that retched 10 min after receiving its capsule on the twelfth day of the study, and one dog in the group given IV that retched about 4 h after being given its capsule on the ninth day of the experiment. No dogs died, and all gained weight at about the same rate as during a preliminary observation period. No alterations that seemed to be related to ingestion of the oximes were found in hematologic values, blood chemistry, urinalysis, organ weights, organ:body weight ratios, or gross and microscopic appearances of tissues and organs removed at necropsy.

An additional study of daily intravenous injection into rats and dogs of IV at 35 mg/kg 5 d/wk for 4 wk used groups of 10 rats and four dogs--two males and two females.[56] The only visible signs of toxicity observed in the rats were wheezing, hyperpnea, and “swelling in the throat.” Wheezing, recorded 11 times during the total of 200 rat-days, was the most common sign among the animals given IV; it was recorded once in the control group and was the only abnormal observation for that group. “Swelling in the throat” was recorded four times among the rats given IV, and hyperpnea three times.

The mean consumption of food and the mean rate of gain of body weight were significantly lower in rats given IV than in the control group, which was not given sham injections. Hematologic measurements disclosed no significant differences between the two groups of rats. The mean liver and kidney weights were reduced in the rats given IV; the mean adrenal weight in these animals was identical with that in

controls. When organ:body weight ratios were calculated, the ratios for liver and kidney in rats given IV did not differ significantly from those in the control rats. The ratio for the adrenals in rats given IV was above that for the control group. One concludes that the anorexia resulting from injection of IV into the rats affected the weights of the liver and kidneys in proportion to its effect on the overall body weight, but did not alter the size of the adrenals.

The only sign of toxicity recorded for the dogs given IV was retching, which was recorded six times during the 80 dog-days of observation within the second, third, and fourth weeks of the experiment, but not during the first week. The dogs given IV lost 2.34% of their original mean body weight during the experiment, whereas the control dogs lost only 1.68%. One bitch among the dogs given IV had increased alkaline phosphatase and transaminase activities in its serum after the fourth week of the experiment, but not after the second week; microscopic study of sections of this animal's liver revealed degenerative changes in the periphery of the lobules. The livers of the other three dogs in this group were free of significant pathologic findings, so that the changes in the liver of one of the bitches may have been unrelated to administration of the oxime. No other significant pathologic findings were reported, except for the finding of intestinal parasites (hook worms, round worms, tape worms, or whipworms) in most of the dogs. Hematologic, blood chemical, and urinanalytic studies revealed no significant differences between the control group and that given IV other than those mentioned above for one bitch.

The toxic effects of 2-PAM I and III on rabbits and dogs, given intravenous injections 5 d/wk for 6-8 wk, have been evaluated.[57] Both rabbits and dogs received daily doses of 2-PAM I at 30 mg/kg and of III at 10 mg/kg. Two dogs and two rabbits received intravenous injections of physiologic saline on the same schedule followed for injecting oximes. Groups of three dogs and three rabbits were given the oximes. All animals were observed for signs of toxic effect after the injections and were weighed weekly. Hematologic and blood chemical studies were performed on the dogs. Rectal temperatures of the dogs were measured once each week. All animals were subjected to necropsy at the end of the study. Samples of abdominal and leg muscles and of blood from the dogs were analyzed for oximes.

All rabbits gained weight during the 6-wk periods of injection of 2-PAM I (0.26-0.84 mg/kg) and of III (0.18-1.14 mg/kg). The dogs ate well and seemed to be in good physical condition during the 8-wk period of injection of 2-PAM I. Two dogs maintained their original body weights, and the third gained about 0.5 kg. The dogs neither lost nor gained weight during the 6-wk period of injection of III.

Neither the rabbits nor the dogs gave visible evidence of toxic effects during the experiment.

The only indicator that suggested an effect on the composition of the blood of the dogs was the white-cell count after 8 wk of injection of 2-PAM I. This increased by a mean of 27.6%, whereas it decreased by 5.5% in the control group during the same period. If the plasma concentration of an oxime 5 min after intravenous injection was taken as the initial value, at 30 minutes it had fallen to 21.8% (2-PAM I) or 28.3% (III) and at 60 min it had fallen further to 10.7% (2-PAM I) or 14.4% (III). It is apparent, therefore, that III is removed initially from the plasma somewhat more slowly than 2-PAM I, but that the difference is not great and that there may be an increase in the rate of removal of III somewhere between 30 and 60 min after the initial value. The percentages of the initial concentrations of 2-PAM I and III remaining in the plasma at 155 min after estimation of the initial values differed by only about 0.1%

Abdominal and thigh muscles examined 20 h after the last intravenous injection of oxime contained no detectable amount of either oxime. Samples of muscles collected 30 and 90 min after the last dose of oxime contained 2-PAM I at higher concentration than III. At both these times, thigh muscle contained a higher concentration of 2-PAM I than abdominal muscle, but a lower concentration of III. Dogs and rabbits appear, therefore, to tolerate repeated daily intravenous doses of 2-PAM I at 30 mg/kg or of III at 10 mg/kg during a period of 6-8 wk when the daily doses are suspended during each weekend. Because in this and the other studies reviewed the animals were killed at or soon after the end of the period of administration of an oxime, there has been no opportunity to judge whether repeated administration of an oxime may initiate some alteration in normal structure or function that will result eventually in a definite lesion. No truly chronic study of the toxicity of an oxime has been found. Thus, possible cryptic toxic effects of this type of compound have never been assessed.

SIDE EFECTS OF OXIMES IN MAN

Hopff and Waser[58] have listed mechanisms whereby reactivators of inhibited cholinesterase could be harmful to persons to whom they are administered in treatment of intoxication by anticholinesterase compounds. The following is a slightly modified version of their list:

- The reactivator may affect enzymes other than those involved directly in the actions of the inhibitor of cholinesterase.
- The reactivator may itself affect some part of the active center of cholinesterase.

- The reactivator may form, either with the inhibitor or with its residue in inhibited cholinesterase, a stable secondary inhibitor of cholinesterase.
- The reactivator or a stable complex between the reactivator and the inhibitor may affect important functional systems of the body other than those related to cholinesterase.
- The reactivator may be metabolized to harmful products.

The last three mechanisms of action, and possibly the other two as well, are involved in the causation of the side effects that have been reported to occur either in normal subjects to whom reactivators had been administered during research projects or in patients who had been given reactivators as therapy for intoxication by pesticidal anticholinesterases or other inhibitors of cholinesterases.

Some side effects seem to be common to all six reactivators with which this report is concerned. For example, complaint of a bitter, metallic, salty or musty taste has been made by people who have been given any of the six oximes, whether by mouth or by injection. Intravenous injections of any of the oximes, if the solutions were too concentrated or were given too rapidly, have resulted in pain along the vein. Dizziness, nausea possibly progressing to vomiting, blurred vision with impaired accommodation, and muscular weakness have been complained about often. Convulsions have been reported after V.[59,63] Moderately marked increases in systolic and diastolic blood pressures with increased pulse pressures and tachycardia have been reported to follow intravenous administration of 2-PAM I, I, and II.[59,63,69,72,73] The increases after II were not as great as those after I. The two bisquaternary bis-oximes (III and IV) may produce initial increases in blood pressure, but these are followed by marked and prolonged hypotension, the pulse pressure being reduced progressively after administration of the oxime.[72,77] Obidoxime given by mouth did not alter blood pressure.[81]

Another symptom of some practical importance is gastrointestinal distress, evident especially when oximes were given repetitively during several days. This symptom has been particularly bothersome with II and III,[72,80] but has been reported after large doses of I also.[76] III and IV have induced symptoms that suggested local anesthetic effects: sensations of heat or coolness in the nose and throat, circumoral numbness, and facial paresthesia.[72,73,77,80] III has given rise to icterus, petechial bleeding with increases in prothrombin time and sedimentation rate, increases in serum alkaline phosphatase, glutamic-oxaloacetic transaminase, and glutamic-pyruvic transaminase activities, an increase in serum bilirubin concentration, and a fine macular rash on the face and arms.[72] IV may have resulted in cholestatic hepatosis,[73] but 11 persons (six men and

five women) given two intramuscular injections of 250 mg of IV 2 h apart on one day had no significant increases in their serum alkaline phosphatase, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, glutamate dehydrogenase, or sorbitol dehydrogenase activities thereafter.[78] Also, four oral doses making a total of 7.36 g of IV on one day induced no significant alterations in serum glutamic-oxaloacetic and glutamic-pyruvic transaminase activities in 10 people who were given that dose, although four of them complained of some side effects.[81] Facial paresthesia and headache were the principal complaints of the 13 subjects, in a group of 24, who mentioned symptoms after oral doses of 1.84-7.36 g of IV.

Sidell et al.[80] reported that intramuscular injection of I or sodium chloride into normal human subjects resulted in increases in serum creatine phosphokinase activity. When the concentrations of the solutes in the solutions were expressed as milliosmols per liter, sodium chloride and I yielded nearly parallel relationships between the increase in serum creatine phosphokinase activity and the amount of solute administered, I being 2.4-3.5 times as active as sodium chloride in inducing an increase in activity. The data furnished by these investigators indicate that, when the volume of solution injected into muscle was constant, the release of creatine phosphokinase from the muscle was related directly to the osmolarity of the solution; when the osmolarity was kept constant, the amount of creatine phosphokinase released was a function of the amount of material injected into the muscle. A graph of the increase in serum creatine phosphokinase activity as a function of the amount of I injected extrapolates to a point close to the origin, whereas the line of the increase in serum creatine phosphokinase activity as a function of the concentration of sodium chloride injected extrapolates to zero at a concentration of 4%, indicating that I had an effect on the integrity of cellular membranes beyond that due simply to osmotic relationships. The report by Wedd and Burgess[61] that intramuscular injection of a 25% solution of II produced no more damage than intramuscular injection of a 7.5% solution of sodium chloride suggests that II may not have the extra damaging effect on cellular membranes that I seems to have. In this regard, consideration should be given to the possibility that the biochemical indicator of effect used by Sidell et al. may be more sensitive than the histologic indicator used by Wedd and Burgess.

Obidoxime has been stated[81] not to have any local irritant action when injected intramuscularly; it did produce facial paresthesia, headache, a sensation of coolness in the mouth, generalized weakness, nausea and vomiting, pallor, pyrosis, and sore throat in 13 of 24 subjects given tablets of IV in doses of 1.84-7.36 g. Administration of IV in tablets with enteric coatings probably did not alter the incidence of side reactions--only four subjects and two doses of the oxime were used in this part of the study.

MODIFICATIONS OF NORMAL FUNCTIONS BY OXIMES

The oximes were developed originally as reactivators of inhibited cholinesterase by evolution from hydroxylamine through hydroxamic acids to oximes. Kinetic studies with hydroxamic acids indicated that the hydroxamate ions were the reactivating agents.[82] On the assumption that the anionic site in inhibited cholinesterase is still operational, so that a positively charged site in a reactivator could be used as a directing group to guide the active group in a molecule of a reactivator to the inhibited esteratic site of the enzyme, compounds containing quaternized amino groups were made. This chemical change in, for example, pyridine-2-aldoxime also lowered the pK of the resulting pyridinium oxime and increases its ability to move into tissues. Methyl nicotinium hydroxamic acid iodide and later 2-PAM I--both having a quaternized nitrogen atom removed by about two carbon atoms from a hydroxylated nitrogen atom--were found to be effective reactivators of cholinesterase inactivated by organophosphorus inhibitors.[2,4,82] Although the truth of the basic assumption was not demonstrated until 1959,[84] the general success of quaternization in increasing the reactivating potencies of hydroxamic acids and oximes with pyridine skeletons made it almost certain before then. Oximes were found to be much more active than the corresponding hydroxamic acids.[83]

At about the same time, hydroxamic acids and oximes were found to react directly with organophosphorus compounds.[84,85] 2-PAM I was found to react in vitro with sarin with marked deviation from first-order kinetics; that suggested that the reaction actually consists of (at least) two sequential reactions. Green[86] showed that quaternized pyridine aldoximes react with an organophosphorus (OP) compound in three steps:

- Formation of a phosphorylated or phosphonylated oxime by reaction of the OP compound with the oximate ion.
- Hydroxylation of the phosphorylated or phosphonylated oxime to produce an N-alkylcyanopyridinium salt.
- Hydrolysis of the N-alkylcyanopyridinium compound to an N-alkylpyridone and hydrogen cyanide.

The second-order rate constant for the reaction between sarin and either 2-PAM I or II was found to be 170 L/mol per minute. If a phosphorylated or phosphonylated oxime that does not enter rapidly into the second step above is formed, that product may be an inhibitor of cholinesterase.[87,88] Hydrolysis of sarin in the presence of 200-fold concentrations of V and II took place more rapidly in plasma from rats with the former oxime than with the

latter.[89] In human plasma, sarin was hydrolyzed more rapidly in the presence of II than in that of V.

The protective activity of IV against lethal intoxication by OP compounds has been thought to be exerted principally by promoting reactivation of inhibited cholinesterase.[90] IV has been found to produce either activation or inhibition of acetylcholinesterase, depending on the simultaneous concentrations of that oxime and of acetylcholine in the vicinity of the enzyme.[91] With acetylcholine at 5 x 10^{-4} M, concentrations of IV below about 3 x 10^{-4} M produced activation of cholinesterase; greater concentrations of the oxime inhibited cholinesterase. Increasing the concentration of acetylcholine to 10^{-2} M decreased progressively both the activating and inhibitory actions of IV. With the substrate at 10^{-2} M, the activity of the enzyme was not altered appreciably by IV at 5 x 10^{-6} M to 7 x 10^{-3} M. These findings suggest that acetylcholine and IV react with different, but interdependent, receptors on the molecule of acetylcholinesterase; the data contain no evidence of competition between IV and acetylcholine for a common receptor.

Holmes and Robins[45] reported that 2-PAM I overcame neuromuscular blockade induced by DFP, TEPP, or sarin, but this could be demonstrated with the isolated phrenic nerve-diaphragm preparation from the rat only after washing away excess OP compound. Intravenous injection of 2-PAM I overcame slowly neuromuscular blockade induced by OP compounds. The oxime, they found, also had a direct toxic action on muscle, reducing the ability of muscle to shorten and decreasing the ability of muscle fibers to conduct impulses. The same investigators reported[92] that V (intraperitoneally at 150 mg/kg) had little, if any, effect on a sarin-induced blockade of neuromuscular transmission.

Wills and Kunkel[93] studied a group of 46 compounds--d-tubocurarine chloride, decamethonium bromide, gallamine triethiodide, a series of quaternized bisoxamide salts, a group of tertiary and quaternary anticholinergic compounds, and a group of hydroxamic acids and oximes--for their ability to antagonize a sarin-induced blockade of neuromuscular transmission to the cat's gastrocnemius-soleus muscle group during indirect stimulation at a frequency of 1 shock every 2 s. They identified d-tubocurarine chloride, two of the oxamide derivatives, and a quaternary anticholinergic and antihistaminic compound as particularly active antagonists of sarin's effect. 2-PAM I and 2-PAM benzyl bromide had comparatively low activity. V had only slight, if any, activity in this test. When the frequency of indirect stimulation was raised to 40/s, 2-PAM I became much more effective in antagonizing blockade of neuromuscular transmission than an atropinium salt that had had about the same activity as 2-PAM I against blockade at the lower frequency of stimulation.

Fleisher[94] reported that both sarin and VX increased the sensitivity of the isolated frog's rectus abdominis to external application of acetylcholine and at the same time decreased the activity of cholinesterase in the external surfaces of the muscle cells. Sarin at 5 x 10^{-7} M reduced the threshold concentration of acetylcholine for inducing contraction of the muscle to 8% of that required before application of sarin. The same concentration of VX reduced the threshold concentration of acetylcholine to 6.7% of that needed previously. Contemporaneously, the activity of cholinesterase in the external surfaces of the muscle cells was reduced to 8.1% and 0% of that before application of sarin and VX, respectively. Addition of 2-PAM I at 5 x 10^{-4} M to the baths in which the muscles were suspended had little effect on the activity of the enzyme in homogenates of the muscles, but restored 75% and 91%, respectively, of the activity of cholinesterase in the external surfaces of the muscles exposed to sarin and VX. At the same time, the concentration of acetylcholine required to induce contraction of the muscles was raised to 53.3% and 58.3% of the original threshold concentrations, respectively, for the muscles exposed to sarin and to VX.

Rats poisoned by subcutaneous injection of VX at twice the LD_{50} were kept alive for 20 min with artificial ventilation of the lungs when necessary.[95] At 20 min after the dose of VX, some rats were given intraperitoneal injections of either atropine sulfate (7 mg/kg) or atropine sulfate plus 2-PAM I (17.5 mg/kg). Untreated rats were killed 20 min after injection of VX; samples of parotid gland, gastrocnemius muscle, and brain were collected for examination for cholinesterase activity. The treated rats were killed 3 h after treatment. Organ samples were collected and analyzed for cholinesterase activity. Reactivation of cholinesterase was calculated as 100 times the ratio of the difference between cholinesterase activities 3 h after therapy and 20 min after VX to the difference between cholinesterase activities in unpoisoned rats and in poisoned rats 20 min after VX; it is shown in Table 3 for the two modes of therapy. Because atropine has never been found to have reactivating activity in vitro, the reactivation that occurred in the rats treated with atropine sulfate is assumed to be spontaneous. It is apparent from Table 3 that addition of 2-PAM I to atropine increased cholinesterase reactivation by 40.5% in the parotid gland, by 127.8% in the gastrocnemius muscle, and by 8.2% in the brain. The especially large change in cholinesterase activity in skeletal muscle suggests that this may be the principal site at which 2-PAM I antagonizes inhibition of cholinesterase.

The finding by Fleisher et al.[47] that 2-PAM I facilitated response by skeletal muscle to acetylcholine and depressed responses to decamethonium and carbamylcholine, whereas III had only the depressant actions, suggests that 2-PAM has an activity that is not duplicated in III. The nature of this difference is not entirely clear.

TABLE 3 Recovery of Cholinesterase Activity in Parotid Glands, Gastrocnemius Muscles, and Brains of Rats Poisoned with Subcutaneous VX at 40 μg/kg and Treated 20 Min Later with Intraperitoneal Atropine Sulfate at 7 mg/kg or with Intraperitoneal Atropine Sulfate and 2-PAM I at 17.5 mg/kg[a]

	Reactivation, % of inhibition by VX		
Therapy	Parotid Gland	Gastrocnemius Muscle	Brain
Atropine sulfate	39.0	25.2	8.5
Atropine sulfate + 2-PAM I	54.8	57.4	9.2

[a] Samples of tissue were removed 3 h after therapy, or 20 min after VX if no therapy was administered. Groups of rats contained a mean of 8 (6-15) animals each.

Because 2-PAM adds to the depressant effect of d-tubocurarine on the response of skeletal muscle to indirect stimulation, as well as to those of decamethonium and carbamylcholine, the facilitating action cannot be based on depolarizing activity. Fleisher et al. suggested that the facilitating component of the action of salts of 2-PAM depends on the ability of this oxime to inhibit cholinesterase, rather than on a direct depolarizing action. The finding that salts of 2-PAM depress the response of eserinized muscle to acetylcholine and to the inhibiting action of d-tubocurarine points to the possession by 2-PAM of not only a cholinesterase-inhibiting action but also a competitive interference with acetylcholine uptake by the motor endplate analogous to that of d-tubocurarine itself. The accentuation by salts of 2-PAM of blockade of neuromuscular transmission by such depolarizing compounds as decamethonium and carbamylcholine would be analogous to that of d-tubocurarine in increasing Phase II blockade by depolarizing compounds. Although III is a more potent inhibitor of acetylcholinesterase in vitro than the salts of 2-PAM, its effects on muscle seem to be those of a competitor with acetylcholine for access to the motor endplate.

Edery[96] studied the effects of V on neuromuscular blockade induced by ethyl pyrophosphate or neostigmine methylsulfate in the cat, finding only an insignificant effect. The skeletal muscle responses to both direct and indirect excitation were altered by V in the absence of any other active chemical. The effect of V on the response of indirectly stimulated muscle to d-tubocurarine was an increase in the blockade of neuromuscular transmission similar to that of the salts of 2-PAM and of III.

Table 3 shows that cholinesterase inhibition in brain is affected only slightly by a dose of 2-PAM I that produces significant reactivation of the enyzme in muscle and parotid gland. Indeed, there has been considerable controversy about the ability of oximes to enter the CNS and reactivate inhibited cholinesterase there. Several clinical observations suggest that comparatively small doses of oximes have significant effects on cerebral function. For example, Schuchter et al.[97] reported a case of poisoning by parathion treated initially with atropine alone. After administration of 30.5 mg of atropine sulfate during the 14 h after admission to hospital, the apnea, convulsions, and arrhythmic tachycardia that had characterized the patient's condition on admission had stopped. The patient was still unconscious, however, with markedly constricted pupils. At that time, intravenous injection of 0.5 g of 2-PAM I in a 1% solution relieved both residual effects immediately, and no further treatment was required. Several similar reports are available in the medical literature; they suggest that, even though the quaternized oximes may not be able to cross the blood-brain barrier in large amounts, either the small quantities that penetrate that barrier are capable of affecting the function of crucial parts of the brain or the oximes

affect the functions of peripheral structures (perhaps receptors of sensory input) that modify the functions of crucial parts of the brain. The latter possibility is related to the proposal of Erdmann et al.[98] that restoration by 2-PAM I of the righting reflex after its abolition by parathion is effected by the oxime's modifying inhibition of cholinesterase in some peripheral site important for activity of the reflex.

Rajapurkar and Koelle[99] reported that intravenous V at 40 mg/kg, but not at 4 mg/kg, induced reactivation of cholinesterase in the surfaces of cells of the cat's superior cervical ganglion after the animal had been given DFP at 3.7 mg/kg 20 min earlier; there was no significant reactivation of the cholinesterase in the ganglion as a whole (after homogenization). These findings suggest that, even when an oxime is able to make contact with the surfaces of nerve cells, it is not able to penetrate into the neurons; this is similar to the situation for muscle cells described by Fleisher.[94].

Schaumann[100] found that pretreatment of mice with 2-PAM I reduced inhibition of acetylcholinesterase in brain by paraoxon much more effectively than those by DFP and 217-AO. The finding of some protection against all three OP compounds could depend on direct reaction between the last two inhibitors and the oxime, with a reduction in inhibition of the enzyme. A similar consideration applies to the report by Bisa et al.[101] that IV protected serum and brain cholinesterase from inhibition by paraoxon administered later at twice the LD_{50}. Although the same intraperitoneal dose of IV (7 mg) was found to protect the cholinesterase of rat serum and brain only incompletely from inhibition by DFP at 5 times the LD_{50}, that of serum recovered its normal activity by 20 h after the dose of DFP, whereas that of brain required 26 h for recovery.

On comparison of cholinesterase activities in the brain on various days after gavage with parathion at 35 mg/kg and after exposure of a homogenate of brain in vitro to 10^{-3} M 2-PAM I, the reactivation accomplished by the oxime was found[100] to decrease progressively as spontaneous reactivation increased:

	Brain Cholinesterase, % of normal	
Day	Before 2-PAM I	After 2-PAM I
0	6	100
1	20	59
4	44	79
8	63	76

In vitro, addition of IV and DFP simultaneously to red-cell cholinesterase[90] protected the enzyme against inhibition by DFP to a greater extent than the reactivation that the same concentration of the oxime (10^{-3} M) was capable of effecting after exposure of the enzyme to the same concentrations of DFP (1-3 x 10^{-7} g/ml). In guinea pigs, simultaneous injections of IV (intramuscularly at 100 mg/kg) and DFP (subcutaneously at 1.5 mg/kg) limited inhibition of red-cell cholinesterase to 20%, whereas the same dose of DFP alone inhibited it by about 65%. Half that dose of IV injected intramuscularly 90 min after the same dose of DFP stopped inhibition of red-cell cholinesterase by DFP and returned the activity of that enzyme to 93% of its normal value by 24 h after the dose of DFP. The red-cell cholinesterase of guinea pigs that were not given oxime after the dose of DFP had risen to only 87% of its normal value by the twenty-first day after injection of DFP.

In dogs poisoned with soman (intravenously at 30 μg/kg) and treated with I at 104 mg/kg (intravenously 3 1/2 min after soman), the large dose of I stopped aging of inhibited cholinesterase and reactivated 24.0% and 35.6% of the red-cell and diaphragm cholinesterase activities, respectively. It failed to reactivate brain cholinesterase. Indeed, the brain acetylcholinesterase activity after the treatment with I was lower than that just before the injection of I. The last finding indicates the inability of I to cross the bloodbrain barrier in significant quantities.

Apparently on the other side of the picture is a report by Meeter[104] that intraperitoneal injection of either III or IV at 40 mg/kg, given to rats when their body temperatures had fallen 2-2.5°C after intravenous injections of DFP at 1.2 mg/kg, blunted the fall, which might proceed in untreated rats to a decrease of nearly 6 °C. These doses also shortened the return of body temperature to its normal value. Larger doses of the same oximes blunted the fall less than the smaller dose, but had more effect on the rate of recovery of body temperature to its normal value. From the fact that atropine also is a good antagonist of the hypothermic action of OP compounds, whereas an equivalent dose of methyl atropinium nitrate has essentially no antagonistic activity, the hypothermia seems to result from some effect of an OP compound in the CNS. On this basis, one supposes that the moderate doses of III and IV antagonize some effect of OP compounds on the CNS that leads to hypothermia and that larger doses of the same oximes exert initially a toxic action that may actually increase hypothermia. As the concentration of the oxime in the body falls back toward that established by the modest dose, the antagonistic activity becomes evident. It would be useful to know whether oximes are able to increase the partial antagonism of inception of hypothermia that atropine has been found to exert.

A dose of IV given intramuscularly to atropinized guinea pigs 0.5 h after a sublethal dose of sarin produced less reactivation of retinal and brain cholinesterase than an equimolar dose of I.[105] A reactivation of 23.9% of the inhibited cholinesterase in the retina by I was accompanied by one of only 3.7% in the brain. If IV produces less reactivation of inhibited cholinesterase in the brain than I, any central mechanism related to the induction of hypothermia by OP compounds must be either very sensitive to the concentration of acetylcholine within the brain or more permeable to the quaternized oximes than the brain in general.

Atropine has been found[106] to reduce markedly the increase in the concentration of total brain acetylcholine in rats later given paraoxon at 0.4 mg/kg. Rats given a dose of paraoxon and than treated with IV intraperitoneally had brain concentrations of free and total acetylcholine that were essentially the same as those in rats given paraoxon alone, but no tremors or convulsions were observed. These animals survived; those given paraoxon alone all died in convulsions within 3-8 min.

Bajgar et al.[107] found that acetylcholinesterase activity in the pontomedullary area of the mouse brain was possibly a direct linear function of the dose of IV (2.5-35.0 mg/kg) injected intramuscularly with a constant dose (21 mg/kg) of atropine sulfate 30 s after an intramuscular dose (400 μg/kg) of sarin. They found no significant relation between the dose of IV and acetylcholinesterase activity in mesencephalon, diencephalon, and basal ganglia in the same experiments. Mortalities in the groups of mice given various doses of IV seemed to be inversely related to the dose of the oxime and, therefore, to cholinesterase activity in the pontomedullary region. Vasi et al.,[108] using armin as the OP compound, found that IV (intraperitoneally at 25 mg/kg 5 min after subcutaneous injection of armin at 0.4 mg/kg) resulted in a cholinesterase activity in the pontomedullary region of the rat's brain 45.3% of that in control animals, whereas this region in rats given armin alone contained only 18.7% of the cholinesterase activity of controls. The decrease in cholinesterase activity was accompanied by increases in the total acetylcholine concentration in the pontomedullary region of 52% in the rats given armin alone and of 25% in those given both armin and IV.

2-PAM I seems to be less effective than IV in reactivating brain cholinesterase after its inactivation by paraoxon or other OP compounds.[109] There was early evidence that 2-PAM I produced more reactivation of cholinesterase in the pontomedullary region and the area postrema that had been inhibited by paraoxon than in the cerebellum and the cerebral cortex[110] and that it could prevent the appearance of grand mal-like discharges in EEGs of rabbits after doses of sarin that evoked such discharges in rabbits not protected

with 2-PAM I.[111] This report by Longo et al. is not completely satisfying in indicating penetration of a quaternary oxime into the brain, inasmuch as the protective effect could arise from direct reaction between the oxime and sarin in the blood; in that case, only a part of the sarin administered would be available to affect cholinesterase in the brain and other tissues. The protective effect of the dose of 2-PAM I (30-50 mg intravenously in rabbits weighing 2-3 kg) was found to be overcome by doubling or tripling the dose of sarin. This finding suggests that the protective action of the 2-PAM I was due primarily to direct reaction with sarin.

In the final study to be mentioned in regard to the possible penetration of pyridinium oximes into brain, anesthetized, atropinized cats were given intravenous injections of sarin at 27 μg/kg.[112] Thirty minutes later, to allow clearance of unreacted sarin from the tissues, some cats received saline injections into one common carotid artery and others received similar injections of I at 15 mg/kg. The cholinesterase activity of cerebral cortex was measured. In animals given I, nearly 20% of the cholinesterase in their cerebral cortices that had been inhibited by sarin was calculated to have been reactivated by the oxime.

The only functional changes noticed in cats and dogs (anesthetized with sodium pentobarbital) after intravenous 2-PAM I at 5-60 mg/kg were stimulation of respiration (principally increased depth) and an increase in pulse pressure.[113] The mean blood pressure, after a small dip immediately after the injection, returned approximately to the control value. With repeated doses of 5 and 10 mg/kg, the heart rate decreased after each dose and then returned gradually to its normal value. After a cumulative dose of 100 mg/kg, there was marked lowering of the heart rate and a decrease in the voltage of the T-wave of the ECG. Isolated hearts from rabbits perfused with 2-PAM I at 1.3-2.7 g/L in the Ringer-Locke solution underwent no alteration of normal activity other than a slight decrease in the rate of beating, even when the perfusion was continued for more than an hour. In cats, intravenous injection of 2-PAM I at 40 mg/kg or more markedly increased the peristaltic activity of the intestines. Atropine stopped this effect.

Supramaximal indirect stimulation at 30/min of the cat's gastrocnemius-soleus muscle group was blocked by close intra-arterial injection of 2-PAM I at 80 mg/kg. By the intravenous route, the oxime at 80-100 mg/kg induced a persistent increase in the tension developed during a twitch. An intravenous dose of 300 mg/kg resulted in a brief increase in the tension developed during a twitch followed by a decrease in the tension lasting for about 2 min. This dose resulted also in a sequence of changes in blood pressure: brief hypertension, hypotension with increasing pulse pressure, and slight hypertension with greatly increased pulse pressure. Respiration, recorded from the

pressure in the side arm of a T-shaped tracheal cannula, went through a sequence of changes similar to those in blood pressure: a brief increase in depth of respiration, a brief decrease in depth of respiration, increasing depth of respiration with an increased rate, and greatly increased depth of respiration at a rate somewhat below the original rate. Mydriasis and muscular fasciculation were observed also after this large dose of 2-PAM I.

Blockade of neuromuscular transmission produced by an intravenous dose of d-tubocurarine chloride at 0.3 mg/kg, but not that due to 0.5 mg/kg, was antagonized by intravenous 2-PAM I at 5 mg/kg. Edrophonium bromide (intravenously at 0.2 mg/kg) was a more potent antagonist of d-tubocurarine than this dose of 2-PAM I. Partial blockades of neuromuscular transmission induced by intravenous injections of decamethonium bromide at 20 μg/kg, neostigmine bromide at 1 mg/kg, or succinylcholine chloride at 50 μg/kg were intensified by intravenous injection of 2-PAM I at 5 mg/kg, in about the same way in which they were enhanced by intravenous doses of edrophonium bromide at 0.2 mg/kg. Intravenous doses of 2-PAM I at up to 150 mg/kg had no effect on the muscle response to direct stimulation in anesthetized cats.

Doses of 2-PAM I larger than 40 mg/kg produced brief blockade of the cardiac response to peripheral vagal stimulation and of the nictitating membrane's response to preganglionic, but not to postganglionic, stimulation of the cervical sympathetic trunk. Reactions in which postganglionic adrenergic effectors participate--such as mydriasis, pressor response to injected acetylcholine by atropinized animals, pressor response to injected epinephrine, and pressor response to bilateral occlusion of the common carotid arteries--were augmented definitely by intravenous injection of 2-PAM I at 20 mg/kg or more. No significant effect of 2-PAM I on the EEGs of curarized cats was recorded.

In summary, 2-PAM I was found by Kunkel et al.[113] to act as a depolarizing compound at the neuromyal junction and to have acetylcholinomimetic properties in large doses. It had some ganglionic blocking activity, but no direct effect on the CNS was detected.

A later paper presented the results of a study of the mechanism of the sympathomimetic cardiovascular actions of I.[114] Cats and dogs anesthetized with allobarbital-urethane were used for measurements of pressure near the bifurcation of the descending aorta and of blood flow with electromagnetic probes. Intravenous injection of I at 20 mg/kg was found to produce an immediate, sharp increase in blood pressure lasting for about 25 s and followed after a lag of about 6 s by an increase in blood flow. A slow drift downward of the peak systolic pressure followed. Repetition of the dose of I after an hour yielded responses similar to those after the first dose, but

with a somewhat larger and more persistent immediate effect on blood flow. Calculation of the peripheral resistance and graphing of simultaneous plots of peripheral resistance, peak systolic blood pressure, blood flow, and contractile force of the heart (from Walton strain-gauge arches attached to the outer surface of the ventricles) revealed that the peak systolic blood pressure paralleled more faithfully the peripheral resistance than it did any of the other variables and that the peripheral resistance increased more abruptly after a third dose of I at 30 mg/kg than after the second such dose, which in turn induced a larger increase than the first dose. The blood flow decreased to 55% of the original value during the 76 min between the peak flow after the second dose of I and the third dose. It increased only slightly after the third dose.

A dose of I at 30 mg/kg increased the effects of intravenous doses of epinephrine at 5 g/kg and of dl-norepinephrine at 10 μg/kg on both blood flow and blood pressure. Intravenous phenoxybenzamine at 15 mg/kg plus tolazoline at 2 mg/kg prevented almost completely the actions of I on blood pressure and blood flow. Intravenous reserpine at 2 mg/kg increased markedly the effects of I at 30 mg/kg on blood pressure and peripheral resistance, but converted the usual immediate, small, temporary increase in blood flow into an immediate, small, temporary decrease. These various responses would be expected from either a mild sympathomimetic amine or an inhibitor of the breakdown of endogenous catecholamines. Indeed, I at 10^{-4} M, was found to inhibit the monoamineoxidase of the rat's liver. If the dose of I used in these experiments were distributed into the same fraction of the body water as that estimated for the human body,[115] the concentration in the plasma would be about 9 times that stated above as the effective concentration for inhibiting the monoamineoxidase. It is possible that inhibition of monoamineoxidase by I plays a part in inducing the effects of the oxime on blood vessels and blood pressure. It is possible also that I interferes with reuptake of catecholamines by nerve endings; this possibility seems not to have been explored.

Another study of the effects of I on the cardiovascular system[116] concluded that, in dogs anesthetized with sodium pentobarbital, the response of blood pressure to intravenous administration of I is a resultant of two separate effects: a direct myocardial stimulation that was stopped with dichloroisoproterenol and a stimulation of vascular smooth muscle that results in a slight increase in renal arterial pressure and a slight decrease in renal arterial flow. Neither atropine nor dichloroisoproterenol affected these vascular effects. Injections of I into a jugular vein or a renal artery had no consistent effect on catecholamine concentrations in plasma taken from a femoral artery or a renal vein. In seven experiments in which I at 21-35 mg/kg was injected into a jugular vein, the mean blood pressure increased from 176/125 ± 22/11

to 186/124 ± 53/39 mm Hg, and the mean concentration of catecholamines in the plasma of femoral arterial blood went from 1.7 ± 0.7 to 1.9 ± 1.3 μg/L. In three experiments in which 100 mg of I was injected into the renal arteries of canine kidneys that weighed a mean of 62.3 g (body weights not stated), renal blood flow changed from a mean of 233 ± 2.9 ml/min to 227 ± 8.2 ml/min. Mean catecholamine concentrations in renal arterial blood changed from 1.5 ± 0.3 to 2.1 ± 0.4 g/L of plasma, and those in renal vein blood from 1.5 ± 0.5 to 2.0 ± 0.3 μg/L.

Another study of the hypertensive action of I was made by DiPalma and associates.[117] Dogs anesthetized with allobarbital-urethane and cats anesthetized with urethane or by section of the cervical spinal cord during anesthesia with ether were used. In 11 dogs, intravenous injection of I at 20 mg/kg induced an increase in mean blood pressure from 160/103 ± 28/22 to 201/130 ± 28/23 mm Hg at 2 min after injection; by 20 min after injection, the mean blood pressure was 185/119 ± 35/27 mm Hg. Phenoxybenzamine (intravenously at 1 mg/kg) and phentolamine (intravenously at 1 mg/kg) consistently blocked the hypertensive action of I when they were injected a few minutes before I. Hexamethonium (intravenously at 1-2 mg/kg 10 min before I) did not prevent the increase in blood pressure, but did eliminate the initial period of bradycardia and the initial spike of hypertension; slow development of an increase in blood pressure still occurred. Pentolinium tartrate (at 2 mg/kg), succinylcholine chloride (at 1 mg/kg), guanethidine (at 1-3 mg/kg), and d-tubocurarine chloride (at 1.2 mg/kg) were ineffective in preventing the increase in blood pressure.

Dogs given norepinephrine by infusion at 0.185-2.04 μg/kg per minute for long periods (9-35 h) until their blood pressures became approximately the same as they had been before the infusions began were then given intravenous injections of I at 20 or 40 mg/kg, tyramine at 0.1 or 0.5 mg/kg, or nicotine at 0.5 mg/kg.[118] The concentration of norepinephrine in carotid arterial blood was estimated before and at intervals after the injection of one of the test compounds. Except after the lower dose of tyramine, when the maximal increase in the concentration of norepinephrine in the blood appeared at 5 min after the injection, the peak concentration of norepinephrine in carotid arterial blood occurred at 3 min after injection. The release of norepinephrine induced by I at 40 mg/kg was about 1.35 times that produced by the lower dose. The release of norepinephrine induced by tyramine at 0.5 mg/kg was 1.47 times that by I at 40 mg/kg. The release by nicotine at 0.5 mg/kg was 1.59 times that by the larger dose of I. The action of I in inducing release of norepinephrine from tissues in which it has been stored, although it is slightly more prolonged (20 min vs. 15 min) than those of the classical releasers of norepinephrine, is much weaker than those of tyramine and nicotine.

Still, release of catecholamines may play a part in the genesis of hypertension after injection of a pyridinium oxime.

Dogs anesthetized with sodium pentobarbital were used in experiments in which the lungs were mechanically ventilated after the dogs' chests had been opened to place electromagnetic flowmeters around the ascending aorta and catheters into the left atrial appendage.[119] Dose-response curves for doses of I at 5-40 mg/kg in the absence and presence of blockade of β-adrenergic receptors (phentolamine), of blockade of β-adrenergic receptors (propranolol), or of depletion of catecholamines (reserpine) were constructed. The heart rate was decreased progressively by intravenous I at 5 and 10 mg/kg; larger doses of 20 and 40 mg/kg yielded serially smaller effects on heart rate. Phentolamine (1 mg/kg) increased the effects of the two lower doses of I on heart rate, but antagonized those of the two larger doses, converting the usual slight bradycardiac effect of 40 mg/kg into a mild tachycardiac one. Both propranolol (0.5 mg/kg) and reserpine (0.5 mg/kg during 3 d) tended to increase the bradycardiac effect of all doses of I. The increase in peripheral resistance induced by I reached significance only after the largest two doses; this effect was antagonized by phentolamine and was increased by both propranolol and reserpine. The stroke volume of the heart was increased progressively by the four doses of I used; this effect was antagonized to some extent by all three drugs and especially by the two blockers of adrenergic receptors. Cardiac output was increased progressively by increasing doses of I and also was antagonized to some extent by all three drugs. Propranolol and reserpine had especially marked antagonistic actions. Arterial pressure, which is a resultant of cardiac output and peripheral resistance, was increased progressively by the four doses of I. The hypertensive action was antagonized especially by reserpine and phentolamine, propranolol having only a minor antagonistic action, according to Barnes et al.[119]

Taken together with the results obtained by DiPalma's group,[117,118] the finding by Barnes et al.[119] of the particularly large effect of prior treatment with reserpine on the response of the arterial pressure to I seems to confirm the involvement of catecholamine release in this hypertensive response. It is clear, however, that I also has a direct inotropic effect on the heart, in that the increase in stroke volume was blocked only partially by any of the three possible antagonists used by Barnes et al. In view of the fact that none of the possible antagonists was able to prevent more than about 68.5% of the increase in peripheral resistance induced by I, this oxime may well have direct stimulant effects on vascular smooth muscle.

III, unlike I, was found[49] to lower blood pressure and produce bradycardia in cats anesthetized with sodium pentobarbital. Partial

heart block and inversion of the T wave of the ECG followed the administration of large doses of this oxime. Moderate doses (5 and 10 mg/kg) produced no noticeable changes in peristaltic or respiratory activities; III at 20 mg/kg induced temporary decreases in both these activities. Doses of 40-80 mg/kg produced respiratory failure and spastic contraction of intestinal muscles. Neuromuscular transmission of either single or repetitive stimuli was not affected by doses of 5 or 10 mg/kg. A dose of 20 mg/kg resulted in a marked, temporary decrease in the tension developed during indirect tetanic stimulation, but was without effect on the twitch response of the gastrocnemius-soleus muscle group. Doses to and including 40 mg/kg did not alter responses to bilateral occlusion of the carotid arteries and to intravenous injections of acetylcholine or epinephrine at 3 μg/kg; 40 mg/kg partially blocked the cardiovascular response to stimulation of the peripheral vagi. This oxime was found to be an effective adjunct to atropine in both prophylaxis and treatment of intoxication by sarin and VX in anesthetized cats and dogs.

Bay[120] made a more extensive study of the efects of III on blood pressure, respiration, and ganglionic and neuromuscular transmission. Intravenous doses of 25 mg/kg or more decreased systolic and diastolic pressure in anesthetized cats and dogs. In the cat, a dose of 30 mg/kg slightly increased the pressor effect of epinephrine at 5 µg/kg and slightly decreased the depressor effect of acetylcholine at 3 µg/kg. III doses of 2.5-40 mg/kg were found to yield linear log dose-response curves for inhibition of effects induced by preganglionic stimulation of sympathetic and parasympathetic ganglia; the effect on the response to stimulation of preganglionic neurons by sympathetic ganglia was 1.4-2.2 times that on the response to similar stimulation by parasympathetic ganglia (the larger factor applies to the lower doses of the oxime). The transient hypotension was attributed largely to the action of III in blocking ganglionic transmission and partially to a direct relaxant action on vascular smooth muscle. Moderate doses of III temporarily increased the breathing rate and transiently decreased the tension developed during an indirectly stimulated twitch response by the gastrocnemius muscle. Doses of III greater than 50 mg/kg abolished the indirectly stimulated twitch response of the muscle and reduced its ability to maintain a tetanic response to repetitive indirect stimulation. The response of skeletal muscle to direct stimulation was not altered. Neostigmine, edrophonium, and K^+ ions antagonized, whereas d-tubocurarine increased, the effects of III on the neuromyal junction, which seemed to arise from competition with acetylcholine for access to the receptor.

In cats anesthetized with sodium pentobarbital, an intravenous dose of III at 25 mg/kg was found to be able to block almost comletely the cardiac response to stimulation of the peripheral right vagus nerve.[121] Small doses (2-8 mg/kg) did not inhibit peristaltic activity; in

fact, an increase in the rate of contractions was seen. II and V had effects on response by the heart to stimulation of the vagus that were similar quantitatively to that of III, but were weaker. Intestinal contraction induced by vagal stimulation was blocked by III, but not by twice as large a dose of V. Lindgren and Sundwall[121] found that the response of the nictitating membrane to stimulation of the cervical sympathetic chain was almost unaffected by III; thus, they differed with Bay on the relative sympatholytic and parasympatholytic activities of III. These investigators concluded that III probably exerts its vagolytic action by competition with acetylcholine.

Wellhöner et al.[122] reported that intravenous injection of III at 25 mg/kg led to marked hypotension in rats, guinea pigs, rabbits, and cats. The hypotensive effect was particularly marked in old cats that had high blood pressures initially; it was not altered by bilateral vagotomy, evisceration, or removal of the carotid bodies, but was reduced or abolished by decapitation. These investigators suggested that the hypotension may result from some effect of the oxime on the CNS. They found also that prior doses of III prevented the hypertensive action of norepinephrine.

Kunkel et al.[50] found that both I and III at 15 mg/kg blocked the response of the heart to stimulation of the vagus nerve. Although I increased the depressor action of acetylcholine, III did not alter it. I increased the effects of injected epinephrine on blood pressure and contraction of the nictitating membrane, but decreased the response of the nictitating membrane to stimulation of the cervical sympathetic trunk. These findings fairly well localized the blocking action of this oxime to the ganglion itself. In contrast, III had no effect on the pressor action of injected epinephrine, partially blocked stimulation of the nictitating membrane by injected epinephrine, and blocked the response of the nictitating membrane to preganglionic stimulation of the superior cervical ganglion. On the basis of these observations, one would think that III affected the receptors in both the ganglion and the nictitating membrane. The pressor effect of acetylcholine in atropinized dogs was increased by I, but not by III. III decreased the pressor effect of bilateral occlusion of the carotid arteries in cats, whereas I did not. It is possible, therefore, that III affects the inferior petrosal ganglion, which includes the somas of the pressoreceptors in the carotid sinus, or the reticular formation of the pons and medulla, which contains the vasomotor area. Previously, the pontomedullary area has been mentioned as the part of the brain that is the most readily accessible to pyridinium oximes.[107,108,109 and 110]

Erdmann and Engelhard[123,124] reported that in vitro IV was a more potent reactivator of acetylcholinesterase inhibited by DFP or

paraoxon than I or III. IV also reacted directly with these OP compounds, particularly paraoxon, at high rates. IV was found to enter the CSF more rapidly and to a greater extent than I and to be excreted in the urine comparatively slowly (11% during 6 h). The maximal concentration of IV in the blood of the rabbit occurred about 20 min after an intramuscular injection. Removal of the oxime from the blood between 1 and 4 h after an intravenous injection amounted to about 80% of the oxime present 1 h after the injection. After a lethal dose of IV, there was somnolence, paralysis of the extremities, and terminal paralysis of respiration. A single intraperitoneal dose of IV at 150 mg/kg resulted in diffuse fatty infiltration of the liver, which disappeared within 1-2 d. No other important pathologic effects were reported.

Dilute solutions of IV (36 mg/100 ml) were not irritating to the eyes.[124] An isotonic solution containing 10 times that concentration of IV was not hemolytic. A solution containing 3.6% of IV was not irritating to the hairless skin inside the ear of the rabbit. IV at 10^{-4} M increased the ability of acetylcholine to stimulate the rectus abdominis muscle of the frog; a concentration of 3×10^{-3} M paralyzed neuromuscular transmission in the isolated rat phrenic nerve-diaphragm preparation. The isolated jejunum of the rabbit was stimulated fleetingly by IV, but this oxime relaxed segments of jejunum that had been caused to contract by acetylcholine or carbamoylcholine. Intravenous injection of IV into a cat at 15 mg/kg decreased the response of the heart to stimulation of the vagus nerve and completely blocked contractions of the nictitating membrane in response to stimulation of the cervical sympathetic chain. An intravenous dose of 50 mg/kg decreased blood pressure and respiratory activity. When mechanical ventilation of the lungs was provided, cats survived doses of 200 mg/kg. Repetitive administration on 3 successive days of 50-70% of the LD_{50} resulted in muscular weakness, swayback, and exophthalmos. Daily injection during 30 d of 30% of the LD_{50} had no evident deleterious effect on the health of the animals.

IV has been found[125] to antagonize stimulation of the isolated ileum by carbamoylcholine and ethyl arecaidinate (a homologue of arecoline) and to cause parallel shifts of the dose-response curves of each of these agonists. This oxime was considered to be a functional antagonist of cholinergic agonists, rather than a competitive one, because various mixtures of IV with the competitive anticholinergic drug, N-methylatropinium nitrate, shifted the dose-response curves of the agonists by amounts that were not proportional to the sum of the individual displacements by the two antagonists. The parasympatholytic action of IV is suggested, therefore, to arise from contact between IV and a specific receptor for that molecule different from the molecule(s) with which the agonists and the N-methylatropinium salt interact, but functionally interdependent with it. This interdependence may involve an allosteric mechanism, but that has not been demonstrated.

METABOLISM OF OXIMES

2-PAM I in dilute hydrochloric acid (0.1-0.4 N) is hydrolyzed in a first-order reaction.[126] The rate of acid hydrolysis at a constant H^+ ion concentration had a C_{10} of about 2 when the temperature was increased from 37.8°C to 47.6°C. The hydrolysis of 2-PAM I was not catalyzed by hydroxyl ions, but was rapid at high pH. The syn form of the oxime was converted to the anti form more rapidly at a pH of 13 than at one of 7 or 1.

Within the range of pH from 1 to 13, 2-PAM I was found to have its maximal stability with respect to hydrolysis in aqueous solvents at a pH of 1, whereas III was most stable at a pH of 3.[127] Even at the indicated pHs, 30% of 2-PAM I and 20% of III were hydrolyzed before equilibrium was reached. After the oximes apparently reached an equilibrium with their hydrolytic products, degradation of the oximes continued at a low rate. Ellin[128] suggested the following sequence of reactions during degradation of 2-PAM I in aqueous solution: the oxime is dehydrated to N-methylpyridinium-2-nitrile (A), which is converted to N-methylpyridinium-2-hydroxylate (B) and then to N-methylpyridinium-2-one, hydrogen cyanide being released during the conversion of A to B.

Creasey and Green[129] reported that II is soluble in water to the extent of 1 g in 2 ml; like I, it is more stable in aqueous solution at low pH, the pH of maximal stability being 4-5. When II in a solution at a pH of 4.5 had been freeze-dried and stored in a sealed glass container for 6 mo, no cyanide was detectable in the container when it was opened. Similar treatment of a solution at a pH of 6.0 resulted in detection of cyanide by odor when the container was opened. Heat sterilization of an aqueous solution of II was found to result in the formation of small amounts of cyanide and in a change in the color of the solution from pale yellow to orangebrown. A homogenate of liver from the rat was found to decompose II slowly; other biologic samples (whole blood, kidney, skeletal muscle, urine, and feces) did not.

Kondritzer et al.,[130] in searching for a salt of 2-PAM that would be more soluble than 2-PAM I in water, made a number of other salts. Most of these were considerably more soluble in water than 2-PAM I. One of the most soluble was the lactate, with a solubility of 1 g/ml. Unfortunately, this salt was found to be quite unstable in aqueous solution with respect to heat. I was less soluble in water than the lactate, but more than 13 times as soluble as the iodide. Furthermore, solutions of I at a pH of 3.5-4.3 could be autoclaved at 120°C for 15 min with only a 4% loss. Solutions of this salt stored at 50-70°C for 1-3 mo contained at least 78% of the original oxime and no more than 0.1% of hydrogen cyanide. When the aged solutions were examined for lethality to experimental animals, their lethal activities were exactly those expected on the basis of their oxime concentrations.

Barkman[131] compared the stabilities of I and II at various pHs and storage temperatures. Storage of a solution of I at a pH of 3 and 50°C for a year resulted in some increase in pH and in loss of 37-60% of the oxime in the original solution. Similar storage of a solution of II at the same pH and at 45°C for 2 yr resulted in loss of 80% of the oxime. Storage of solutions of these two salts at the same original pH, but at a temperature of only 25°C, resulted in loss of only 5% of I during 1 yr and of 4.8% of II during 2 yr. The pH increased by 1.16 during storage of the solution of II at 45°C for 2 yr, but by 0.19 during storage of that of I at 50°C for 1 yr. The principal products of degradation of these oximes were found to be N-methylpyridinium-2-aminocarbonyl and N-methylpyridinium-2-nitrile. These end products have low toxicities, so the deteriorated solutions of I and II were less toxic than freshly made ones (see also Barkman, et al.[132]) If the temperature of storage of solutions of either of these 2-PAM salts can be kept below 25°C, the solutions should have shelf-lives of several years.

When stored at 10-20 °C, solutions of III had their greatest stabilities at an initial pH of 6.[133] When the temperature of storage was 30°C or more, the initial pH that provided the greatest stability of the oxime in an aqueous solution was 5. With that initial pH, the estimated half-life of III in solutions stored at 40°C was 9.6 yr. The half-life of I in solutions at the pH of greatest stability, initially 4, for storage at 40°C was estimated to be 9 yr. In a solution containing also atropine and benactyzine at a pH of 2.8, 1.4% of the protective potency due to III was lost after 1 yr at 25°C.[134]

There seems to be little basis for choice among I, II, and III on the grounds of relative stability in solutions, so long as the storage temperature can be held below 40°C and the initial pH of the solution is adjusted to the value that yields the greatest stability for the particular oxime. For the salts of 2-PAM in solutions with pHs above those of maximal stability, the sequence of hydrolytic products may be N-methylpyridinium-2-aminocarbonyl, N-methylpyridinium-2-nitrile, N-methylpyridinium-2-hydroxide, and N-methylpyridinium-2-one, hydrogen cyanide being a side product. At pHs at or below those of maximal stability, the end products of hydrolysis of N-methylpyridinium-2-formyl oxime are N-methylpyridinium-2-aldehyde and hydroxylamine. Information on the storage stabilities of IV and V has not been found, other than a note that, when stored at 37°C at an unspecified pH, both 2-PAM I and V had half-lives greater than 7 mo.

After oral administration of I to dogs at nearly 100 mg/kg in an aqueous solution, the concentration of oxime in the plasma 1 h later was as great as was measured at any later time.[135] By 5 h after the dose, the plasma concentration had decreased to 51.3% of that at 1 h; by 13 h, it was only 18% of that at 1 h. When approximately the same

dose was given in tablets, the peak plasma concentration did not occur until 2 h later; it was more than 2.3 times the plasma concentration found 1 h after administration of the solution. This last finding suggests that the oxime is absorbed rapidly from a solution introduced into the gastrointestinal tract, so that the peak plasma concentration of oxime after oral administration of the solution may have passed before the first blood sample was taken 1 h after administration of the solution. By 5 h after administration of the tablets, the plasma concentration was slightly more than 39% of the peak concentration, but still nearly twice that measured at the same time after administration of I in solution. By 13 h after the tablets had been given, the plasma concentration was only 7.6% of the peak concentration and was the same as that measured at the same time after the solution had been administered. Inclusion of atropine in the preparation of the oxime was found not to influence absorption of the oxime. Administration of a nearly lethal dose of sarin to rabbits before the intramuscular injection of II or III nearly doubled the mean time (9 min vs. 5 min) for attainment of the peak plasma concentration.[136] The concentration of III in the blood was nearly twice that of II when equal doses of the two oximes were administered. However, Erdmann and Okonek[137] found that introduction of parathion into the lumen of a dog's intestine increased the absorption of IV from the lumen. In the rat, oral administration of disodium EDTA with IV nearly doubled the rate of absorption of the oxime. The peak plasma concentration of oxime at 1 h after administration of that mixture ranged from 6.9 to 17.4 μg/ml.

Sundwall[138] regarded a concentration of oximate ion in the blood of 4 μg/ml as minimal to overcome the bradycardia and apnea induced by lethal doses of isopropoxymethylphosphorylthiocholine in an anesthetized cat. Kunkel et al.[139] found that three of six rabbits given intramuscular injections of atropine sulfate at 6 mg/kg 55 min before intravenous injection of soman at 1.5 times the LD_{50} lived for 7 d thereafter. When the atropine was accompanied by I at 15 mg/kg, yielding a plasma concentration of oxime of about 4.8 μg/ml, two of six rabbits lived for 7 d. When the dose of atropine sulfate was increased to 10 mg/kg and the time between prophylaxis and poisoning was shortened to 5 min, only one of six rabbits challenged with soman at 2 times the LD_{50} lived for 7 d. When I at 15 mg/kg accompanied the same dose of atropine, the plasma concentration of the oximate ion just before soman was administered was about 25.4 μg/ml, and three of six rabbits given intravenous injections of soman at 2 times the LD_{50} lived. Larger doses of I, up to 45 mg/kg, yielded a plasma concentration of oxime of about 50 μg/ml and increased survival after soman at 2 times the LD_{50} to four of six. Accepting that lethal intoxication with soman is unusually difficult to prevent or treat, one nonetheless sees in these results an indication that the plasma concentration of oxime is important in determining the severity of intoxication by OP compounds that can be withstood.

Jager et al.[59,115] reported that infusion of 2-PAM I into dogs at 0.5 mg/kg per minute resulted after about 2 1/2 h in a relatively stable serum concentration of oxime of about 40 µg/ml. Ligation of the renal pedicles resulted in a rapid increase, during the 30 min thereafter, in the serum concentration of oxime to nearly 120 µg/ml. In one dog in which the infusion was continued for another 90 min, the serum concentration rose to nearly 140 µg/ml. In nephrectomized rats given intravenous 2-PAM I at 50 mg/kg during 2 min, the serum concentration of oxime decreased during an hour at about two-thirds the rate at which it decreased in control rats. Furthermore, the extrapolated serum concentration of oxime in the nephrectomized rats at zero time was one-third greater than that in the control rats. Evidently, therefore, the kidneys are important organs for the removal of 2-PAM salts from the circulating blood.

These investigators found also[59,115] that homogenates of rat liver were able to degrade both 2-PAM I and V if oxygen was present during the incubation; anaerobic incubation left the concentrations of the oximes in mixtures with the homogenate the same as they had been before incubation. Dultz et al.[23] found that dog kidneys took up from the blood more than 5.4 times as much 2-PAM I as V and that V disappeared from the serum of nephrectomized rats at essentially the same rate as from that of normal rats. Excretion into the urine seems to be a much more important route for removal of 2-PAM salts than of V from the body.

On comparing various organs of the dog other than the kidneys for ability to take up bloodborne 2-PAM I and V, Dultz et al.[23] found that V was abstracted from the blood by the brain slightly more than 11 times as readily as 2-PAM I, whereas liver, spleen, skeletal muscle, and cardiac muscle removed the two oximes in ratios between 1.0 and 1.7. Fat took up 4.5 times as much 2-PAM I from the blood as V. Because of its comparatively great ability to enter the brain, V has been considered by some people to be an especially good antagonist of inhibition of brain cholinesterase by OP compounds.

Kalser[41] found that excretion of the label in intravenously injected 2-PAM I having ^{14}C in the methyl group attached to the N atom in the pyridine ring was largely in the urine of mice; the feces accounted for less than 5% of the total excretion. The label was excreted rapidly (38.7% in 145 min) in the urine of one cat. The urine of mice was believed to contain at least six chromatographically separable metabolites of 2-PAM I, in addition to the unchanged oxime, whereas the urine of the one cat seemed to contain only unchanged oxime. Only 0.18% of the label was found in the expired air of mice during 6 h after intravenous injection of labeled oxime. The label disappeared from the blood of the cat rapidly: by 2.5 h after injection of the oxime, only about 17.5% of the peak concentration of the oxime

in the plasma remained there. Correspondingly, about 38.8% of the label had been excreted in the urine during the same time. In the cat, comparatively large concentrations of the label were found in the gall bladder and bile, the urinary bladder, the small intestine, the feces, and the liver.

Crook et al.[57] reported that the plasma concentration of III after its injection into a dog at 10 mg/kg followed essentially the same course as that of 2-PAM I injected intravenously at 30 mg/kg. Inasmuch as the plasma concentrations at approximately the same times after injection of the two oximes were almost identical, despite the difference between the doses, one must assume that III has a much smaller volume of distribution than 2-PAM I and that either the kinetics of removal of 2-PAM I from its larger volume of distribution were correspondingly greater than those of III or the 2-PAM I in extravascular components of its volume of distribution was held there tenaciously. The fairly rapid changes in the 2-PAM I concentrations found by Kalser[41] in various organs and tissues of the mouse argue against the validity of the latter possibility.

Enander, Sundwall, and Sörbo[140,141 and 142] found that either oral or intramuscular administration of II to rats at 500 or 100 mg/kg, respectively, increased by many times the urinary excretion of thiocyanate. From the amount of thiocynanate excreted above the base line, the quantity of hydrogen cyanide produced in metabolism of the oxime was calculated to be 0.1 mg/kg--a little more than one-third the LD_{50} for rats by intraperitoneal injection. Urine from rats given 120 μmol of II intramuscularly or 400 μmol by mouth contained 3.9-7.8% N-methylpyridinium-2-nitrile methanesulfonate. When this compound was injected intramuscularly into rats at 90 mg/kg, thiocyanate was excreted in the urine in increased amounts. Also present in the urine was a metabolic product that yielded cyanide on acidification of the urine, similar to a cyanide-yielding metabolite of II found earlier.

Studies with N-[^{14}C]methylpyridinium-2-aldoxime iodide administered to rats by intramuscular injection at 40 or 100 mg/kg or by mouth at 100 mg/kg revealed that about 87% of the label was excreted in the urine during the 24 h after injection, but that only 52% was excreted during the same time after oral administration. The label excreted in the urine during this period was largely (80-90%) in the form of unaltered oxime. Other metabolic products identified in the urine were N-methylpyridinium-2-nitrile and N-methylpridinium-2-carboxylic acid. Two other peaks in the radio-chromatograms of the urine were thought to represent N-methyl-α-picolinium amine and the cyanide-yielding metabolite mentioned earlier, but the identities of the compounds were not established, because there was too little of the substances represented by these radiochromatographic peaks. No N-methylpyridinium-2-one was found. Enander et al.[142] presented a scheme for the metabolic transformations undergone by 2-PAM whereby the

oxime is converted to either the nitrile, the aminocarbonyl derivative, or the aldehyde. The aminocarbonyl compound and the aldehyde were considered to be converted to the carboxylic acid as the end product. End products thought to be derived from the nitrile are the pyridone and the unidentified cyanide-yielding metabolite.

Way criticized this work as being superficial and inept and developed a chromatographic method for removing from a reaction mixture or solution all compounds containing a pyridinium group. The pyridinium-containing compounds could then be resolved at pHs permitting the molecules to exist without degradation. Using this method, Way et al.[143,144,145 and 146] identified N-methylpyridinium-2-nitrile, N-methylpyridinium-2-one, and N-methylpyridinium-2-aminocarbonyl-4-one as metabolic products of 2-PAM in the perfusate from isolated rat livers. They also found, but did not identify, an N-methylpyridinium-2-O-conjugate, which was not hydrolyzed by either β-glucuronidase or phenol sulfatase. When exposed to 0.3 N NaOH, the conjugate released N-methylpyridinium-2-one. N-methylpyridinium-2-ethoxy iodide was synthesized and found to yield the pyridone on exposure to 0.3 N NaOH. However, the metabolic product and the synthetic compound were found to have different mobilities on paper chromatograms with various solvent systems. The synthetic compound may be a model of the natural conjugate, but is not identical with it.

Way presented a scheme for the metabolism of 2-PAM wherein the oxime is converted to either a 2-nitrile-4-one or a 2-acetiminomethyl or a 2-phosphoryliminomethyl compound. The acetimino or phosphorylimino compound is converted to the 2-nitrile, which is changed into either the 2-cyanohydrin or the 2-nitrile-4-one derivative. The 2-nitrile-4-one is metabolized to the 2-aminocarbonyl-4-one. The 2-cyanohydrin is proposed as the precursor of the 2-one derivative and also of the 2-O-conjugate through a 2-cyano-O-conjugate. Both reactions of the cyanohydrin would release CN^- ion.

Way et al.[145] found N-methylpyridinium-2-nitrile in human urine as a metabolite of 2-PAM. Kramer[147] reported that five men given 2-PAM I at 2 g/day by mouth on 2 consecutive days while they were on a closely supervised regimen of dietary and other intakes excreted in their urine not only unaltered 2-PAM, but also a material that had a bright blue fluorescence when exposed to UV light (366 nm). This material was shown not to result from simple exposure to urine by incubation of 2-PAM I in normal human urine under several different conditions for up to 7 d. It was thought possibly to be a derivative of N-methylpicolinic acid.

Berglund et al.[148] studied the handling of II by the dog's kidneys and found that the amount of the oxime excreted always was greater than the amount filtered through the glomeruli. At a plasma concentration of II in one dog of about 6.2 μg/ml, the difference between the

amount excreted in the urine and the amount filtered was about 1.2 mg/min in a total excretion of about 1.75 mg/min. This plasma concentration of the oxime gave the Tm for tubular secretion in that animal (the Tm was reached in other dogs at plasma concentrations of II of 6-8 μg/ml). Loading dogs with ammonium chloride (6 μg/d by mouth for 3 d) before the experiment increased excretion of the oxime without altering appreciably its filtration. Conversely, induction of alkalosis by intravenous infusion of 0.6 M sodium bicarbonate decreased excretion of the oxime without changing significantly its filtration. Alkalinization of the urine by administration of acetazolamide had no effect on the urinary excretion of the oxime. Probenecid (priming with 25 mg/kg followed by infusion at 40 mg/kg per hour) lowered the urinary excretion of the oxime from 1.66 mg/min to 1.61 mg/min and tubular secretion from 0.96 mg/min to 0.88 mg/min.

These differences are not statistically significant. Apart from a conclusion that the tubular epithelium has a more effective transfer system for the oximate form of II than for the N-oxide form, the meaning of these findings is obscure.

The only other oxime on which any metabolic information was found is III. Way et al.[149] perfused III labeled with ^{14}C in the 1 and 3 positions of the 3-carbon chain between the pyridinium moieties through isolated rat livers. The only metabolite that they reported finding in the perfusate was 1-(4-aldoximinopyridinium)-3-(4-cyanopyridinium) propane ion, which was identified by comparison with the authentic synthetic compound. Later, DeMiranda et al.[150] found the same compound as the principal metabolite in the urine of rats that had been given doses of III. III seems to be treated metabolically as though it were one molecule of 2-PAM attached to a large inert group.

In an attempt to find a way of extending the protective action of oximes, Stern and Boskovi [151] explored the possibility that an inhibitor of drug processing enzymes, diethylaminoethyl diphenylpropylacetate (SKF 525A), can increase the ability of oximes to antagonize the lethal actions of tabun. In the scheme used, SKF 525A and atropine were injected into experimental animals 40 min before tabun was injected; oximes were injected 10 min before tabun. SKF 525A approximately doubled the protective activity of mixtures of atropine with 2-PAM I, III, and a mixture of 2-PAM I and V. Later, Milosevi and Terzi [152] compared other inhibitors of microsomal enzymes with SKF 525A for potency in increasing the ability of III to prevent the lethality of paraoxon. The principal difference between III alone and with SKF 525A was that SKF 525A significantly prolonged the time between injection of III and of paraoxon at which the oxime would remain protective. For example, injection of III at 25 mg/kg 30 min before paraoxon yielded 30% survivors in the group. When the same dose of III was injected with SKF 525A at 50 mg/kg 60 min before the same dose of paraoxon, all the animals lived. These investigators found also that

one of a group of related inhibitors of microsomal enzymes, diethylaminoethyl phenyldiallylacetate (CFT 1201), was at least as effective as SKF 525A in a much smaller dose.

The same people carried the study of CFT 1201 further.[153] When rats were given CFT 1201 1 h before intravenous injection of III, the blood concentration of III was only 21.6% greater than when III was administered alone. When mice were given oximes subcutaneously and CFT 1201 intraperitoneally simultaneously 15 min before a challenge with paraoxon at 3 times the LD_{50}, the protective dose of 2-PAM I was 24.3% of that when 2-PAM I was given alone, and that of III was 20% of that when III was injected alone. CFT 1201 and SKF 525A alone had almost no protective actions against tabun or paraoxon.

STUDIES WITH HUMAN SUBJECTS

Jager et al.[59,115] injected 2-PAM I intravenously into five human subjects during 2-4 min at 15 mg/kg and I into five other subjects at the same dose. One patient with a plasma concentration of urea nitrogen of 165 mg/100 ml was given the same dose of 2-PAM I. During 4 h after these doses, essentially all the 2-PAM I left the serum of the five normal subjects, whereas the serum concentration of this oxime in the azotemic patient decreased by only about 8.6%. The mean concentration of V in the serum of normal subjects decreased by about 13.5% during 4 h. Correspondingly, three subjects given 2-PAM I excreted in their urine a mean of 80.9% of their doses of the oxime during the 6 h that followed the injections, whereas two subjects given V excreted a mean of only about 6.8% of their doses of that oxime during the 6 h after administration of the oxime. Urinary excretion of 2-PAM I by the azotemic subject seems not to have been measured.

During a continuous intravenous infusion of V into a subject at 0.27 mg/kg per minute samples of spinal fluid taken at 60 and 150 min after the start of the infusion contained a mean of 68.3% ± 2.4% of the serum concentration of the oxime at the same time.[115] A subject given an infusion of 2-PAM I for 60 min at 0.73 mg/kg per minute had a serum concentration of the oxime of 2.8 mg/100 ml at the end of the infusion, but had no detectable concentration of that oxime in the spinal fluid. These findings corroborate the idea that V may have readier access than the pyridinium oximes to the brain.

In 1964, Calesnick[154] and Calesnick et al.[155] reported that, when I was injected intravenously into normal male and female volunteers at 15 mg/kg (three subjects), both systolic and diastolic blood pressures were increased immediately after the end of the infusion (usual duration, 15 min). The hypertension lasted for 1.5-4 h and was accompanied by a slight increase in heart rate. The plasma concentration

of oxime at the end of the infusion ranged from 12.3 to 17.3 μg/ml. Two subjects given similar infusions of I at 30 mg/kg had no immediate increases in blood pressure, but the systolic and diastolic pressures rose slowly--e.g., to about 32 and 44 mm Hg above the original pressures, respectively, at about 35 min after the end of the infusion in one subject; subnormal blood pressures developed about 3 h later. The subjects given the smaller dose of I also developed subnormal pressure after the hypertensive phase of the response. The mean plasma concentration of I at the end of the infusions at 30 mg/kg was 17.6 ± 0.3 μg/ml. Infusions of I at 45 mg/kg into two subjects resulted in marked increases in systolic and diastolic blood pressures immediately after completion of the infusions. The hypertensive response lasted for about 1.5 h and was followed by the development of subnormal blood pressure about 2.5 h later. Increases in the voltage of the T waves and in the length of the PR interval of the ECG were reported after this dose. Administration of a second dose of I after the blood pressures had returned to the control values yielded a second hypertensive response. Ephedrine intensified the hypertensive response to I, whereas phentolamine and reserpine reduced it.

Injection of I intramuscularly at 15 mg/kg into two subjects gave a plasma concentration of oxime of 4 μg/ml, but had no effect on blood pressure.[154] Two other subjects given intramuscular doses of 30 mg/kg developed hypertension about 1.5-2 h after the end of the infusions. A subject who inhaled aerosols of 10% and 50% aqueous solutions of I did not develop hypertension or detectable oximemia. Oral administration of 1 or 2 g every 6 h for 5 d to eight subjects resulted in no significant changes in blood pressure or heart rate.

Infusion of II at 45 mg/kg into one subject resulted in a hypertensive response immediately after the infusion was completed.[154] Oral doses of 2 and 4 g of II had no detectable effect on blood pressure in another subject.

When 4 g of III were given by mouth to one subject, there were slight decreases in systolic and diastolic blood pressure 6 h later. Infusions of III at 15 or 30 mg/kg into one subject produced immediate, brief hypertensive episodes at the end of the infusions, followed by a period of subnormal pressures lasting from about 22 min to more than 363 min after the end of the infusion for the systolic pressure; the diastolic pressure did not fall below normal until about 117 min after the end of the infusion, but was still below normal at 363 min after completion of the infusion.

More detailed data from these experiments and from others in which I and 2-PAM maleate were administered orally and by intraduodenal tubes, to compare rates of absorption of the two salts by the two methods of administration, were given by DiPalma and Calesnick.[156] As a result of these studies, Calesnick et al.[157] concluded that I

was more satisfactory than II or III, in that the last two oximes caused marked gastrointestinal disturbances that limited both the amounts of these oximes that could be administered and the durations of their administration that could be tolerated. This limitation was especially marked with III, which also caused nervousness, malaise, dizziness, paresthesia of the face and arms, rash, and icterus in some subjects.

Details of studies in which propranolol and phentolamine were found to antagonize the hypertensive effect of infusions of I at 25-45 mg/kg were given in the same report by DiPalma and Calesnick.[157] Doses of 5 mg of phentolamine, probably as the methane sulfonate although they did not identify the salt used, and of 4 or 5 mg of propranolol were used in four subjects (one woman and three men) aged 23-32 yr. In three subjects given I by intravenous infusion at 25 and 30 mg/kg during 15 min, phentolamine was the more potent antagonist of the hypertensive effect of I; in one subject given I at 45 mg/kg, propranolol was a better antagonist than phentolamine. It is of some interest that the adrenergic blocking agent, phentolamine, usually had a greater antagonistic action against the hypertensive effect of I than the nonselective β-adrenergic blocking agent, propranolol. This suggests that the principal action of I is directly on the vascular smooth muscles--a posssibility that is supported by the finding that the hypertensive action of I usually does not involve a marked change in heart rate.

Kondritzer et al.[158] administered I, 2-PAM lactate, 2-PAM dihydrogen phosphate, II, 2-PAM I, and III to human subjects as aqueous solutions. The subjects were primed with 400 ml of water during the hour before they drank the solution of oxime and drank 100 ml of water during each of 5 consecutive hours after taking the oxime. The peak plasma concentrations of oxime occurred at about 2 h after drinking of a solution of 2-PAM I that contained a mean dose of 71.4 mg/kg, at about 2.5 h when the dose was increased to 114.3 mg/kg, and at about 3 h when the dose was increased further to 142.9 mg/kg.

The lactate gave a greater peak concentration of oxime in the plasma than the same dose of the dihydrogen phosphate, but required a longer time to induce it (3 h vs. 2 h); the peak concentrations differed by 18.9% of that established by the dihydrogen phosphate, although the molecular weight of that salt was greater than that of the lactate by only 3.4% of its own molecular weight. When I and II were administered in equimolar doses, the peak plasma concentrations of oxime were reached ater 2 h. With doses of 0.31 mmol/kg, II gave a peak concentration greater than that given by I by about 4.6 M. When the dose was 0.12 mmol/kg, the peak concentration established by I was about 1.5 μM above that established by II. These two salts are probably absorbed and eliminated from the plasma at closely similar rates.

The biologic half-lives of the five salts of 2-PAM calculated from both the data on plasma concentrations at various times after ingestion and those on urinary excretion are given in Table 4. The table demonstrates that the iodide was cleared from the plasma more slowly than the other salts and that the dihydrogen phosphate was cleared from the plasma a little more rapidly than the others.

The only undesirable effects observed during these studies were signs and complaints of iodinism by the subjects given 2-PAM I and decreases in red-cell and plasma cholinesterase activities of about 20% in the subjects given III. Less of III was excreted in the urine (3% during 24 h) than of the monoquaternary compounds (27% in 14 h).

Sidell et al.[76] gave oral doses of 3-9 g of I to a total of 28 men. Although these doses were not adjusted to the body weights of the subjects, there was a general tendency for the mean peak plasma concentration of oxime to increase as the dose increased. The mean peak concentration varied from 4.20 µg/ml after 3 g of I was ingested by four subjects to 9.15 µg/ml after 9 g was taken by two subjects. The time for attainment of the peak concentration varied from 30 min to 3 h, without any discernible relation with dose or any other variable in the experiment. The mean half-life of the oxime, calculated from concentrations measured in the plasma at various times, was 2.66 h in 25 subjects; the mean half-life calculated from the amounts of oxime excreted in urine at various times was 2.44 h in 21 subjects. In five experiments in which the mean peak plasma concentration of oxime of 6.1 µg/ml was attained 2 h after ingestion, the mean 6 h after ingestion was 2.0 µg/ml--slightly less than 32.8% of the mean peak concentration.

When these investigators[76] administered oral doses of I every 6 h for 48 h, the plasma concentration of oxime proceeded as a series of peaks at 3, 9, 15, 21, 27, 33, 39, 45, and 51 h. There was a tendency for each peak to be slightly greater than the preceding one. When the interval between doses was shortened to 4 h, the peak concentrations were about 1.3 times those when the same dose was given every 6 h.

TABLE 4 Biologic Half-Lives of 2-PAM Salts Given Orally to Man

2-PAM Salt	Half-Life, h Concentration in Plasma	Based on Urinary Excretion
Iodide	2.0 (9)	2.2 (5)
Chloride (I)	1.7 (7)	1.7 (7)
Methane sulfonate (II)	1.7 (10)	1.6 (10)
Lactate	1.7 (9)	1.7 (9)
Phosphate	1.3 (5)	1.5 (5)

[a] Figures in parentheses are numbers of subjects.

Sidell et al.[159] found that, when doses of 600 mg of I and 2 mg of atropine sulfate were injected intramuscularly simultaneously but separately into the two arms of a man, his heart rate rose more sharply and to a higher value than when the two compounds were injected in one solution. The mixed solution seemed to produce less pain than intramuscular injection of I alone. On the basis of this study, there is no evident disadvantage in mixing atropine and I in a single solution for intramuscular injection. There is a question, however, whether the doses of atropine and I should be linked in a fixed ratio, as they would be if these two compounds were provided only as a mixed solution.

Swartz and Sidell[160] administered I to subjects under various conditions of ambient temperature and exercise (walking on a treadmill). Resting at 21°C was the baseline condition. The renal clearances of creatinine, p-aminohippurate, and I were measured during rest at 40.6°C, during walking at 3 mph during 20 of each 30 min in a period of 3 h at 21°C, and during walking at 3 mph during 20 of each 30 min in a period of 3 h at 40.6°C. Whereas the renal clearance of creatinine was decreased progressively by exercise, by exposure to an increased ambient temperature, and by exercise at the higher ambient temperature, the clearances of both p-aminohippurate and I were increased during rest at the higher ambient temperature. Walking on the treadmill in the heated room, however, decreased the clearances of p-aminohippurate and I even more than walking on the treadmill at the lower temperature. That the effects on these two renal clearances were similar is not astonishing when one considers that tubular secretion is the most important part of the mechanism of clearance of both substances from the plasma. It is likely that exposure to the higher temperature at rest resulted in increased blood flow to the kidneys by vasodilatation, that exercise at the lower ambient temperature decreased blood flow to the kidneys by shunting of blood to the active muscles and possibly to the skin to permit dissipation of the extra heat produced during the exercise, that the greater heat load imposed by the combination of higher ambient temperature and exercise resulted in an even larger shunting of blood away from the kidneys to the skin, and that the resultant of vasodilatation in the active skeletal muscles and in the skin was a sharply decreased blood flow to the kidneys and, consequently, lower tubular secretion of p-aminohippurate and I.

After being found to be healthy on a thorough physical examination accompanied by a broad range of laboratory examinations, 22 men were used in studies of the renal clearance of I, after intravenous injection at 5 mg/kg under a variety of conditions.[161] Alkalinization of the urine to a pH above 7.5 by administration of bicarbonate and acidification of the urine to a pH below 5.0 by administration of ammonium chloride both reduced urinary excretion of I. When 200 mg of thiamine was injected intramuscularly 20-30 min before intravenous injection of I, urinary excretion of I during the 5 h after

its injection was decreased to the greatest extent (by almost 24%). Thiamine resulted in an increase in the total volume of distribution of I of about 47.4%, the expansion of the central volume being about 13.3% greater than that of the peripheral volume. The mechanism of the changes in the volume of distribution of I is not apparent.

During the first 3 h after intravenous injection of I that followed administration of thiamine, urinary excretion of the oxime was about 12.7% below that during the corresponding period of the control experiment; during the remainder of the run, it was 62.2% above that during the same period of the control experiment. Inasmuch as intravenous injection of 900 mg of sodium p-aminohippurate with I decreased by only 6.3% the urinary excretion of I during the first 3 h after its administration, the tubular transport mechanisms for I and for p-aminohippurate probably are different.

Josselson and Sidell[162,163, and 164] extended study of the effect of thiamine on the pharmacodynamics of I in the human body. They found initially[162] that an infusion of thiamine at 100 mg/h during 2.5 h led to a greater plasma concentration of oxime after intravenous injection of I at 5 mg/kg than when the injection of I was not accompanied by an infusion of thiamine. The renal clearance of I and the urinary excretion of I were greater during the first 1.5 h of infusion of thiamine than during the corresponding period after injection of I without infusion of thiamine. The investigators concluded that thiamine decreased the peripheral volume of distribution of I in this study. However, their published value for the standard deviation of the mean for this variable during the infusion of thiamine was 0.01 times the real value; that may have contributed to this unjustified conclusion. The sole barely significant change in the volume of distribution of I was an increase in the central volume of distribution. The most significant changes in the men given thiamine infusions were a decrease in the removal of I from the central compartment of the volume of distribution, a decrease in the movement of I from the central to the peripheral compartment, and a decrease in the value of the exponential constant for the rapid phase of the decrease in the plasma concentration of I.

Infusions of thiamine at two different rates during intravenous injections of a constant dose of I produced sequentially slower removal of I from plasma.[163] The half-time for the rapid phase of removal of I from plasma was 4.2 min in the control runs; it was increased to 8.4 min during the infusion of thiamine at both rates. The half-time for the slow phase of removal of I from plasma was 59.8 min in the control runs; it was increased to 87.6 min and 91.2 min, respectively, by the lower and higher rates of infusion of thiamine. The rate constant for removal of I from the central compartment was 2.94 in the control runs and was decreased to 1.32 by infusion of thiamine at the lower rate; the higher rate of infusion of thiamine

(twice the lower one) induced no significant further reduction of this rate constant. The rate constant for movement of I from the peripheral compartment to the central one was 4.95 in the control runs; it was decreaed to 2.49 by the lower rate of infusion of thiamine, but rose to 3.21 with the higher rate of infusion. The last value had a very large standard deviation and consequently was not significantly different from either the rate during the control runs or that during infusion of thiamine at the lower rate. The higher rate of infusion of thiamine, but not the lower one, decreased significantly the peripheral and total volumes of distribution of I. The higher rate of infusion of thiamine markedly prolonged side effects of the oxime, including lethargy, but was tolerated well by the subjects.

Infusions of 1 g of I, with and without 200 mg of thiamine hydrochloride, into five men during 30 min produced hypertension in two subjects during the infusions that included thiamine.[164] In the trials without thiamine, the only effects detected by the investigators or reported by the subjects were transient blurring of vision, diplopia, and a sensation of expansion of the eyeballs, never lasting for more than about 3-5 min, in four of the five subjects. During the infusions that included thiamine, in addition to the hypertension in two subjects, one other subject had an increased heart rate, and all complained of the same visual effects that were reported during the control infusion. These were more marked and lasted for up to 2 h, instead of for a few minutes as during the control runs. Four of the men also complained of fatigue and drowsiness; two complained of "vitamin gustatory sensation."

The two men who had hypertension after the infusion of I with thiamine had mean increases in systolic blood pressure of 34 mm Hg and in diastolic pressure of 24 mm Hg. The mean maximal increase in heart rate of these two men and of the one other man who had only an increase in heart rate was 25 beats/min. In all five men, the mean plasma concentration of oxime was always greater in the experiments in which the infusion included thiamine than in those in which the infusion contained I alone, by about 1-11 μg/ml. During the first 1.5 h after the beginning of the infusions, those with I alone excreted in their urine a mean of 73% of the total 24-h excretion of the oxime, whereas when the infusions included thiamine only 34% of the 24-h excretion of the oxime took place during the first 1.5 h after the beginning of the infusions. During the first 3 h after the beginning of the infusions, 89% of the total 24-h urinary excretion of I given alone occurred, compared with only 55% of that of I accompanied by thiamine. During the remainder of the 24 h after the start of the infusions, 11% of the cumulative urinary excretion of I not accompanied by thiamine appeared in the urine, compared with 45% of that of I mixed with thiamine. The total urinary excretion of I

during the 24-h collection period was 83.6% of that infused alone and 72.3% of that given with thiamine. It is unfortunate that these investigators never measured the excretion of thiamine alone and accompanied by I, because such information would allow one to judge whether I and thiamine use the same transport system across the tubular epithelium. This is a distinct possibility suggested, but not proved, by these studies.

Walker[135] reported the results of giving 12 subjects I orally in tablets at a mean of 58.6 mg/kg (experiment performed by G. Marier, P. Dussault, and J.M. Orr). Blood samples taken at intervals thereafter contained the following mean concentrations of I: 1 h, 6.48 µg/ml; 1.5 h, 6.33 µg/ml; 2 h, 6.75 µg/ml; 3 h, 5.77 µg/ml; 5 h, 3.03 µg/ml; and 7 h, 1.60 µg/ml. The mean time to the maximal plasma concentration of I was 1.67 h, and the mean value of that concentration was 7.28 µg/ml. The mean half-life of I in the plasma after reaching the maximal concentration was 125 min.

Sundwall[138] found that plasma concentrations of II established by intravenous and intramuscular injections of that oxime became approximately identical about 1 h after the injections, although the courses during that hour were quite different--one descended, at first sharply and then progressively more slowly, and the other ascended to a maximum and thereafter descended slowly. An intramuscular injection of a 25% solution of II caused some pain at the site of injection; similar administration of a 12.5% solution did not elicit pain. When 10 subjects got intramuscular injections of II at 30 mg/kg, three gave evidence of rapid absorption of the oxime, its plasma concentration rising to about 20 µg/ml within 5 min. The other 7 men seemed to have slower absorption of the oxime, its plasma concentration rising to 12-27 µg/ml after 20-30 min. In one subject, the peak concentration in the plasma was not achieved until 90 min after the injection. On oral administration of II at 45 mg/kg in gelatin capsules, the peak plasma concentration of about 5 µg/ml was reached after a mean (six subjects) of 130 min.

Sidell et al.[75] used two types of tablets containing II--one designed to disintegrate rapidly in the gastrointestinal tract and the other intended to provide slow, sustained release of oxime. The time courses of the plasma concentrations of the oxime with the two types of tablets were fairly similar, the peak concentrations being attained at the same time after ingestion. The peak concentrations after the rapid-release tablets were somewhat above those produced by the same total amounts of II in the sustained-release tablets and the descent from the peak concentration was less precipitous after the sustained-release tablets than after the rapid-release ones. Oral doses of I (form not specified) produced peak plasma concentrations of oxime sooner after ingestion than after ingestion of the tablets of II; the peak plasma concentration from a given dose of I usually was larger than that from a similar dose of II in the form of

either of the tablets. The mean half-time for elimination of II from the body, starting at the peak plasma concentration, was 144 min for the rapid-release tablets and 135 min for the sustained-release tablets. The mean biologic half-life, calculated from analyses of urine, were 132 min for the rapid-release tablets and 138 min for the slow-release ones.

Holland et al.[165] performed a similar study, using a single dose of 4 g of II, whereas Sidell et al.[75] had used doses of 3, 5, 7, and 9 g. The two types of tablets used in these two studies were made by Glaxo Laboratories Ltd. Holland et al. used a total of 51 subjects and found that the rapid-release tablets yielded more rapidly (by about 1 h) a slightly larger peak plasma concentration of oxime (means for 42 subjects: 6.63 and 6.32 μg/ml for the rapid-release and slow-release tablets, respectively) and that the decrease from the peak concentration after the slow-release tablets was slower than that after the rapid-release ones. They concluded that use of a mixture of the two types of tablets would be preferable to use of only one type in facilitating both a rapid increase in plasma concentration of oxime to an effective point and a relatively prolonged maintenance of such a concentration. In a later paper, Holland and Parkes[166] reported that an intramuscular injection of 500 mg of II is needed to establish an effective plasma concentration. They found that three such doses given 20 min apart could be tolerated by normal people without complaints of visual disturbances. When intramuscular injection of II was added to an oral prophylactic regimen with II (4 g every 6 h), complaints of a feeling of enlargement of the eyeballs, blurred vision, and difficulty in accommodation after sudden head movement were expressed. Such complaints were more common from the subjects who received the larger doses of II.

Sidell et al.[167] used 20 subjects in a study of tablets of II prepared by Philips-Duphar in the Netherlands. Fourteen subjects took the tablets on empty stomachs; the other six ate a breakfast of eggs, bacon, and toast about 30-45 min before they took the tablets, which were coated with a methacrylate ester. Doses of 2, 4, 6, and 8 g of II were taken by the fasted subjects, and doses of 4 and 6 g by those who had broken their fasts. Peak plasma concentrations of the oxime were reached within 2-3 h after the tablets were ingested. The ingested doses resulted in the following order of decreasing peak plasma concentrations of oxime: fed, 6 g; fasted, 8 g; fasted, 6 g; fed, 4 g; fasted, 4 g; and fasted, 2 g. The only surprise in this list is the high positions held by the two groups of fed subjects.

Urinary excretion of II by the fed subjects was greater than that by the fasted subjects. The plasma concentration of II was a linear function of the logarithm of the dose of oxime for both groups of subjects, but the slope of the line for the fed subjects was greater than that for the fasted subjects. Although the difference was never

large, the mean volume of distribution of II in the fasted men was greater than that in the fed ones. The mean first-order rate constant for absorption of II by the fasted subjects was greater than that for the fed subjects, and the mean first-order rate constant for elimination of II was greater for the fasted subjects than for the fed ones. Comparison of the plasma concentrations of II at various times after ingestion of the Netherlands tablets with those after ingestion of the English tablets reveals that comparable doses of II in the English tablets produced the lower plasma concentrations according to the data provided by Sidell et al.,[75] but the higher concentrations according to the data of Holland et al.[165] Values measured after the rapid-release English tablets were used for these comparisons. Because more closely similar methods were probably used in the two studies by Sidell et al., comparison of the two sets of tablets based on the data of the two reports by Sidell et al.[75,167] may be more valid than that based on the data of Holland et al.[165] and of Sidell et al.[167]

Simon et al.[81] presented data that indicate, by a small extrapolation, that an oral dose of 4 g of IV in tablets would induce a peak plasma concentration of oxime in a human subject of about 6.2 μg/ml. This is about the same figure found by Sidell et al.[167] after 4 g of II in the Netherlands tablets, somewhat less than that reported by Holland et al.[165] after 4 g of II in the English tablets, and almost 1.5 times that found by Sidell et al.[75] with the English tablets.

Sidell and Groff[77] gave 10 men intramuscular injections of IV at 2.5, 5.0, 7.5, or 10 mg/kg. These doses produced mean peak plasma concentrations of the oxime of 12.1, 19.8, 34.9, and 39.3 μg/ml. These concentrations were achieved after 20-30 min. The logarithm of the peak concentration was a linear function of the logarithm of the dose injected. The half-time of removal from the plasma was about 81.3 ± 4.3 min. The half-time of urinary excretion was about 84.5 ± 10 min. Dose-related increases in the heart rate and systolic and diastolic blood pressures were also produced by IV. A comparison of the dose-plasma concentration relationships for IV and I reveals that the plasma concentration achieved by a given dose of oxime is 4.7-8.0 times as great for IV as for I. An apparently all-or-nothing symptom complex induced in some subjects by IV consisted of a feeling of warmth in the upper body that gradually became localized circumorally, a feeling of warmth in the throat that according to three of nine complainants was associated with a taste similar to that of menthol, numbness in and around the mouth and a definite hypoalgesia of that area in response to pricking with a pin, and a feeling that the eye-balls had enlarged and become heavy. Most of these symptoms subsided within 1-2 h.

In a later report,[79,168] Sidell and Groff told of giving a 25% aqueous solution of IV to 13 men by mouth, using doses of 1, 3, 5, 7,

and 9 g. The smallest dose produced a plasma concentration of the oxime so low that measurements of it were of questionable significance; the results from that dose were not analyzed further. The peak plasma concentrations produced by the doses of 3, 5, 7, and 9 g were 1.9, 3.19, 4.41, and 5.56 µg/ml, respectively. Both I and II produced larger plasma concentrations with a given dose than IV; the ratio between the plasma concentrations produced by a given dose of II and the same dose of IV varied between 1.44 and 2.0, and the same ratio for I and IV varied between 1.44 and 2.6. The half-time for removal of IV from plasma was about 159 min, and that for urinary excretion of IV was about 288 min. The latter time is not only considerably longer than the corresponding times for I and II, but also much longer than that for removal from the plasma. The last discrepancy may indicate metabolic breakdown of the compound in the kidney. Karyotypic study of three of the subjects found no unusual number of gaps and breaks or increased mitotic index.

Sidell et al.[169] compared I, II, and IV with respect to absorption, distribution, and excretion in human subjects. The oximes were injected intravenously, I and II at 5 mg/kg and IV at 0.5 and 1.0 mg/kg. The lines for the plasma concentration of oxime at various times after the injections of I, II, and the larger dose of IV were nearly identical. The smaller dose of IV yielded plasma concentrations 0.5-5.0 µg/ml below those produced by the larger dose. The renal clearances of I and II were 606 and 644 ml/min, respectively, whereas that of IV was 95 ml/min. Although no independent measure of glomerular filtration was reported, the last value for renal clearance is well below the generally accepted mean for glomerular filtration in the human kidney of 126 ml/min and could be smaller than glomerular filtration in the particular subjects used in the study. If the clearance of IV really is smaller than the glomerular filtration, that would mean that the kidneys have a tubular reabsorption mechanism for IV, whereas the tubular epithelium secretes I and II into the tubular lumina. The volumes of distribution of I and II were 815 and 775 ml/kg, respectively, whereas that of IV was only 173 ml/kg. The central compartment accounted for 33.2% and 25.3% of the total volumes of distribution of I and II, respectively, and for 58.0% of that for IV. The half-times for removal of oxime from the plasma were 79 min for I, 85 min for II, and 72 min for IV. Half-times for urinary excretion of the oximes were not given, but values for I and II can be estimated from curves in the report of the cumulative percentages of the doses excreted in the urine during various times. The half-times for urinary excretion estimated from these curves were 39 min for I and 48 min for II. After intramuscular injection of IV, the half-time for its urinary excretion was about 85 minutes;[77] the half-time for excretion after intravenous injection might be smaller by about 20 min, the minimal time required for reaching the peak plasma concentration of the oxime after intramuscular injection. The half-time for urinary excretion of IV may be about 1 h after intravenous injection.

GENERAL COMMENT

None of the oximes used in experiments with human subjects by the personnel of the Biomedical Laboratory at Edgewood Arsenal or of its contractors has given evidence of being dangerously toxic in the single doses used. The studies detailed in the body of this review have shown that the lethalities of the various oximes are not due solely to the content of the oximate radical in these compounds. The bispyridinium bisoximes (III and IV) seem to be more curaremimetic and more disturbing of normal gastrointestinal function than the monopyridinium monoximes (2-PAM I, I, and II) and the ketoxime (V). The most marked effect of both the monopyridinium monoximes and the bispyridinium bisoximes on functions in subjects poisoned by OP compounds is on neuromuscular transmission. The bispyridinium bisoximes are absorbed from the gastrointestinal tract less rapidly than the monopyridinium monoximes, but are more efficient in yielding a comparatively high plasma concentration of oxime than the monopyridinium monoximes, by virtue of having smaller volumes of distribution within the body and being excreted less rapidly by the kidneys.

The oximes, although they are metabolized to some extent, are removed from the body largely by excretory processes. Particularly for the monopyridinium monoximes, the kidneys are important excretory organs. Renal insufficiency has been found to increase the toxicities of these oximes significantly, but to have no definite effect on that of the ketoxime.

Most of these compounds are removed from the human body so rapidly that there is comparatively little hazard of cumulative toxicity from administration of reasonable doses of the oximes. The principal exception is V, which has been found to act synergistically with depressant barbiturates[170] and to induce significant changes in CNS function when repetitive doses were administered. The discovery that IV also appears in the CSF comparatively rapidly after its administration suggests that its potential for exerting toxic effects on CNS function during a course of repetitive administrations should be evaluated carefully. III also has been found to produce bothersome, but apparently not particularly dangerous, effects after repetitive doses.

Whereas the most striking cardiovascular response to the monopyridinium monoximes is hypertension, that to the bispyridinium bisoximes is prolonged hypotension after an initial, short hypertensive response. The hypertension and tachycardia that have been induced by initial

doses of oximes have been found not to persist during a series of repetitive administrations. Furthermore, sympatholytic compounds have been found to be effective antagonists of these actions that could be used to control bothersome cardiovascular symptoms after the use of oximes.

The principal lack in the available information perceived in this review is the absence of any significant attempt to determine whether a delayed toxic effect becomes evident after administration of these compounds. Although hydrogen cyanide is a metabolic product of the monopyridinium monoximes and a nitrile has been found in the urine of rats given III, the production of these compounds has not been great enough to cause obvious toxic effects near the times of administration of the oximes. There is a slight possibility that repetitive administrations of oximes during sufficient periods could induce damage to crucial organs by continued production of low concentrations of hydrogen cyanide and nitriles in the body. Studies of the toxicities of these compounds during long-term administrations should be designed to afford information not only on chronic toxicity itself, but also on the possibility that oximes may have some degree of carcinogenic or mutagenic potenital. Their teratogenic potential should also be evaluated, although the existing information[171] suggests that there will be no effect of that sort.

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