AD NUMBER: AD0342332
CLASSIFICATION CHANGES
TO: Unclassified
FROM: Confidential
LIMITATION CHANGES
TO: Approved for public release; distribution is unlimited.
FROM: Distribution authorized to U.S. Government Agencies and their Contractors; Administrative/Operational Use; 1 Aug 1963. Other requests shall be referred to Army Chemical Research and Development, Edgewood Arsenal, MD, 21010.
AUTHORITY
U per USAEA ltr dtd 21 Jan 1970; ST-A per USAEA notice dtd 25 May 1970

CONFIDENTIAL

(1)

CRDL Special Publication 1-44

(6)

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

(8)

by the

Directorate of Medical Research

and

Directorate of Weapons Systems

August 1963

U. S. ARMY CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
Edgewood Arsenal, Maryland

CONFIDENTIAL

Chemical Research and Development Laboratories Special Publication 1-44

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

17

Tasks: 4C08-02-024-01 and
4C08-03-016-017

APPROVED:

VAN M. SIM, M. D.
Deputy Director of Medical Research

LaMONTE A. TUCKER
Colonel, CmlC
Director of Weapons Systems

(U) FOREWORD

This work was conducted under Task 4C08-02-024-01, Experimental Medicine and Clinical Investigations (U), and Task 4C08-03-016-017. This is a continuing project.

The research reported herein was performed under the immediate supervision of a qualified biological scientist. The experimental animals were at all times treated in accordance with acceptable hospital practices. The research conforms to the provisions of AR 70-18, dated 20 November 1961, and to the appendix thereto.

Acknowledgments

Grateful acknowledgment is made to Dr. Van M. Sim, Dr. Bernard McNamara, Dr. Benjamin Witten, Maj. James S. Ketchum, and Capt. Herbert Rakatansky for their assistance in making this publication possible.

Notices

This document contains information affecting the national defense of the United States within the meaning of the Espionage Laws, Title 18, U.S.C. sections 793 and 794. The transmission or revelation of its contents in any manner to an unauthorized person is prohibited by law.

Disposition

When this document has served its purpose, DESTROY in accordance with AR 380-5. DO NOT return to U. S. Army Chemical Research and Development Laboratories.

(U) CONTENTS

		Page
I.	INTRODUCTION	5
II.	CHEMICAL AND PHYSICAL DATA	5
III.	BIOMEDICAL EVALUATION	21
	A. EA 1476	21
	B. EA 2233	42
	C. Human Data	52
IV.	WEAPONS SYSTEMS AND METHODS	60
V.	PROTECTIVE MEASURES	62
VI.	SPECIFICATION DATA	62
VII.	AVAILABILITY STATUS	62
VIII.	USE CONCEPTS	62
IX.	CONCLUSIONS	62
	LITERATURE CITED	63

(C) SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

I. (C) INTRODUCTION.

(U) The psychophysiologic changes in man caused by the resin obtained from the hemp plant, Cannabis sativa, have been known for about 3,000 years. The physiological and medicinal properties of this resin were first mentioned in Chinese writings dating back to 1000 B.C. Many names are used for the various physiologically active hemp extracts and preparations; e.g., hashish, marihuana, charas, bhang, and ganja.

(U) Investigators who have worked with marihuana, or with one of the derivatives of its active principle, have found that it characteristically produces a feeling of euphoria and relaxation, followed by lassitude and increased daydreaming, sleepiness, uncommunicativeness, and eventual recovery within 6 to 24 hours. Large doses may lead to mental confusion and apprehension, together with more vivid and more overwhelming sensory experiences that take precedence over reality and constitute, in effect, a temporary psychosis. Synthetic compounds, related to the parent substance Cannabis, have been studied: tetrahydrocannabinol and substituted synthetic derivatives. The effects of these are basically similar, with differences in potency. This information is available in the open literature and references may be found in Wikler's The Relation of Psychiatry to Pharmacology.

(C) In the light of the pharmacologic actions of these compounds, great interest has been attached to the possible use of one or more of these substances as an agent in the chemical armamentarium of the Army. This report, therefore, represents a summary of the known data, physicochemical, toxicologic, and pharmacologic, pertaining to EA 1476, EA 2233, and their isomers.

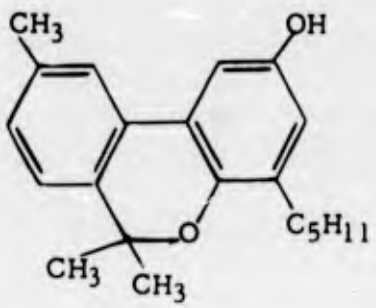
II. (C) CHEMICAL AND PHYSICAL DATA.

(U) Chemical investigations of the physiologically active resin obtained from Cannabis sativa have been going on for over a century. In 1857, T. Smith and H. Smith[1] showed that the Cannabis resins were not alkaloidal in nature. In 1896, Woods, Spivey, and Easterfield[2] isolated a high boiling, red oily, physiologically active fraction from the petroleum ether extract of Indian charas. Since this fraction had a constant boiling point, they assumed it was a homogeneous material and gave it the name cannabinol. In later investigations, Woods, Spivey, and Easterfield[3] isolated a crystalline acetate from this constant boiling fraction in a yield of 20% to 25%. The remainder of the

material, which could not be crystallized, was assumed to be a mixture of at least two components by these authors. After Woods, Spivey, and Easterfield[3] found that the constant boiling fraction from the charas extract was not a single component, they referred to this material as "red oil" because of its appearance. These investigators transferred the name cannabinol to the yellow, viscous liquid they obtained by the hydrolysis of the crystalline acetate. These same investigators,[3] Woods, Spivey, and Easterfield indicated that the physiological activity of "red oil" was caused by cannabinol. Cahn,[4] however, proved that, although cannabinol is very toxic, it does not produce the characteristic intoxicating effects of the Cannabis resins.

(U) Czerkis,[5] Frankel,[6] Casparis,[7] and Bergel[8] in the following years, tried to isolate cannabinol, but their attempts were unsuccessful until 1930, when Cahn[4, 9, 10, 11] again obtained this compound from Cannabis indica. He demonstrated by oxidative degradation studies that cannabinol was a hydroxy-n-amyl-6, 6, 9-trimethyl-6-dibenzopyran, structure (I), and tentatively placed the n-amyl and hydroxyl groups in the 2 and 4 positions, respectively.

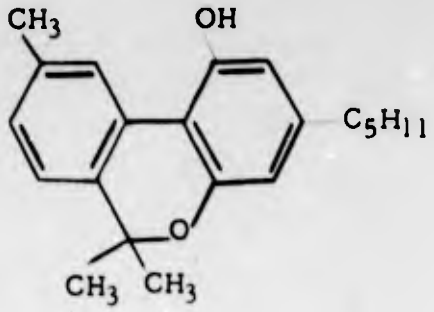
(U)



(I)

(U) The structure determination of cannabinol was completed almost simultaneously in this country by Adams and his coworkers[12-17] amd by Todd and his coworkers[18-22] in England. These two groups proved independently by synthesis that cannabinol was 1-hydroxy-3-n-amyl-6, 6, 9-trimethyl-6-dibenzopyran, structure (II).

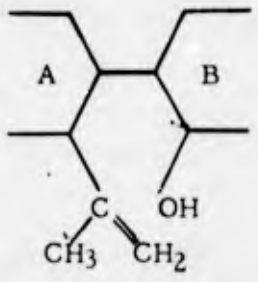
(U)



(II)

(U) Cahn[4] had suggested in 1933 that, in addition to cannabinol, "red oil" contained a dihydric phenol. He based his assumption on the fact that "red oil" gave the characteristic violet color of dihydric phenols in alkaline solution. He indicated that this dihydric phenol might contain an isopropenyl group, as in structure (III). This was suggested to Cahn[4] by the fact that Bergel[8] had shown that "red oil" could be catalytically hydrogenated. Cahn[4] stated that structure (III) was very similar to the dibenzopyran system which it would yield by ring closure.

(U)

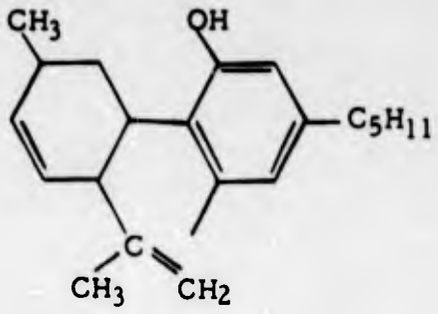


(III)

(U) Cahn's[4] assumptions were proved by Adams, Hunt, and Clark[23] in 1940, when a second pure compound was isolated from the "red oil" derived from Minnesota wild hemp in the form of the crystalline bis-(3,5-dinitrobenzoate). Ammonolysis of this ester with liquid ammonia yielded a crystalline substance called cannabidiol, because of the presence of two phenolic hydroxyl groups. Todd and Jacob[19] isolated cannabidiol from Eygptian hashish, but

in much lower yields than Adams and his coworkers[23] had obtained from Minnesota wild hemp. The structure of cannabidiol was proved by Adams and his coworkers,[23-30] except for the position of the alicyclic double bond, which was tentatively placed into the 7(8)-position as represented by structure IV.

(U)

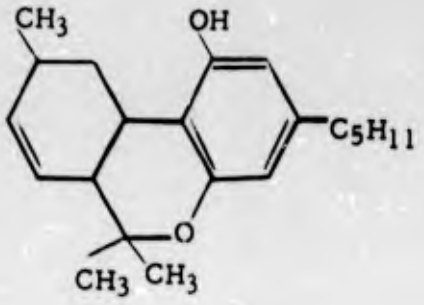


(IV)

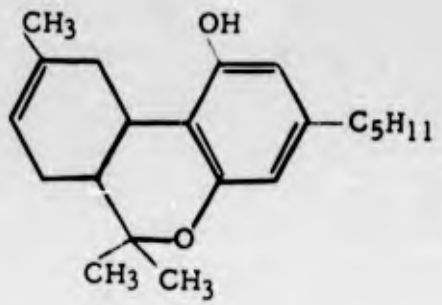
(U) Todd, Ghosh, and Wilkinson[21] and Adams, Cain, and Wolff[24] showed that the alicyclic double bond of cannabidiol was not conjugated with the aromatic ring by ultraviolet spectroscopy. Todd and his coworkers[21] observed a strong adsorption band at 2300 Å (t_{max} 10,000) in the ultraviolet spectrum of cannabidiol, which indicated that the alicyclic double bond was conjugated with the exocyclic isopropenyl double bond; however, the work of Adams and his coworkers[30] on the acid isomerization of cannabidiol indicated that the alicyclic double bond was not conjugated with exocyclic double bond.

(U) The acid isomerization of cannabidiol yielded two physiologically active, isomeric tetrahydrocannabinols. The alicyclic double bonds of these isomers were not conjugated with the aromatic ring. Sulfur dehydrogenation of the two isomeric tetrahydrocannabinols yielded the same product that was identical with an authentic sample of cannabinol.[28] This clearly proved that the treatment of cannabidiol with acid had resulted in a ring closure to form the pyran. Structures (V) and (VI) represent the configurations assigned to the two isomeric tetrahydrocannabinols by Adams and his coworkers.[30]

(U)



(V)

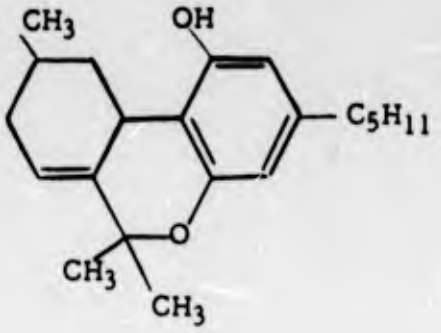


(VI)

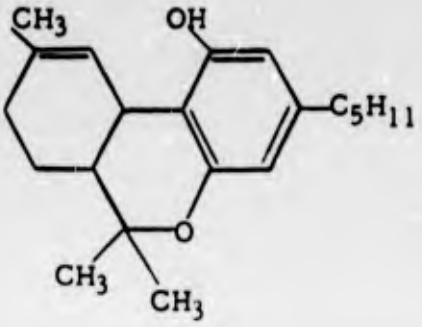
(U) The conditions of the isomerization determine, according to Adams' interpretation, which of the two isomers [structures (V) or (VI)] is formed. When Cannabidiol was refluxed with dilute ethanolic hydrogen chloride, a tetrahydrocannabinol resulted with an optical rotation of α_D-130°C. A more vigorous treatment with p-toluenesulfonic acid in refluxing benzene yielded a tetrahydrocannabinol with an optical rotation of α_D-265°C. Treatment of the low-rotating isomer, with p-toluenesulfonic acid in refluxing benzene, yielded a mixture of the high- and low-rotating isomers with a maximum rotation of α_D-200° to 225°C.[30] This led to the assumption that migration of the double bond preceded ring closure in the isomerization of cannabidiol. Complete conversion of the low-rotating to the high-rotating form was not possible, because of the increased difficulty of migration of the alicyclic double bond in the cyclized compound.

(U) The above data served as evidence for the assignment of the alicyclic double bond to the 3(4)-position in the cannabidiol, structure (IV). Since the low-rotating form of tetrahydrocannabinol, structure (V), was produced under mild conditions, Adams and his coworkers[30] assumed that the alicyclic double bond had not migrated and had the same position as in cannabidiol. The high-rotating form was regarded as the result of a migration of the alicyclic double bond into the more stable 8(9)-position, as this would provide the driving force for the migration. The 8(9)-position, with the double bond having termini on a tertiary and a secondary carbon atom, was considered to be more stable than the 7(8)-position where both termini are on secondary carbon atoms. The 6a(7)- and 9(10)-position [structures (VII) and (VIII), respectively] are ruled out, although the termini of the double bond are on secondary and tertiary carbon atoms, because, under the vigorous conditions of the p-toluenesulfonic acid treatment, the double bond would migrate into the most stable 6a(10a)-position or 10(10a)-position, respectively, in conjugation with the aromatic ring.

(U)



(VII)



(VIII)

(U) The fact that the two isomeric tetrahydrocannabinols Adams and his coworkers[28] isolated from the acid isomerization of cannabidiol had the same type of physiological activity as "red oil" indicated that the component responsible for the intoxicating effects of Cannabis resins was tetrahydrocannabinol.

(U) Several investigators have isolated tetrahydrocannabinols from the various Cannabis resins. In 1940, Haagen-Smit and his coworkers[31] isolated a crystalline compound of high physiological activity from the "red oil" derived from Minnesota hemp. The activity was 100 times greater than the activity of the crude "red oil," as shown by the dog ataxia tests used by these investigators. The high physiological activity of this crystalline material definitely established that it was not cannabinol or cannabidiol. This material was assumed to be a tetrahydrocannabinol, primarily on the basis of physiological activity, since very little supporting evidence was obtained by these investigators.

(U) More recently, Korte and Sieper[32] isolated a very small amount of a crystalline tetrahydrocannabinol from German hemp (Cannabis sativa non indica) by counter-current distribution methods. These investigators indicated that this material may be the same as Haagen-Smit and his coworkers[31] isolated, since the melting point of 120° to 125°C is very close to the melting point of 128°C Haagen-Smit reported for his crystalline tetrahydrocannabinol. The ultraviolet and infrared spectra of the crystalline tetrahydrocannabinol that Korte and Sieper[32] isolated were not identical with the infrared and ultraviolet spectra of a synthetic tetrahydrocannabinol (mp, 128°C), prepared by these investigators. This synthetic tetrahydrocannabinol will be discussed in more detail later.

(U) Todd and Jacob[33] isolated a very small amount of a substance from hashish, which they called "cannabol." They showed that this material was a cyclized isomer of cannabidiol. Based on the work of Adams,[30] Todd and his coworkers[21] indicated that "cannabol" was probably a tetrahydrocannabinol, which was isomeric to the tetrahydrocannabinols isolated from the acid isomerization of cannabidiol.

(U) Wollner, Matchett, Levine, and Loewe[34] separated a homogenous tetrahydrocannabinol from the "red oil" derived from Indian charas. This material, which was isolated as the acetate, showed a high physiological activity as manifested by the response in dogs.

(U) Tetrahydrocannabinol acetate was easily hydrolyzed to the free phenol by acid, alkali, or ammonia in alcohol solutions. The deacetylated product in each instance had a physiological potency of about 60% that of the acetate. A change in the specific rotation from α_D-214°C for the acetate to α_D-193°C for the free phenol indicated that during the hydrolysis a partial racemization had occurred, because neither the optical rotation nor the physiological activity could be restored to its initial value by reacetylation. The acetate of tetrahydrocannabinol and its hydrolysis product were compared to the two isomeric tetrahydrocannabinols isolated from the acid isomerization of cannabidiol. The following table summarizes some of the physical data for these materials.

(U)

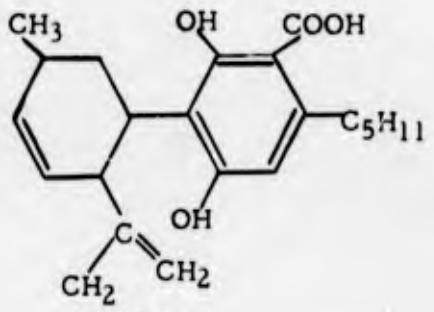
Compound	Relative physiological activity*	Optical rotation °C
Tetrahydrocannabinol acetate	14.7	-214
Hydrolysis product of tetrahydrocannabinol acetate	8.0	-193
Tetrahydrocannabinol from the alcoholic hydrogen chloride isomerization of cannabinol	8.7	-130
Tetrahydrocannabinol from the p-toluenesulfonic acid catalyzed isomerization of cannabinol	8.7	-265

* The activity of the above materials was determined by the dog ataxia test. The physiological activities are compared to 1-hydroxy-3-n-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran, structure (XII), as the standard.

(U) Recent work by Schultz and Haffner[35] indicated that tetrahydrocannabinol may exist in the fresh Cannabis extracts in very low concentrations or may be completely absent. These investigators propose that tetrahydrocannabinol is an artifact that is formed by cyclization of a compound related to cannabidiol during the purification of the Cannabis resins. They suggest that tetrahydrocannabinol may be formed by the same cyclization process in the isolated Cannabis resins by standing for long periods of time at high temperatures.

(U) By working at low temperatures with the exclusion of light, Schultz and Haffner[35] isolated a sedative principle from Cannabis sativa and from Cannabis indica. This material showed a sedative action in mice at doses from 0.05 to 0.1 mg/gm and was lethal to 50% of the animals at doses of 0.25 to 0.5 mg/gm. These investigators showed that the sedative principle was cannabidiol carboxylic acid, structure (IX).

(U)



(IX)

(U) Schultz and Haffner[35] found that structure (IX) could be isolated from fresh extracts of Cannabis sativa in yields up to 50% of the total resin, but the yields were much lower from old extracts of Cannabis sativa and from fresh extracts of Cannabis indica. Cannabidiolcarboxylic acid, structure (IX), could be decarboxylated at room temperature in concentrated ammonium hydroxide or by heating to 100° in vacuo to form cannabidiol, structure (IV). Since structure (IX) decarboxylates at relatively low temperatures in vacuo, it is not unusual that none of the previous investigators had isolated (IX). With the exception of the work of Korte and Sieper,[32] all the other investigators previously mentioned did their work on Cannabis resins, which were subjected to an initial purification by high-temperature distillation at low pressures.

(U) Krejci, Gorak, and Santavy[36,37,38] isolated a material from the leaves of hemp grown in central Europe, which these authors identified as the diacetate, structure (IX). The two melting points of 80° to 100°C

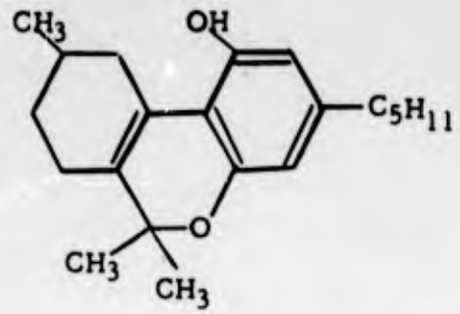
and 127° to 128°C reported differ considerably from the melting points of 96° to 97°C and 115° to 118°C reported by Schultz and Haffner.[35] The sedative action and antibacterial action of the compound isolated by Krejci, Gorak and Santavy[37] are very similar to that of the compound Schultz and Haffner[35] isolated.

(U) From the work of Schultz and Haffner,[35,39] it appears that the essential components of Cannabis resins were cannabinol, cannabidiol, cannabidiolcarboxylic acid, and tetrahydrocannabinol. These investigators indicated that structure (IX) is probably the precursor of the other three components. Decarboxylation and ring closure of structure (IX) would form the tetrahydrocannabinol, structure (V). Dehydrogenation of structure (V) would result in the formation of cannabinol, structure (II). Decarboxylation of structure (IX) without ring closure would form cannabidiol, structure (IV), as was shown experimentally by these investigators.

(U) Schultz and Haffner[39] found that the hashish principle of the resin derived from German hemp, which contains very little tetrahydrocannabinol, could be increased by heating the resin to 50°C for 48 hours. These data offered an explanation for the wide variation in physiological activity of extracts of hemp grown under different climatic conditions. The extracts of hemp grown in warm climates are much more active than the extracts of hemp grown in colder climates. As pointed out by Schultz and Haffner,[39] it appeared that in warm climates, the thermally unstable cannabidiolcarboxylic acid cyclizes and decarboxylates to form tetrahydrocannabinol. In colder climates, structure (IX) is more stable and only a small quantity of tetrahydrocannabinol is found in extracts of hemp grown under these conditions.

(U) Since it appeared that tetrahydrocannabinol was the physiologically active principle responsible for the marihuana-like effects of the hemp extracts, a large amount of work was done on the synthesis of tetrahydrocannabinols. From the consideration of the structures of natural tetrahydrocannabinols [structures (V) and (VI)] proposed by Adams,[30] it was apparent that many isomers were possible. Depending on the source, the tetrahydrocannabinol in "red oil" may be composed of one or all of the possible isomers. Wollner, Matchett, Levine, and Loewe[34] pointed out that the various isomers would vary widely in physiological activity. This was substantiated by the synthesis of 1-hydroxy-3-n-amyl-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran, structure (IX), and optically inactive isomer of the naturally occurring tetrahydrocannabinols [structures (V) and (VI)] by Adams and Baker[17] and independently by Todd and his coworkers.[21]

(U)

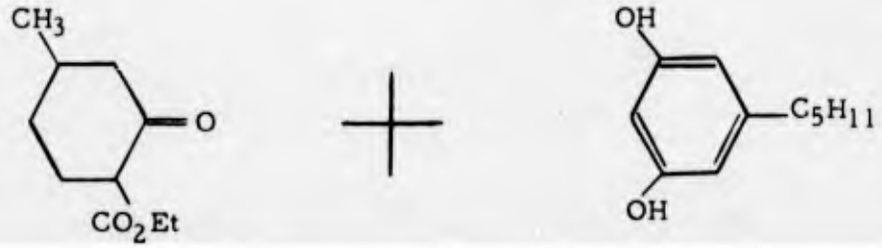


(X)

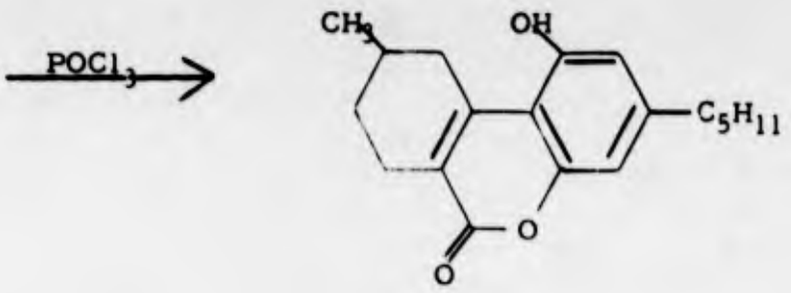
(U) Structure (X) differs from the natural tetrahydrocannabinols in two respects. First, the alicyclic double bond of structure (X) is conjugated with the aromatic ring. Second, structure (X) contains only one asymmetric carbon atom. The natural tetrahydrocannabinol, which has structure (V), has three asymmetric atoms and one with structure (VIII) has two asymmetric atoms. The physiological activity of structure (X) is of the same type as the natural material, but the potency is considerably less than that of the natural material.

(U) The first method for preparing the synthetic isomer of structure (X), reported by Adams and Baker[17] and by Todd and his coworker,[21] is illustrated by the reaction sequence I. Ethyl 5-methylcyclohexanone-2-carboxylate, structure (XI), was condensed with olivetol, structure (XII), in the presence of phosphorus oxychloride. The resulting pyrone, structure (XIII), was treated with excess methylmagnesium iodide to yield the tetrahydrocannabinol, structure X.

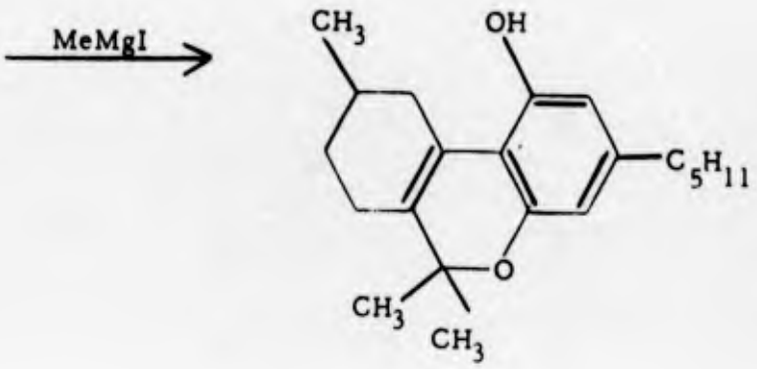
(U) Reaction I:



(XI) (XII)



(XIII)



(X)

(U) This synthetic tetrahydrocannabinol, structure (X), was reported by Adams and Baker[17] to be a colorless viscous oil having marihuana-like activity. Recently, Korte and Sieper[32] succeeded in separating the non-crystalline product prepared by the method of Adams into two crystalline components. These investigators purified the product from the Grignard reaction by counter-current distribution over an 87-tube train. The predominant isomer, which melted at 62° to 63°C, was assigned structure (X). The second isomer, which melted at 128°C, was present in very small amounts. The ultraviolet spectrum of this isomer was the same as the ultraviolet spectrum of crystalline tetrahydrocannabinol that these investigators isolated from Cannabis sativa. The structure of the high-melting isomer was not definitely established, but Korte and Sieper[32] proposed

that this material differed from the low-melting isomer in regard to the position of the alicyclic double bond.

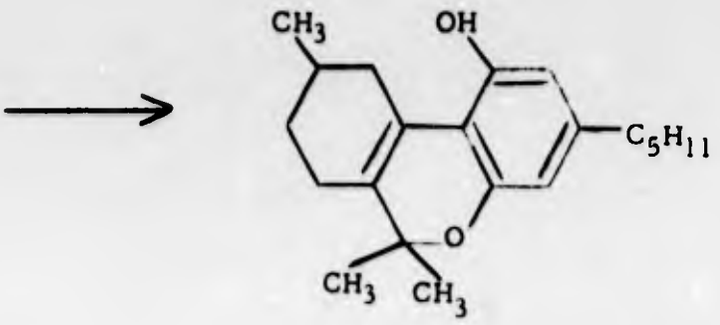
(U) The physiological activity of the synthetic tetrahydrocannabinol, represented by structure (X), had not been expected by Adams and his coworkers.[40,41] The compound was initially prepared to prove the structure of cannabinol (II). Sulfur dehydrogenation of structure (X) does in fact yield a compound identical with an authentic sample of cannabinol.[15] The added bonus of physiological activity enabled these investigators to potency.[42] The condensation of ethyl d and l-5-methylcyclohexanone-2-carboxylate with olivetol yield optically active pyrones, which were converted by treatment with excess methylmagnesium iodide to the two optically active tetrahydrocannabinols, d- and l- structure (X), respectively. Both antipodes of the synthetic tetrahydrocannabinol were physiologically active, but the d-isomer was only 40% as active as the racemate and the l-isomer was four to five times more active than the d-isomer.[42] The higher activity of the levorotatory form is of interest, because the tetrahydrocannabinols derived from cannabidiol and directly from "red oil" were levorotatory.

(U) Todd and his coworkers[43] prepared the d-isomer of structure (X) by a method similar to that used by Adams. The physiological activity of the d-isomer measured by the Gayer test in rabbits was only 12% to 16% as active as the racemate of structure (X).

(U) Todd and his coworkers[44] and Adams and his coworkers[45] prepared a synthetic tetrahydrocannabinol by the synthetic route outlined by reaction sequence II. These investigators found that pulegone structure (XIV) would condense with olivetol [structure (XII)] in the presence of an acid catalyst to form structure (X).

(U) Reaction II:

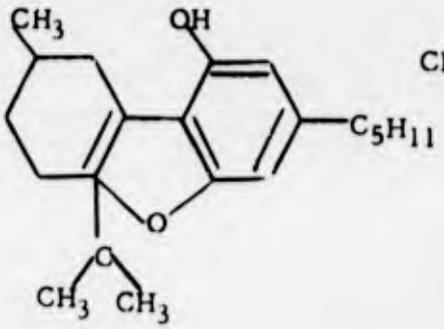
(XIV) (XII)



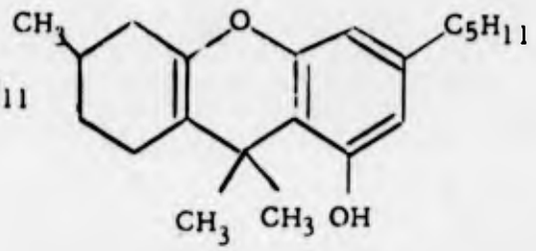
(X)

(U) This method of preparing structure (X) has a serious disadvantage. A large number of side reactions occur under the conditions of the condensation and only about half of the product has structure (X). Some of the side products have been assigned structures (XV) and (XVI) by Adams and his coworkers.[46]

(U)



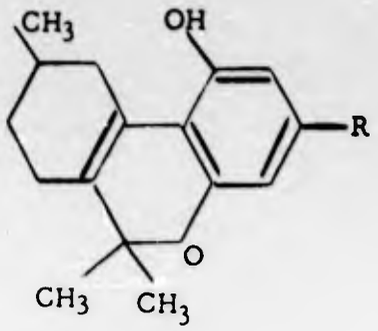
(XV)



(XVI)

(U) The activity of the tetrahydrocannabinol represented by structure (X) led Adams and his coworkers[40, 41, 47, 48, 49, 50, 51] to the preparation of a large number of homologs of structure (X). These compounds all had the general structure represented by structure (XVII).

(U)



(XVII)

(U) The homologs were all prepared by the method illustrated by reaction sequence I, using the appropriate alkyl resorcinol in place of olivetol. The length and branching of the 3-alkyl side chain were varied over a wide range. The resulting tetrahydrocannabinol homologs all caused ataxia in the dog and differed only in their potency.

(U) Todd and his coworkers[52, 53] prepared several homologs of structure (X) by the method outlined in reaction sequence I. These homologs were all active as illustrated by the Gayer test in rabbits and differed only in their potency. The data Todd and his coworkers[52, 53] obtained for the physiological activity of the homologs of structure (X) did not compare favorably with those reported by Adams and his group. Since the two groups of investigators used different methods for testing the physiological activity of structure (X) and the homologs of (X), Todd and his coworkers[52] ran some comparative experiments on the dog-ataxia and Gayer-rabbit tests. These investigators reported that the results from the methods of testing for physiological activity were only roughly comparable. Todd[52] suggested that the two methods may not be measuring the same type of activity. The dog-ataxia test appeared to be much more sensitive than the Gayer-rabbit test. Adams and his coworkers[30] proved that motor incoordination in the dog (dog-ataxia test) was a satisfactory criterion for determining whether the tetrahydrocannabinol would have marihuana-like activity in human beings.

(C) The activity of the synthetic tetrahydrocannabinol analogs structure (XVII) increases dramatically by lengthening the R groups to 6- and 7-carbon chains, with additional branching in the α- and β-positions to the aromatic nucleus. The potencies, as measured by the dog-ataxia test, of a series of homologs prepared by Adams et al.,[50, 51] are listed in the following table with the synthetic tetrahydrocannabinol, structure (XVII) ($R = C_5H_{11}$), serving as a unit standard.

(C)

R of Structure (XVII)	Potency	Literature citations
C_5H_{11}	1.0	50
$-CH(C_2H_5)-CH(CH_3)-CH_3$	3.40 ±1.10	50
$-CH(CH_3)-CH(CH_3)-C_2H_5$	3.80 ±0.32	50
$-C(CH_3)(CH_3)C_3H_7$	4.18 ±0.34	50
$-C(CH_3)(CH_3)-C_6H_{13}$	21.8 ±1.91	50
$-CH(CH_3)-CH(CH_3)-C_4H_9$	39.8 ±8	51
$-CH(CH_3)-CH(CH_3)-CH_2-CH(CH_3)-C_2H_5$	36.0 ±9.5	51
$-CH(CH_3)-CH(CH_3)-C_5H_{11}$	512.0 ±72.6	50
$-CH(CH_3)-CH(CH_3)-C_6H_{13}$	19.0 ±9.5	51

(C) Outstanding by far in its potency was the 3-(1,2-dimethylheptyl) analog (EA 1476) of tetrahydrocannabinol structure (X) (EA 1477). Further lengthening of the R group of structure (XVII) by one carbon atom to a 1,2-dimethyloctyl side chain, resulted in a 25-fold decrease of the activity as compared to EA 1476.

(U) Numerous variations of the basic structure (XVII) in the cyclohexene moiety of the molecule, as well as the replacement of both methyl groups in the 6-position by hydrogen resulted in partial and even complete loss of activity in comparison to natural tetrahydrocannabinol, as the studies of Todd et al.[52,53] demonstrated.

(C) The most active compound in the series of the synthetic tetrahydrocannabinol analogs, the 3-(1,2-dimethylheptyl) analog EA 1476, was prepared by Adams and coworkers[50] from benzoic acid in an overall yield of approximately 3%. A careful re-investigation of each of the 12 different steps of the Adams synthesis by Reeves et al.,[54] led to an increase of the overall yield to 7.6%.

(C) Since the large-scale production of EA 1476 by the classical Adams route is uneconomical, alternate approaches to the synthesis of the key intermediate, 5-(1,2-dimethylheptyl) resorcinol, were investigated. The most successful solution toward an economical production of the intermediate rescorcinol homolog was found to be the conversion of 1,3,5-trichlorobenzene in one step to 3,5-dimethoxyphenyl chloride in 70% yield. This intermediate is then converted by the way of its Grignard reagent by reaction with 2-methylheptanonitrile and subsequent treatment of the resulting ketone with methylmagnesium halide to 2-(3,5-dimethoxyphenyl)-3-methyl-2-octanol, which, by successive dehydration, reduction, and cleavage, yields the desired resorcinol.[55]

(C) Utilizing this alternate route in combination with the suitable, subsequent steps of the Adams synthesis, EA 1476 can be prepared in an overall yield of approximately 23% from trichlorobenzene.

(C) The boiling point of EA 1476, reported by Adams et al.,[50] is 170° to 173°C at 0.04 mmHg Reeves and coworkers[54] reported a boiling point of 185° to 195°C at 0.025 mmHg.

(C) EA 1476, prepared by either one of the routes described, is an extremely viscous resin of a pale yellow color when freshly prepared. It is sensitive to light and atmospheric oxygen and discolors rapidly when stored under ordinary conditions without special precautions. This marked instability of the molecule, also shared by natural tetrahydrocannabinol, results in a slow degradation, with the formation of highly colored fragmentation products that is apparently caused by the presence of a free phenolic hydroxyl group.

(C) Since the acetate of natural tetrahydrocannabinol is reported to have a physiological activity similar to that of the free phenolic compound, EA 2233, the acetate of EA 1476, was expected to exhibit a somewhat greater stability toward the degrading effects of light and air than EA 1476. The stability is indeed increased by the esterification of the free phenolic group of EA 1476 with acetic acid while its activity is retained. The resulting acetate, EA 2233, is a very pale yellow, viscous resin that can be stored in ordinary containers in the refrigerator without discoloration for a prolonged period of time.

III. (C) BIOMEDICAL EVALUATION.

(U) The biological evaluation of EA 1476 and EA 2233 is divided into two major areas; i.e., (a) toxicologic studies in animals and (b) clinical studies in man. These data are further divided into two major subdivisions according to compound (EA 1476 and EA 2233) and into the categories of acute and of chronic. For clarity, the majority of references are given in the body of the report.

A. (C) EA 1476.

1. (C) Acute Administration.

a. (C) Mice.

(1) Toxicity Screening Branch Data Sheet, 14 December 1961.

The samples of EA 1476, one labeled "purified" and the other labeled "crude" have been evaluated intravenously, using the standard dose-response screening technique. The results from these two samples are almost identical and are as follows:

Compound	LD50 mg/kg	MED50 mg/kg	LD50:MED50
EA 1476 (purified)	>10.0	0.32	>31.3
EA 1476 (crude)	ca. 10.0	0.32	31.3

Toxic signs occurring for each compound were similar for a given dose. Those seen at the MED50 for each compound were decreased activity, slow deep respiration, and hunched posture.

(2) Contract DA-18-108-CML-3968.

The Fifth Progress Report, 10 February 1964.

LD50 mg/kg	19/20 confidential limits mg/kg	Route	Number of animals
390	260-585	ip	40

b. (C) Rats.

(1) Toxicity Screening Branch Data Sheet, 29 February 1960.

Dose mg/kg	Reaction fraction	Toxic Signs
0.32	2/2	Decreased activity
	1/2	Ataxia; general weakness; labored breathing
0.10	1/2	Decreased activity
0.032	1/2	Decreased activity; ataxia; general weakness; labored breathing
0.01	-*	Decreased activity

(2) Hazelton Laboratory Monthly Progress Report No. 2, August 1960.

Dose mg/kg	Route	Number of animals	Toxic signs
0.1	iv	4	Slight mydriasis for 1 hr
1.0	iv	4	Slight mydriasis for 1 hr
10.0	iv	4	Slight mydriasis for 1 hr; slight exophthalmos for 24 hr

Margin of safety = >100.0 mg/kg

(3) Fourteenth Tripartite, U. S. Progress Report on Medical Aspects, September 1959.

The following presents comparison data of the analgestic action of oral EA 1476 to morphine in rats:

* (U) None was given.

Drug	Oral dose mg/kg	Analgesia %	Molar rates drug:nalorphine	Nalorphine antagonism sec
Morphine	75.0	71-100	32 - 2/1	0-75
EA 1476	150.0	20-87	10 - 2/1	0
EA 1476	200.0	42-100	- 1/1	10-12

Definitions: Analgesia = reaction time >15 sec.
Antagonism = reaction time reduced to <7.5 sec.
Maximum control reaction time = 7.5 sec; mean = 5.0 sec.

Analgesia is based on the response of the rat tail to artificial heat generated by a hot wire.

c. (C) Rabbit.

(1) Toxicity Screening Branch Data Sheet, 3 November 1961.

Dose mg/kg	Reaction fraction	Toxic signs
10.0	1/1	Ataxia, mydriasis; generalized weakness
3.2	1/1	Hypersensitivity to touch and sound
1.0	1/1	Nystagmus; prostration; loss of corneal reflex
0.32	2/2	Nystagmus; loss of corneal reflux
	1/2	Ataxia; mydriasis
0.10	2/2	Loss of corneal reflex
	1/2	Mydriasis
0.032	4/4	Loss of corneal reflex
0.010	3/4	Loss of corneal reflex
0.0032	0/4	None

(2) Hazleton Laboratory Monthly Progress Report, August 1960.

Dose mg/kg	Route	Number of animals	Toxic signs
0.01	iv	4	Slight mydriasis
0.10	iv	4	Slight mydriasis; slight lacrimation
1.0	iv	4	Slight mydriasis; moderate lacrimation

Safety ratio = >100.0 mg/kg

d. (C) Cat.

(1) Hazleton Laboratory Monthly Progress Report, August 1960.

Dose mg/kg	Route	Number of animals	Toxic signs
0.01	iv	4	None
0.10	iv	4	Slight mydriasis
1.0	iv	4	Moderate mydriasis; moderate ataxia; slight piloerection

Safety ratio = >10.0 mg/kg

(2) Contract DA-18-108-CML-5596 Report, 26 May 1959.

Preliminary studies in six cats were done at doses from 1.0 to 6.0 μg/kg. No behavioral changes were noted below dose levels of 10 μg/kg. Two delayed deaths occurred in the 1.0 to 6.0 μg/kg range, but these were attributed to "poor nursing care."

e. (C) Dog.

(1) (C) Hazleton Laboratory Monthly Report, 2 August 1960.

Dose mg/kg	Route	Number of animals	Toxic effect
0.01	iv	1	Slight mydriasis
0.10	iv	1	Marked mydriasis

Safety ratio = >10.0 mg/kg

Dose mg/kg	Route	Number of animals	Toxic effect
		Pharmacodynamics	
0.01	iv	1	None
0.10	iv	1	None
1.0	iv	1	Slight norepinephrine antagonism; slight diphasic blood pressure response
		Conditioned Avoidance Response (CAR) test	
0.01	iv	2	None
0.05	iv	2	None

(2) (C) Hazleton Laboratory Monthly Progress Report No. 4, October 1960.

Sustained Physical Exercise (SPE) test

Species	Dose	Pulse		Temperature		Running time
		Before run	After run	Before run	After run	
	mg/kg	beat/time		°F		min
Dog (control)	-	96	114	102	102	30
Dog	0.25	125	114	102	103	30
Dog (control)	-	162	114	102	102	30
Dog	0.25	150	140	105	104	30

No toxic signs were noted.

(3) (C) Vapor Toxicity Branch Quarterly Progress Reports, July 1961 to December 1962.

Dose mg/kg	Route	Number of animals	Reaction fraction	Toxic effect
			Conditioned Avoidance Response (CAR) test	
0.025	iv	3	None	
0.050	iv	3	1/3	CAR
			3/3	Bradycardia; hyperthermia
			2/3	Ataxia; vomiting; head jerking
			1/3	Salivation
0.10	iv	3	1/3	CAR
			3/3	Bradycardia
			2/3	Hyperthermia; ataxia; mydriasis
			1/3	Hypothermia; hypopnea; vomiting; salivation; prominent nictotating membrane; decreased activity
0.20	iv	3	3/3	CAR; hyperthermia; mydriasis; ataxia; muscular weakness
			2/3	Subconvulsive jerking
			1/3	Collapse
0.30	iv	1	-*	CAR; bradycardia; ataxia; hyperthermia; mydriasis; salivation; analgesia (tail pinch); muscular weakness; collapse
0.35	iv	1	-*	CAR; bradycardia; ataxia; hyperthermia; salivation, mydriasis; subconvulsive jerking; ptosis; collapse
			Sustained Physical Exercise (SPE) test	
0.10	iv	2	1/2	SPE; vomiting: ataxia
0.175	iv	1	-*	None on SPE; ataxia; and hind-leg weakness
0.25	iv	1	-*	SPE; ataxia; salivation; appeared confused

* (U) None was given.

(4) (C) Hazleton Laboratory Monthly Progress Report No. 16, October 1961.

Dose-Regression Study, Mongrel Dogs

(U) Twenty-four dogs (18 females and 6 males) used in this study were acclimated to laboratory conditions for 4 to 6 weeks before dosing. All dogs were vaccinated for canine distemper, infectious hepatitis, and rabies during this time. The dogs were housed individually and fed a basal laboratory diet (Wayne Dog Food) once each day. Water was available at all times. The dogs were randomly divided into the following groups:

Dose mg/kg	Number of dogs	Sex	Weight kg
0.01	3	F, F, M	8.5, 5.5, 7.4
0.10	3	F, F, M	7.9, 8.3, 9.3
0.316	5	F, F, F, F, M	8.0, 10.8, 7.4, 8.5, 8.6,
0.562	5	F, F, F, F, M	10.2, 6.9, 7.3, 8.6, 9.8
1.0	3	F, F, M	9.0, 7.0, 5.4
3.16	3	F, F, M	5.3, 7.3, 10.7
10.0	2	F, F,	4.4, 5.7

(U) All doses were administered intravenously by the way of the cephalic vein. Observations for pharmacotoxic signs were made immediately, 1, 2, and 4 hours after administration and at 24-hour intervals thereafter, until normal appearance and behavior returned.

(C) Table 1 presents a summary of those specific toxic signs that had an apparent dose-response relationship that may be amenable to statistical evaluation.

(C) The most sensitive indicator of effect appeared to be mydriasis, which was moderate at 0.01 mg/kg, was slight to moderate at 0.1 mg/kg, and was marked at all subsequent higher dose levels.

(C) Other signs indicating reasonable dose-response relationships included decreased activity, weakness of limbs, ataxia, prostration, and loss of righting reflex. These signs largely reflect central depression of propioceptive reflexes. Signs revealing possible central stimulation were also apparent and consisted of tremors, subconvulsive jerking, and hyperpnea.

(C)

TABLE I

A SUMMARY OF SELECTED PHARMACOTOXIC SIGNS, TOGETHER WITH REACTION FRACTIONS AND DEGREE OF TOXIC SIGNS, REVEALING DOSE-RESPONSE RELATIONSHIPS IN DOGS FOLLOWING ACUTE INTRAVENOUS ADMINISTRATION OF EA 1476 AT INDICATED DOSAGE LEVELS (C)

Pharmacotoxic signs	Dosage levels													
	0.01 mg/kg		0.10 mg/kg		0.316 mg/kg		0.562 mg/kg		1.0 mg/kg		3.16 mg/kg		10.0 mg/kg	
	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*
Mydriasis	3/3	++	1/3 2/3	+ ++	5/5	+++	1/5 4/5	++ +++	3/3	+++	3/3	+++	2/2	+++
Decreased activity	-	-	0/3	-	1/5 1/5	+ ++	1/5 3/5	++ +++	3/3	+++	3/3	+++	2/2	+++
Weakness of limbs	1/3	++	0/3	-	0/5	-	2/5 2/5	++ +++	3/3	+++	3/5	+++	2/2	+++
Ataxia	-	-	0/3	-	1/5	+	2/5 3/5	++ +++	3/3	+++	1/3 2/3	++ +++	2/2	+++
Prostration	-	-	-	-	0/5	-	2/5	X	2/3	X	2/3	X	2/2	X
Righting reflex absent	-	-	-	-	0/5	-	2/5	+++	2/3	+++	2/3	+++	1/2	+++
Hyperpnea	-	-	-	-	0/5	-	1/5 4/5	+ ++	1/3	+	1/3 1/3 1/3	+ ++ +++	2/2	++
Tremors	-	-	-	-	0/5	-	1/5	++	0/3	-	3/3	++	1/2	+++
Subconvulsive jerking	-	-	-	-	0/5	-	1/5	++	0/3	-	3/3	++	1/2	+++
Hypopnea	-	-	-	-	-		-	-	0/3	-	1/3	+++	2/2	+++
Death	0/3	-	0/3	-	0/5	-	0/5	-	0/3	-	0/3	-	1/2	-

* (U) + = slight; ++ = moderate; +++ = marked; X = all-or-no response.

(C) Other signs occurring, but lacking dose-response relationships, included slight to moderate lacrimation at 0.316 mg/kg, marked absence of pain reflex at 0.562 mg/kg, and retching, moderate relaxation of the nictitating membrane, and slight salivation at 1.0 and 3.16 mg/kg.

(C) The minimum lethal dose was found to be 10 mg/kg where one of two dogs died between 3 and 4 days postinjection. Other than generalized congestion, gross necropsy did not reveal any significant indication of organic injury. Therefore, a significant margin of safety at 1,000 was demonstrated between the minimal effective dose and the lethal dose.

(5) (C) University of Michigan, Fifth Progress Report, Contract DA-18-108-CML-3968, 10 February 1954.

(U) Twenty dogs were injected intravenously with EA 1476 in doses ranging from 0.125 to 100 mg/kg and were observed for toxic signs. The results of these experiments are as follows:

(a) (C) Two Dogs; 0.125 mg/kg.

Both dogs exhibited analgesia as measured by the ear-pinch test and a delayed withdrawal reflex to pressure applied to the paw. These animals were unable to stand after receiving this dose; one for as long as 36 hours. Recovery occurred in 30 hours in one case and 48 hours in the other.

(b) (C) Eight Dogs; 0.25 mg/kg.

Marked central depression, analgesia, ataxia, and lethargy were seen in all dogs. Most dogs showed an initial central stimulation, followed by marked depression and, during the recovery period, a slight hyperexcitability. Although markedly depressed, most dogs could be roused and become very excited for a short time. Several could not be roused, however. Analgesia varied considerably. Two dogs did not respond to a weight of 170 lb placed on their paws or tail. Recovery occurred in 2 to 4 days.

(c) (C) Four Dogs; 1.0 mg/kg.

Predominant signs seen in these dogs were marked depression, analgesia, and hyperexcitability upon stimulation. Two dogs could not be roused from depression. Some dogs appeared to be having hallucinations. All recovered uneventfully in 4 to 5 days.

(d) (C) Two Dogs; 5.0 mg/kg.

Only one of these dogs exhibited hyperexcitability initially and both exhibited deep depression. The animals appeared to be asleep. Both dogs recovered uneventually, one in 3 days and the other in 5 days.

(e) (C) Two Dogs; 10.0 mg/kg.

The signs shown by these dogs were identical to those seen at 5.0 mg/kg. Recovery times were similar.

(f) (C) One Dog; 50.0 mg/kg.

Initial CNS stimulation included clonic and tonic convulsions of short duration. This was followed by marked depression characterized by deep sleep for approximately 48 hours. After 3 days, the animal began an uneventual recovery.

(g) (C) One Dog; 100.0 mg/kg.

This animal was depressed immediately after receiving the drug. Bloody stools and shallow respiration were observed during 24 hours. The animal was found dead at 28 hours.

(U) In addition to the intravenous work stated above, five dogs were evaluated when the agent was given orally. The results are as follows:

(a) (C) One Dog; 0.5 mg/kg.

Ataxia, analgesia, as measured by ear pinch, and deep depression characterized by sleep were the dominent signs seen in this dog. Some emesis, gagging, and increase in nasal and lacrimal secretions were seen between 10 and 24 hours after dosing. This animal appeared to have recovered at 48 hours.

(b) (C) Two Dogs; 1.0 mg/kg.

Both dogs vomited several times during the first hour after compound. They became sedated and depressed but could easily be aroused. Analgesia was of short duration. At 24 hours both animals appeared normal.

(c) (C) Two Dogs; 2.0 mg/kg.

Slight hyperexcitability, salivation, and emesis followed by marked depression, ataxia, and analgesia were seen in these dogs. When roused, both animals became hyperexcited. Marked depression persisted until 36 to 48 hours, when they became more alert. Analgesia, salivation, and increased nasal and lacrimal secretions were still evident at this time. Three days after receiving the drug, the animals appeared to be normal.

(6) (C) Fourteenth Tripartite, U. S. Progress Report on Medical Aspects, September 1959.

A comparison study of EA 1476 with reserpine in dogs was conducted. Gross behavioral changes are often indistinguishable between these two compounds. The results of these studies are as follows:

Observation	Dose	
	Reserpine	EA 1476
	mg/kg	
Tranquilization	0.1	0.025-0.05
Miosis	0.1	±0.050
Bradycardia	1.0	0.05
Hypothermia	1.0	≤1.0
Diarrhea	0.1	100.0
Relaxation of nictitating membrane	0.1	0.10
Respiratory depression	1.0	0.05
Inhibition of carotid occlusion pressor response	1.0	0.05
Inhibition of central vagal stimulation pressor response	1.0	0.50
Increased vascular response to epinephrine	1.0	0.50

The effect of EA 1476 on the mean arterial blood pressure in dogs is also reported in the Fourteenth Tripartite, Medical Aspects Progress Report. EA 1476 produces a gradual and prolonged reduction in the mean arterial blood pressure in dogs lightly anesthetized with sodium pentobarbital (30.0 mg/kg). This action has a long latent period after intravenous injection of the agent. The following table illustrates this fact:

Time	Control: Number of animals	Control: Blood pressure change	EA 1476 (0.05 mg/kg): Number of animals	EA 1476 (0.05 mg/kg): Blood pressure change	P value
hr		%		%	
0.2	5	>0.04	6	<2.1	0.2
0.5	5	<1.4	6	<7.4	0.2
1.0	5	<0.6	6	>15.1	0.05
2.0	5	>0.4	5	<18.4	0.05
3.0	5	>1.2	5	<23.3	0.01
4.0	5	>1.9	5	<22.4	0.01

The mechanism by which EA 1476 kills an animal given toxic doses is partly related to the effect of the agent on the cardiovascular system. Electrocaridographic records indicate clearly that ventricular fibrillation is the mechanism of death in dogs after a dose of 1 mg/kg or more.

f. (C) Monkey.

(1) (C) Fifth Progress Report, University of Michigan, 10 February 1954, Contract No. DA-18-108-CML-3968.

(a) One Monkey; 0.5 mg/kg Intravenously.

The monkey showed immediate signs of depression, which included marked body sag, closing of eyes, complete indifference to handling and no response to pain (no pain-pupil response). This animal could walk when prodded, but exhibited marked ataxia. After 8 hours, the monkey appeared to be fairly agile, but was still depressed. Twenty-four hours later, he was well coordinated, but appeared somewhat confused. The animal appeared normal at 48 hours.

(b) One Monkey; 1.0 mg/kg Intravenously.

The signs observed were essentially the same as those described for 0.5 mg/kg; however, they were more pronounced and of longer duration. Depression persisted for 48 hours after which the monkey appeared more alert. Hematuria and increased lacrimal secretions were observed during this period. Five days after the drug, the monkey still appeared slightly depressed. Eight days after the drug, the animal appeared normal.

(2) (U) Tulane University Final Report, Contract No. DA-18-108-CML-5596, 26 May 1959.

Two monkeys were administered intravenously EA 1476 in doses of 500 and 1,250 μg/kg. Definite "spike-dome" patterns appeared immediately in electrocorticogram leads from the septal region. These effects, characteristic of the schinzophrenic spike, were present for at least 72 hours after injection of the drug.

(3) (C) Hazleton Laboratory Monthly Progress Report No. 16, October 1961.

Dose-Regression Study; Monkeys

(U) The monkeys (Macaco mulatta), used in this study, were quarantined for 10 to 12 weeks in individual cages. Tuberculosis tests, which were carried out several times during this period, were negative. These animals received chloromycetin, tetracycline, and neomycin sulfate prophylactically, at periodic intervals, particularly to counteract episodes of diarrhea.

(C) The 15 monkeys, 5 females and 10 males that were used were divided into groups as follows:

Dose mg/kg	Number of animals	Sex	Weight kg
0.0316	3	F, M, M	2.3, 1.8, 2.2
0.10	5	F, M, M, M, M	2.0, 2.5, 2.3, 2.0, 2.0
1.0	3	M, M, M	2.1, 2.1, 2.2
3.16	3	F, F, F,	2.7, 2.3, 2.1
10.0	1	M	2.0

(C) All injections were made into the saphenous vein. Observations were made immediately after injection, at 1, 2, and 4 hours, and 24-hour intervals thereafter, until the animal regained normal appearance and behavior.

(C) A summary of the pharmacotoxic signs that occurred in an apparent dose-response relationship is presented in table 2.

(C) The most sensitive indicators of effect appeared to be ptosis, decreased activity, and peripheral vasodilation, which were noted at a slight degree in one monkey dosed at 0.316 mg/kg. These signs, in general, increased in severity and in the fraction of animals affected as the dose increased. Other signs of significance and revealing dose-response relationships included hyperpnea followed by hypopnea, ataxia, and poor appetite.

(C) Those signs that did not reveal any dose-response relationships included moderate piloerection in four or the five monkeys at 0.10 mg/kg, moderate mydriasis in one of the three monkeys at 1.0 mg/kg, and moderate subconvulsive jerking in one of the three monkeys at 3.16 mg/kg. The relative absence of pupillary dilatation in monkeys with EA 1476 was unique in that the rat, rabbit, cat, and dog all exhibited mydriasis at doses ranging from 0.01 to 0.10 mg/kg.

(C) The single monkey dosed at 10.0 mg/kg exhibited marked pharmacotoxic signs and died between 28 and 43 hours. Death was apparently caused mainly by respiratory failure, and necropsy revealed moderate congestion of the lungs, liver, and kidneys.

(C) These experiments indicate that the minimal effective dose for EA 1476 in monkeys was found to be 0.0316 mg/kg and the minimal lethal dose to be 10.0 mg/kg. A moderately large safety margin was demonstrated for this compound.

(4) (C) Hazleton Laboratories Monthly Progress Report No. 2, February 1962.

Visual Discrimination Test (VDT)

The detailed data of the effects of EA 1476 in monkeys trained to perform the VDT can be found in Hazleton Laboratories Monthly Progress Report Nos. 4, 26, and 27, dated October 1960, August 1962, and September 1962, respectively. These data have been summarized, graded, and compared with data obtained for EA 2233 and its isomers, and EA 2479 and its isomers in Monthly Progress Report No. 2, February 1963.

EA 1476 has been evaluated in 10 monkeys trained to perform in the VDT at three dose levels as follows:

Dose mg/kg	Number of animals
0.01	3
0.10	4
0.316	3

(C)

TABLE 2

SUMMARY OF PHARMACOTOXIC SIGNS, TOGETHER WITH REACTION FRACTIONS AND DEGREE OF SIGNS REVEALING DOSE-RESPONSE RELATIONSHIPS IN MONKEYS FOLLOWING INTRAVENOUS ADMINISTRATION OF EA 1476 AT INDICATED LEVELS (C)

Pharmacotoxic signs	Dosage levels									
	0.0316 mg/kg		0.10 mg/kg		1.0 mg/kg		3.16 mg/kg		10.0 mg/kg	
	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*	Reaction fraction	Degree of toxic signs*
Ptosis	1/3	+	5/5	++	1/3	+	1/3	+	-	-
					1/3	++	1/3	++	-	-
					1/3	+++	1/3	+++	-	-
Decreased activity	1/3	+	0/5	-	2/3	+++	1/3	++	1/1	+++
							2/3	+++		
Peripheral vasodilation	1/3	+	0/5	-	1/3	+	1/3	+	1/1	+++
					1/3	++				
Hyperpnea	-	-	0/5	-	1/3	+	1/3	++	1/1	+++
					2/3	++				
Hypopnea	-	-	0/5	-	2/3	+	1/3	++	1/1	+++
Ataxia	-	-	0/5	-	1/3	++	1/3	++	1/1	+++
Poor appetite	-	-	0/5	-	1/3	X	1/3	X	1/1	X
Pain reflex absent	-	-	-	-	0/3	-	1/3	+++	1/1	+++
Prostration	-	-	-	-	-	-	0/3	-	1/1	X
Righting reflex absent	-	-	-	-	-	-	0/3	-	1/1	+++
Weakness of limbs	-	-	-	-	-	-	0/3	-	1/1	+++
Fixed pupil	-	-	-	-	-	-	0/3	-	1/1	++
Tremors	-	-	-	-	-	-	0/3	-	1/1	+
Death	0/3	-	0/5	-	0/3	-	0/3	-	1/1	-**

* (U) + = slight; ++ = moderate; +++ = marked, X = all-or-no response.

** (U) None was given.

CONFIDENTIAL

The VDT data are tabulated under the following measures:

(a) Avoidance discrimination ratio (N:100 trials)

(b) Number of correct escape responses (N:100 trials)

(c) Number of incorrect escape responses (N:100 trials)

(d) Duration of escape shock (min x 100)

The final graded conclusions are expressed as 0 equals no effect, + equals slight, ++ equals moderate, and +++ equals marked, are based upon the total duration of shock received by the animal during the experimental run, either in the avoidance or escape periods. The duration of shock required for each test condition to establish the intensity of effect is as follows:

	Duration of shock (min x 100)		
Test condition	Slight	Moderate	Marked
Avoidance period	5-9	10-34	≧ 35
Escape period	2-9	10-34	≧ 35

The result obtained by these criteria are shown in table 3.

2. (C) Chronic Administration.

a. (C) Rats.

Hazleton Laboratories Monthly Progress Report No. 16, October 1961

Forty male albino rats (weight range was from 173 to 204 gm), of the Holtzman Sprague-Dawley strain, were divided at random into the following groups:

Group number	Number of animals	Material tested	Dosage level
1 (control)	10	Ethanol and methyl-cellulose	1.0 ml/kg
2 (low-level)	10	EA 1476	0.1 mg/kg
3 (intermediate-level)	10	EA 1476	1.0 mg/kg
4 (high-level)	10	EA 1476	10.0 mg/kg

All animals received intravenous injections by the way of the tail vein, 5 days/week for 4 weeks.

(C)

TABLE 3

COMPREHENSIVE DATA COMPILATION FOR EA 1476 IN THE VISUAL DISCRIMINATION TEST (U)

Dose	Avoidance discrimination ratio					Number of correct escapes					Number of incorrect escapes					Duration of escape shock (min x 100)					Degree of toxic effect a/
mg/kg																					
0.01	3	5	5	13	37	4	4	5	28	0	2	1	6	21	0	3	1	3	39	0	-b/
0.01	10	11	14	7	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++
0.01	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
0.10	0	0	0	0	0	-	-	1	1	-	-	-	0	0	-	-	-	1	1	-	+
0.10	0	1	0	0	0	-	1	-	1	-	-	1	-	0	-	-	3	-	1	-	+
0.10	0	0	0	0	0	-	-	-	3+3c/	-	-	-	-	0	-	-	-	-	35	-	+++
0.10	0	0	0	0	1	-	1	-	1	1	-	0	-	1	0	-	1	-	1	1	+
0.316	0	0	0	0	0	2	2	-	1	-	0	0	-	0	-	3	2	-	1	-	+
0.316	0	0	0	0	0	2	18+3 c/	60	13	-	0	0	0	2	-	2	252	72	33	-	+++
0.316	0	0	0	0	0	-	-	1	-	-	-	-	0	-	-	-	-	1	-	-	0

a/ (U) 0 = No effect; + = slight; ++ = moderate; and +++ = marked

b/ (U) Subject not adequately trained

c/ (U) Second digit represents number of missed escape trials

CONFIDENTIAL

Before initiation of the study, animals were adapted to laboratory conditions for 14 days. They were housed individually in wire-mesh cages and food (Purina Laboratory Chow) and water were available ad libitum.

Compound was prepared daily at a concentration of 10.0 mg/ml by dissolving the weighed amount in a minimum volume of ethanol and diluting it to the desired volume with 0.5% methyl-cellulose solution.

Gross observations for pharmacotoxic signs were made daily before dosing and at 10 minutes and at 4 hours after administration. Body weights were obtained before the study, daily before compound administration, and at the termination of the study. Food consumption was determined weekly.

Hematological determinations, total erythrocyte counts, and total and differential leukocyte counts were made on five animals from each group initially, at 15 days and at the termination of the study.

Gross necropsies were performed on all surviving animals at the end of the 4-week experimental period. Weights of liver, kidney, and adrenals were measured and organ-to-body weight ratios were calculated.

The following tissues were prepared for histologic examination: brain, spinal cord, thyroids, heart, lungs, liver, kidneys, stomach, duodenum, adrenals, testes, and bone marrow (sternum). Microscopic evaluations were made on these tissues from five animals from the control and high-level groups and only on the brain, spinal cord, liver, kidneys, adrenals, and bone marrow from the low- and intermediate-level groups.

The control and low-level test rats exhibited normal appearance and behavior throughout the 4-week experimental period. After the first injection, all intermediate-level rats exhibited slightly decreased activity. The majority of the animals also exhibited spastic rigidity and excessive urination and a smaller number exhibited ptosis and hypersensitivity to touch. Only one animal exhibited toxic signs after the next two injections. For the remaining 17 days, only slight miosis was observed at periods of 10 minutes and 4 hours, but not at 24 hours.

After the first injection, high-level test rats exhibited marked decrease in activity and spastic rigidity, urination, ptosis, hypersensitivity to touch, miosis, hypopnea, dry mouth, lacrimation, weakness of limbs, and depression of righting reflex to a moderate-to-marked degree at the 10-minute and 4-hour observation periods. A smaller number exhibited chromodacryorrhea.

During the next two injection days, the pharmacotoxic signs were similar but reduced in degree. Thereafter, only a slight-to-moderate degree of miosis was observed at 10 minutes and at 4 hours, but was absent at 24 hours. The marked reduction of pharmacotoxic signs upon repeated injection suggests a very rapid development of tolerence to EA 1476. Body weight gain for low-level test rats was not statistically different from that of the control rats. The intermediate- and high-level rats had a significantly lowered growth rate than the controls. No significant differences in the hematological values for the control and test animals were found. An analysis of variance revealed the following significant differences from control in the organ weight measurements and the organ-to-body weight ratios. For both the intermediate- and high-level groups, liver weights, liver-to-body weight ratios, kidney weights, and kidney-to-body weight ratios were significantly lower.

The lungs from the high-level rats showed histological lesions. This organ was the seat of chronic pneumonitis in several instances, which was characterized by focal infiltration of primarily chronic inflammatory cells, accompanied by proliferation of lipophages in varying degrees. This was seen in both the control and high-level groups; however, the lungs of all five high-level animals contained large vacuoles surrounded by a "collar" of chronic inflammatory cells, sometimes histiocytes, and, occasionally, fibrous tissues. The exact location of the vacuoles was not always ascertained, but occasionally they were situated in the wall of an alveolus or immediately beneath the pleura. This suggests that these vacuoles were not purely dilated bronchioles lined with atrophic epithelium or devoid of any lining. Distinct multinucleated foreign-body giant cells were not found. This distinctive lesion would appear to be related to the test compound. The liver and kidney did not show lesions that were considered to be a significant contribution from compound administration. Heart, adrenals, bone marrow, brain, thyroid, and spinal cord did not show any significant lesions. Spermatogenesis was normal in all animals.

b. (C) Dogs.

Hazleton Laboratories Monthly Progress Report No. 16, October 1961

The eight mongrel dogs used in this study were acclimated to laboratory conditions for a minimum of 3 weeks before initiation of the dosing. During this time, these animals were inoculated for canine distemper, infectious hepatitis, and rabies. They were housed individually, fed once daily (Wayne Laboratory Chow), and given water ad libitum.

A total of 10 injections by the way of the cephalic vein was given in this experiment, five per week over a 2-week period. The dogs were injected at a dose rate of 1.0 ml/min and then randomly divided into the following groups:

Group number	Number of dogs	Sex	Material tested	Dosage level
1 (control)	2	F, M	Ethanol and methyl cellulose	1.0 ml/kg
2 (low-level)	3	F, M, M	EA 1476	0.1 mg/kg
3 (high-level)	3	F, F, M	EA 1476	1.0 mg/kg

Gross observations for pharmacotoxic signs were carried out daily, before dosing and at 10 minutes and at 4 hours after dosing. Body weight and food consumption were also noted daily.

Hematological and biochemical studies and urine analyses were performed on all dogs before and after 1-, 4-, 8-, and 10-injection days. Hematological studies consisted of erythrocyte counts, total and differential leukocyte counts, hemoglobin concentrations, cell volume, sedimentation rates, and prothrombin time. Biochemical studies that were carried out were the bromsulphalein liver-function test, the determination of blood-urea nitrogen level, blood-sugar level, and serum-transaminase activity. Urine analysis included appearance, volume, pH, specific gravity, protein, sugar, bilirubin, occult blood, and microscopic examination of sediment. All blood samples were taken from the jugular vein and the urine samples were collected overnight, utilizing metabolism cages. Dogs were sacrificed by exanguination under thiamylal (Surital Sodium) anesthesia 2 days after the last injection. A complete necropsy was performed on each animal. The weights of livers, kidneys and adrenals were measured and organ-to-body weight ratios were calculated. The following tissues were prepared for histological examination: brain, spinal cord, liver, kidneys, adrenals, stomach, duodenum, gonads, and bone marrow (sternum).

The two control dogs exhibited normal appearance and behavior throughout the experiment. The three low-level dogs showed only minimal and relatively insignificant effects during the course of the study. They had exhibited moderate hyperpnea and slightly decreased activity at the 10-minute observation period after each injection. One dog exhibited retching after the first injection, but not after subsequent injections.

The three high-level dogs exhibited marked pharmacotoxic effects throughout the course of study. These signs included the following: marked

ataxia, decreased activity, absence of pain (pinch), and extensor thrust reflexes; moderate-to-marked hind-limb weakness; moderate bradycardia, hyperpnea, and confused behavior; and slight-to-moderate circling movements. Two of these three dogs exhibited a marked absence of placement reflex and one a moderate catalepsy. Each of these signs were observed to the same intensity at the 10-minute, 4-hour, and 24-hour observation period, with three exceptions: hyperpnea at 10 minutes only; bradycardia at 10 minutes and at 4 hours only; and at 24 hours, after the second- and seventh-injection days, ataxia, hind-limb weakness, and circling movements were slight rather than a moderate-to-marked degree.

All dogs in the high-level group lost a significant amount of weight, which averaged 17%, as compared to the control and low-level groups. The latter two groups averaged a 6% and 9% loss, respectively. It should be noted that no abnormalities in appetite, defecation, or urination were observed with the high-level group.

One high-level dog exhibited the development of low-grade anemia, as reflected by a 25% to 30% decrease in red-cell volume, hemoglobin concentrations, and red-cell count. One low-level and two high-level dogs also revealed elevated sedimentation rates. Otherwise, all hematological, biochemical, and urine-analysis values were essentially within normal limits.

One low-level dog had a significantly lower liver-to-body weight, kidney-to-body weight, and adrenal-to-body weight ratios. All three dogs in the high-level group had a significantly lower liver-to-body weight ratios and two of the three dogs had a significantly lower kidney-to-body weight ratios.

Pathologic alterations were found in the lungs, liver, and gonads of the test animals, which were possibly caused by the compound. Fatty metamorphosis of the liver correlated well with the dose, this being more severe in the high-level dogs. Definite damage to the seminiferous epitheliums was present in all male test dogs and alterations in the ovaries were present in the test females. A possible decrease in the mitotic division was present in the intestinal epithelium. This finding requires a more accurate determination before a definite conclusion of effect can be given. The lungs of five of the six test dogs contained foci of chronic pneumonitis and partial atelectasis in which vacuolated histiocytes were frequently found. One dog had frank lipoid pneumonia.

B. (C) EA 2233.

1. (C) Acute Administration.

a. Mice.

Toxicity Screening Branch Data Sheet, 18 March 1960.

Dose mg/kg	Route	Reaction fraction	
25.0	iv	3/3	Decreased running and walking activities; slow deep respiration; pale skin
		2/3	Prostration
		1/3	Exopthalmos; ataxia; loss of placing and labyrinthine reflexes; subconvulsive jerking; loss of coordination on narrow strip and roto rod
0.40	iv	1/1	Low posture; slow respiration; decreased running and walking activities
0.32	iv	3/3	Decreased running and walking activities; slow deep respiration; miosis
0.32	iv	2/3	Analgesia
		1/3	Ataxia; convulsions; loss of escape reflex
0.10	iv	4/4	Miosis
		1/4	Increased activity
0.032	iv	1/4	Miosis

LD50 = >25.0 mg/kg
MED50 = 0.1 mg/kg

Ratio: $\frac{LD50}{MED50}$ = >250.0

b. Rat.

(1) Toxicity Screening Branch Data Sheet, 24 February 1960.

Dose mg/kg	Route	Toxic effects
1.0	iv	Ataxia; general weakness; miosis; labored respiration
0.32	iv	No effects
0.10	iv	No effects
0.032	iv	No effects
0.01	iv	No effects

MED50 = 1.0 mg/kg

(2) Hazleton Laboratories Monthly Progress Report No. 2, August 1960.

Dose mg/kg	Route	Toxic effects
10.0	iv	Dyspnea; ataxia; decreased activity; lacrimation; dry mouth; urination
1.0	iv	Miosis; ataxia; dyspnea; decreased activity
0.1	iv	Miosis; lacrimation; dry mouth

Safety ratio = >100.0 mg/kg

c. Rabbits.

(1) Toxicity Screening Branch Data Sheet, 24 February 1960.

Dose mg/kg	Route	Toxic effects
1.0	iv	Ataxia (legs stiff); general weakness; fright; coughing; hypersensitive to touch and sound
0.10	iv	Mydriasis; ataxia; general weakness
0.01	iv	Mydriasis

Mydriasis: MED50 = <0.01 mg/kg
Other signs: MED50 = 0.10 mg/kg

(2) Hazleton Laboratories Monthly Progress Report No. 2, August 1960.

Dose mg/kg	Route	Toxic effects
1.0	iv	Mydriasis; rapid respiration; increased activity
0.1	iv	No effects

d. Cat.

Hazleton Laboratories Monthly Progress Report No. 2, August 1960.

Dose mg/kg	Route	Toxic effects
1.0	iv	Dyspnea; mydriasis; ataxia; decreased activity; sensitive to touch; relaxation of nictitating membrane
0.1	iv	Dyspnea; mydriasis; increased respiratory rate

Safety ratio = >10.0 mg/kg

e. Dog.

(1) Hazleton Laboratories Monthly Progress Report No. 2, August 1960.

Dose mg/kg	Route	Reaction fraction	Toxic effects
10.0	iv	*-	Dyspnea; slow respiration; tachycardia; ataxia; weakness of limbs; decreased activity; sensitive to touch and sound; tremors; mydriasis; miosis; labyrinthine reflex absent; righting reflex absent; analgesia; urination; vomiting
1.0	iv	*-	No effects
		Pharmacodynamics	
1.0	iv	*-	Slight hypertension
0.10	iv	*-	Slight hypertension
0.01	iv	*-	No effects
		Conditioned Avoidance Response (CAR)	
0.10	iv	1/2	Slight CAR effect at 4 hr
		1/2	No effect on CAR

* (U) None was given.

(2) Hazleton Laboratories Monthly Progress Report No. 3, September 1960.

Sustained Physical Exercise (SPE)

Dose mg/kg	Route	Toxic effects
0.25	iv	No SPE effects; no toxic effects

(3) Vapor Toxicity Branch Quarterly Progress Reports.

Conditioned Avoidance Response (CAR)

Dose mg/kg	Route	Number of animals	
0.10	iv	1	None on CAR; mydriasis; hyperthermia
0.20	iv	1	None on CAR; bradycardia; mydriasis; head jerks
0.25	iv	1	None on CAR; bradycardia; hypopnea; mydriasis; salivation; ataxia; subconvulsive jerking; muscular weakness; vomiting; prominent nictitating membrane; dry mouth; hypersensitive to sound
0.35	iv	1	CAR; ataxia; subconvulsive jerking; hypersensitive to sound and touch; mydriasis; apprehensive; mild tremors; fasciculations; weakness; dry mouth; confused appearance

f. Monkey.

Hazleton Laboratories Monthly Progress Report No. 2, February 1963

Visual Discrimination Test VDT

The detailed effects of EA 2233 in monkeys trained to perform the VDT can be found in Hazleton Laboratories Monthly Progress Reports Nos. 7 and 28, dated January 1961 and October 1962, respectively. These data have been summarized and graded in monthly progress report no. 2, February 1963.

EA 2233 has been evaluated in 15 monkeys trained to perform in the VDT at four-dose levels as follows:

iv Dose mg/kg	Number of animals
0.01	1
0.10	6
0.316	6
1.0	2

The VDT data are tabulated under the following measures:

(1) Avoidance discrimination ratio (N:100 trials)

(2) Number of correct escape responses (N:100 trials)

(3) Number of incorrect escape responses (N:100 trials)

(4) Duration of escape shock (min x 100)

The final graded conclusion, expressed as 0 equals no effects, + equals slight, ++ equals moderate, and +++ equals marked, is based upon the total duration of shock received by the animal during the experimental run either in the avoidance or escape periods. The results for these evaluations are in table 4.

For comparative purposes, the intensity-grading data for EA 1476 and EA 2233 are summarized as follows:

	EA 1476		EA 2233	
Dose mg/kg	Reaction fraction	Degree of toxic effects	Reaction fraction	Degree of toxic effects
0.01	-*	-*	1/1	0
0.10	3/4	+	1/6	+
	1/4	+++**	5/6	0
0.316	1/3	0	3/6	+
	1/3	+	3/6	++
	1/3	+++		
1.0	-*	-*	1/2	++
			1/2	+++

* (U) None was given.

** (U) A typical response.

(C)

TABLE 4

COMPREHENSIVE DATA COMPILATION FOR EA 2233 IN THE VISUAL DISCRIMINATION TEST (U)

Dose	Avoidance discrimination ratio					Number of correct escapes					Number of incorrect escapes					Escape shock (min x 100)					Degree of toxic effects*
mg/kg																					
0.01	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
0.10	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
0.10	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
0.10	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
0.10	0	0	0	0	3	-	1	-	-	-	-	2	-	-	-	-	1	-	-	-	+
0.10	0	0	0	0	3	-	-	1	-	-	-	-	0	-	-	-	-	1	-	-	0
0.10	0	0	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
0.316	0	0	0	0	0	-	1	3	3	-	-	0	2	2	-	-	1	2	3	-	+
0.316	0	0	0	4	2	-	-	1	2	-	-	-	0	0	-	-	-	2	4	-	+
0.316	0	0	0	0	0	-	1	2	2	-	-	0	0	0	-	-	1	2	3	-	+
0.316	0	0	0	0	0	-	1	5	3	1	-	1	0	1	0	-	16	21	14	7	++
0.316	0	1	0	0	3	1	1	-	1	-	0	0	-	0	-	1	1	-	2	2	+
0.316	0	0	0	0	0	-	-	2	3	-	-	-	1	7	-	-	-	2	4	-	+
1.0	2	0	2	1	0	-	-	2	-	1	-	-	3	-	0	-	-	13	-	1	++
1.0	0	0	0	0	0	-	5	34	64	11	-	0	4	84	4	-	2	115	167	11	+++

* (U) 0 = No effect; + = slight; ++ = moderate; +++ = marked.

CONFIDENTIAL

2. (C) Chronic Administration.

a. Rats.

Hazleton Laboratories Monthly Progress Report No. 15, September 1961

Forty male rats (weighing 156 to 195 gm) of the Holtzman Sprague-Dawley strain were adapted to laboratory conditions for 14 days before the initiation of this study. These animals were individually housed and were given food and water ad libitum.

Solutions were prepared daily at a concentration of 1 mg/ml, by dissolving in a minimal volume of ethanol and diluting to volume with saline. All solutions were administered by the way of the lateral tail vein.

The animals were divided into the following groups:

Group number	Number of animals	Material tested	Dosage level
1 (control)	10	Ethanol and saline	1.0 ml/kg
2 (low-level)	10	EA 2233	0.01 mg/kg
3 (intermediate-level)	10	EA 2233	0.10 mg/kg
4 (high-level)	10	EA 2233	1.0 mg/kg

Observations for pharmacotoxic signs were made each day before dosing, at 10 minutes and at 4 hours after administration, and at the termination of the experiment. Body weights for each animal were determined daily and food consumptions were measured weekly.

Hematological measurements, consisting of total erythrocyte counts and total and differential leukocyte counts, were taken from five animals from each group initially, at 15 days, and at the termination of the study.

Gross necropsies were carried out on all animals surviving the 4-week experimental period and on all animals that died during the study. Weights of the liver, kidneys, and adrenals for each animal were recorded, and organ-to-body weight ratios were calculated.

Microscopic examinations of the brain, spinal cord, thyroids, heart, lungs, liver, kidneys, stomach, duodenum, adrenals, testes, and bone marrow (sternum) from five animals in the control and high-level groups. For the intermediate- low-level groups, microscopic examinations were made of the brain, spinal cord, liver, kidneys, adrenals, and bone marrow.

All control animals and those that received EA 2233 at the low-level dosage exhibited normal appearance and behavior throughout the 4-week experimental period.

Miosis was consistently noted in the intermediate- and high-level groups. Generally, in the intermediate-level group, most animals exhibited slight miosis and the frequency of occurrence gradually, but slightly, progressed during the 4-week experimental period. In this group, miosis was consistently seen at the 10-minute and at the 4-hour observation periods, but there were only a few incidences of miosis at the 24-hour observation period. The high-level group exhibited slight to moderate miosis daily. The intensity increased slightly during the second week and remained essentially unchanged for the remainder of the test period. Miosis was still noted at the 24-hour observation period in 25% to 50% of the animals. The only other signs observed was lacrimation, which was observed intermittently in the high-level group. This group consistently showed lower weight gains than the control and other test groups. As compared to the control, this decrease was 17% during the first week, 23% during the second week, and 30% during the third and fourth weeks. The weekly food consumption of this group was corresponding significantly lower than the control group throughout the experimental period. No significant changes in the hematological values were noted during this study. Lower terminal body weight, liver weight, liver-to-body weight ratios, and kidney weight were obtained for the high-level exposure group. A higher value for adrenal-to-body weight ratio was noted for this group. No significant changes in these values for the other exposure groups were obtained. No gross or microscopic pathology was seen in any of these animals that could be attributed to the effects of EA 2233.

b. Dog.

Hazleton Laboratories Monthly Progress Report No. 15, September 1961.

Preclinical Pharmacology Study.

Eight mongrel dogs used in this study were acclimated to confinement in the laboratory for at least 3 weeks. During this time, they were inoculated against canine distemper, infectious hepatitis, and rabies. The dogs were individually housed, fed (Purina Dog Chow) once daily, and given water ad libitum.

The animals were randomly divided into the following groups:

Group number	Number of animals	Sex	Material tested	Dosage level
1 (control)	2	F, M	Ethanol and saline	1.0 ml/kg
2 (low-level)	3	F, F, M	EA 2233	0.01 mg/kg
3 (high-level)	3	F, F, M	EA 2233	0.10 mg/kg

All injections were made into the cephalic vein at a rate of 1.0 ml/sec. A total of 10 daily injections (5 days/week) was administered over a period of 2 weeks.

Observations were made daily before dosing and at 10 minutes and at 4 hours after administration. Body weights and food consumptions were noted daily. Hematological and biochemical studies and urine analyses were performed on all dogs initially and after 1-, 4-, 8-, and 10-injection days. Hematological studies included erythrocyte counts, total and differential leukocyte counts, hemoglobin concentrations, cell volume, sedimentation rate, and prothrombin time. Biochemical studies included bromsulphalein liver-function test and determinations of blood-urea nitrogen level, blood-sugar level, and serum-transaminase activity. Urine analysis included appearance, volume, pH, specific gravity, protein, sugar, bilirubin, occult blood, and microscopic examination of sediment. All blood samples were taken from the jugular vein and urine samples were collected overnight, utilizing metabolism cages.

Three days after the last injection, all dogs were sacrificed and a complete necropsy was performed on each animal. Weights of liver, kidneys, and adrenals were recorded and organ-to-body weight ratios were calculated. Tissues of brain, spinal cord, liver, kidneys, adrenals, stomach, duodenum, gonads, and bone marrow (sternum) were prepared for histologic examinations.

The two control dogs and two of the three low-level dogs exhibited normal behavior and appearance throughout the experimental period. The third low-level dog showed signs characteristic of canine distemper and the relevance of the results from the animal is questionable.

Two of the three high-level dogs exhibited a marked degree of defensive hostility after three daily injections, which persisted throughout the experimental period. This behavior was observed at each observation period (10 minutes and 4 and 24 hours). A slight cumulative effect was suggested since this behavior was not exhibited until the third-injection day.

Body weights were not significantly altered throughout this experiment. All dogs, except one in the low-level group, ate well and experienced no difficulty with defecation or urination throughout the study. All hematological and biochemical studies and urine-analysis values were within normal levels indicating that no effect can be attributed to the compound. No significant changes in body weights, organ weights, and organ-to-body weight ratios were obtained in these animals. It was of possible significance that the livers of all three high-level dogs showed considerable glycogen storage, which may be related to EA 2233. No other pathologic changes, either gross or microscopic, which could be related to the administration of the compound were found.

C. (C) Human Data.

1. (C) EA 1476.

Thirty five volunteers (Directorate of Medical Research, these Laboratories) were administered per os agent EA 1476 (racemate) in a dose range of 7.0 to 55.0 μg/kg body weight. Objective studies of arterial blood pressure, heart rate, body temperature, and motor performance were made. Subjective symptoms were also recorded; e.g., unusual dreams, blurring of vision, and dryness of the mouth. These data are summarized in table 5.

At the lower doses (0.5 to 1.0 mg), fatigue, thirst, and headache were experienced. In the intermediate-dose range (1.5 to 3.0 mg), postural hypotension was prominent, with temporary blurring or actual loss of vision upon standing. Weakness was also noted along with giddiness and a general slowing of motor activity. At the higher doses (3.5 to 4.0 mg), the subjects manifested marked psychomotor retardation clinically. Postural hypotension was less pronounced, probably because the volunteers stayed in bed and were unwilling or incapable of assuming an erect position.

(C)

TABLE 5

REACTION FRACTION OF 35 MEN TO EA 1476 (U)

Toxic effects	Dose							
	0.5 mg	1.0 mg	1.5 mg	2.0 mg	2.5 mg	3.0 mg	3.5 mg	4.0 mg
Decrease in blood pressure, >25/15	1/4	1/4	2/4	4/5	2/4	1/5	2/4	2/5
Decrease in oral temperature, >1°C	1/4	0/4	0/4	3/5	1/4	1/5	2/4	0/5
Increase in pulse rate, >20/min	1/4	2/4	3/4	4/5	2/4	2/5	0/4*	3/5
Occurrence of dreams	0/4	0/4	1/4	0/5	0/4	2/5	0/4	0/5
Decrease in motor performance	0/4	0/4	2/4	4/5	3/4	3/5	4/4	5/5
Visual disturbance >1° angle	0/4	1/4	1/4	4/5	0/4	2/5	1/4	4/5
Thirst; dry mouth	0/4	3/4	2/4	3/5	4/4	4/5	3/4	3/5

* (U) Pulse in all four dropped

CONFIDENTIAL

Sluggishness, inability to concentrate, and dimness and blurring of vision persisted for as much as 48 hours. Not one volunteer was capable of performing his regular duties when given doses greater than 2.5 mg. No significant change was seen in reflexes, blood count, urinalysis, or EKG. Pulse rate increased when postural hypotension occurred, but showed little change or an actual diminution of the higher dose levels.

2. (C) EA 2233.

(C) In view of the orthostatic-hypotensive action alluded to above, it was deemed necessary to test further the O-acetyl derivative of EA 1476; i.e., EA 2233. By the provisions of the experimental design, 11 subjects were given 13 doses of the compound per os in dose levels ranging from 10 to 60 μg/kg body weight. The compound was given orally in absolute ethanol in a final concentration of 0.76 mg/ml. Parenteral usage was precluded by poor solubility in water-alcohol mixtures. General comments on physiological responses will be made in reference to each dose level. Comments on laboratory and other examinations will follow.

a. (C) Two Men; 30 μg/kg.

At this dose, one of the men became quite light-headed and felt as though he were going to faint. In these two cases, systolic pressure fell 25 to 50 mmHg upon standing, while diastolic pressure fell 10 to 30 mmHg. The pulse rose concomitantly to 100 to 120. Both men were moderately sleepy the evening of the test (12 to 20 hours).

b. (C) Two Men; 40 μg/kg.

These men had symptoms that were slightly different from the preceding test.* They became extremely lethargic. This effect came on in the evening (10 hours), persisted throughout the night, and was still evident, though less pronounced, the second day. The systolic blood pressure fell 20 to 40 mmHg and the diastolic 10 to 25 mmHg in one subject upon standing. The pulse rose to levels of 110 to 130. One subject had minimal lightheadedness upon standing. They both complained of dry mouth and nasal stuffiness to a greater degree than any other subjects, including the ones at high doses.

---

* (U) These men had received 10 μg/kg 2-1/2 weeks previously.

c. (C) Two Men; 50 μg/kg.

One subject had a drop in systolic blood pressure of 40 to 55 mmHg and of diastolic of 10 to 30 mmHg upon standing. His pulse rose to 100 to 110. He did not faint nor did he feel lightheaded. The other subject had a drop of 40 to 55 mmHg systolic and of 10 to 15 mmHg diastolic. His pulse rose to rates of 130 to 155. This man did feel lightheaded and fainted once upon standing. Although both complained of a dry throat, nasal stuffiness, and sleepiness, these symptoms were not as pronounced as in those who had received 40 μg/kg.

d. (C) Two Men; 60 μg/kg.

(C) One of these men had quite severe central effects, which will be described in another section. Although the other man had a marked decrease in performance, he did not report the same subjective responses. Both men experienced drops in systolic blood pressure in the range of 40 to 60 mmHg and of 10 to 20 mmHg diastolic, but occasional readings showed greater change in diastolic. One man was slightly lightheaded and at 20 hours felt faint upon standing. The pulse rise in both was to 120 to 130 upon standing. It should be noted that there seemed to be a slightly greater decrease in blood pressure and increase in pulse after meals, possibly caused by greater pooling in the splanchnic areas at those times.

(C) Other measurements included EKG's of two kinds. Standard 12-lead EKG's were taken periodically. These were without significant change in all cases. Continuous EKG's (lead II) were taken simultaneously with the vital signs; that is, while the patient arose from the supine position, stood for 60 seconds then resumed the supine position. These EKG's demonstrated a lag in the pulse rise of about 6 to 10 seconds after becoming erect. A sinus tachycardia then ensued with rates, as described as above. In general, the rate at the end of the 60-second erect-position period was higher by 5 to 10 than the initial phase of the tachycardia. Upon reclining, there was again a lag of 3 to 6 seconds then abrupt slowing occurred with the rate changing from 120 to 130 to 50 to 60 in the space of 5 to 10 seconds. In one case, there was a complete inhibition of the S-A node with a pair of nodal escape beats (this man had 50 μg/kg). This phenomenon did not repeat. In addition, there was generally inversion of the T-wave in lead II during the tachycardia phase. This is probably of little significance and may be attributed to heart rate and decrease in blood pressure rather than a direct effect of the agent.

(C) Liver-function tests (bile, alkaline phosphatase, SGOT, SGPT, TT) and BUN's, drawn at control, at 8 hours and at 24 hours showed no consistent alterations. Other observations included a decrease in temperature, as measured orally. This drop in temperature occurred at 3 to 10 hours. The magnitude varied from 0.5° to 1°F at lower doses and 2°F at higher doses. The time of onset of symptoms varied from person to person; however, changes in pulse and in blood pressure were observed at 2 hours, with the peak effects on pulse and blood pressure occurring at 6 to 10 hours and even later in some cases. With the larger doses, the peak effect seemed to occur later than with the smaller doses. The major effect of the agent on the cardiovascular system was gone after 24 hours. There were drops in blood pressure and pulse rises, however, which remained for several days, although the subjects felt perfectly well and had no symptoms whatsoever. There were injection and hyperemia of the conjunctivae in all cases; this is consistent with other reports of human administration of Cannabis.

(C) It should be noted that the somnolence induced by this agent had its peak after the cardiovascular effects had reached their peak. The two men who received 40 μg/kg had the longest lethargic period and slept all night and the day following exposures. At this time, there were changes in pulse and blood pressure, but less marked than previously and symptoms attributed to these changes, if any had occurred, had disappeared.

(U) Objective physiological data with significant drug-induced changes are to be found in table 6.

(C) Psychophysical decrement of drug-induced origin was assessed by numerical facility and speed of closure (Texas Battery Test), Purdue Pegboard Test, and the Stromberg Manual Dexterity Test, all tests being administered to the subjects at regularly scheduled intervals throughout the course of the experiments.

(U) In table 7, the mean of the three highest performance scores is compared with the mean of the three lowest scores for each of the tests used. This numerical relationship of dose to psychophysical performance is expressed graphically in the following figure.

2. (C) Human Estimates for EA 1476, EA 2233, and Isomers.

The oral ID50, for both EA 1476 or EA 2233, is 4 mg/70-kg man.

(C)

TABLE 6

PHYSIOLOGICAL DATA WITH SIGNIFICANT DRUG-INDUCED CHANGES IN MAN (U)

Date	Subject	Dose	Arterial blood pressure						Heart rate				Body temperature of experimental subject	
			Mean change in control subject		Mean change in experimental subject		Maximum change in experimental subject		Time of maximum change after dose	Mean in control subject	Mean in experimental subject	Time at maximum rate		Mean	Maximum decrease
			Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic							
		mg/kg	mmHg						hr	beats/min		hr	beats/min	°F	
17 Jan 63	Echols	10	-3	+10	-20	+5	-45	-30	5	74	95	5	100	98.0	1.0
15 Jan 63	Eget	10	+10	+5	-10	+8	-40	+5	5	78	89	5	100	98.1	0.8
15 Jan 63	Butts	20	-9	+1	-24	-17	-40	-24	7	89	124	7	127	97.7	1.8
17 Jan 63	Hallau	20	+11	+11	-40	0	-62	-8	5-2/3	77	133	5-2/3	160	99.2	2.4
22 Jan 63	Hardin	20	-10	+23	-17	+16	-44	+1	18	104	100	18	120	98.5	1.9
24 Jan 63	Fox	30	-10	-5	-27	-7	-50	-20	7-1/3	93	105	7-1/3	92	98.2	1.2
24 Jan 63	Premus	30	-10	+15	-30	0	-60	-10	2-3/4	78	100	2-3/4	118	98.1	1.7
29 Jan 63	Echols	40	-11	-1	-22	-10	-44	-10	5-2/3	90	96	5-2/3	91	97.7	2.1
29 Jan 63	Eget	40	-20	-5	-32	-7	-52	-14	11	98	119	11	108	98.3	3.3
31 Jan 63	VanNess	50	-28	+6	-42	-10	-66	-26	7-5/6	74	103	7-5/6	105	98.2	2.2
31 Jan 63	Watrous	50	+8	+16	-29	-13	-64	-32	5-2/3	90	131	5-2/3	160	98.4	3.4
5 Feb 63	Warec	60	-34	+1	-32	-10	-60	-20	1-1/3	99	106	1-1/3	107	98.0	2.0
5 Feb 63	McDonald	60	+1	+20	-27	-11	-50	-34	8-1/3	98	104	8-1/3	120	98.6	1.9

NOTE: All data on blood pressure and heart rate were obtained 60 seconds in an erect position.

CONFIDENTIAL

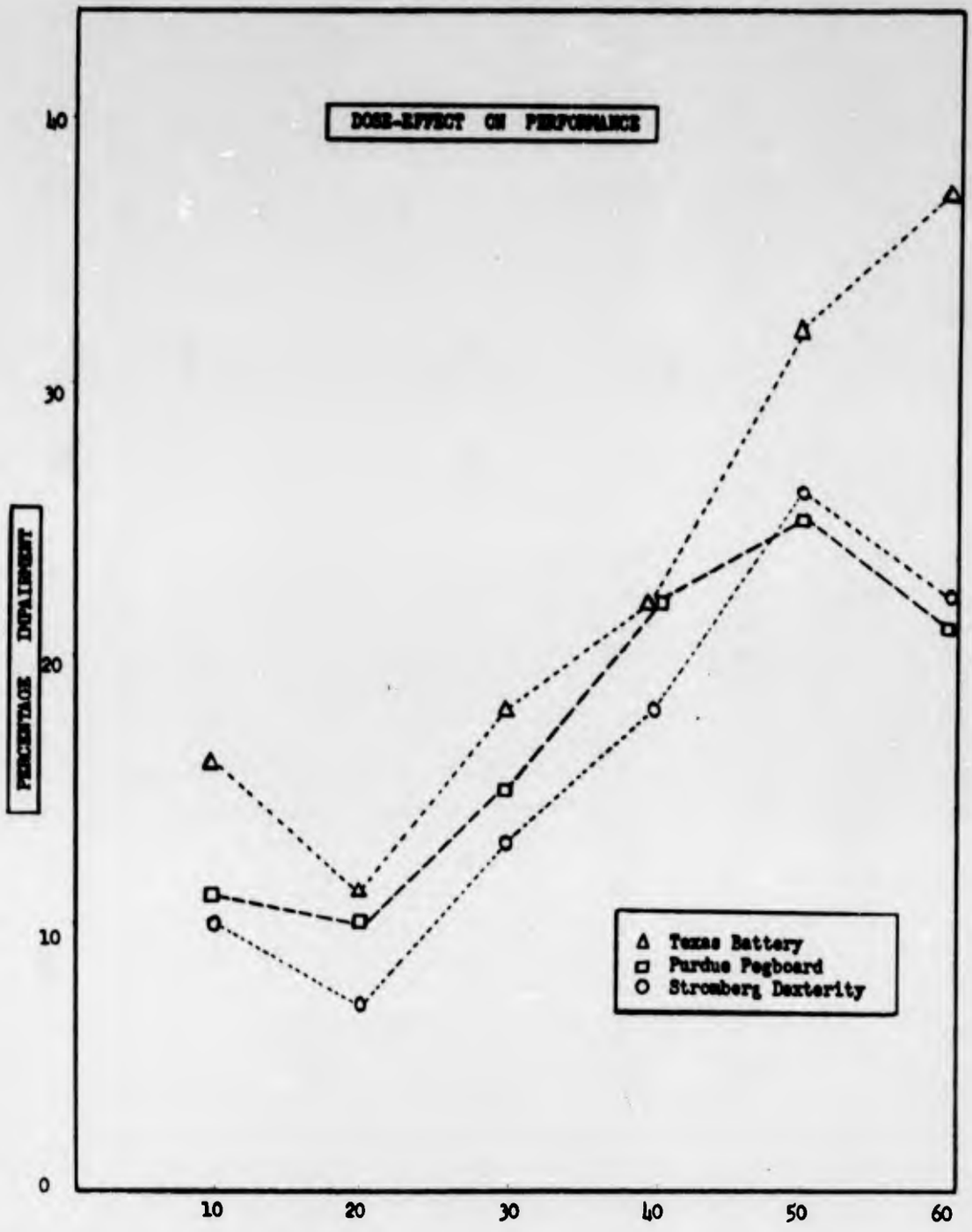
(C)

TABLE 7

A COMPARISON OF THE AVERAGE SCORES IN PERCENT ON TEXAS BATTERY, PURDUE PEGBOARD, AND STROMBERG DEXTERITY TESTS OF THIRTEEN SUBJECTS (U)

Subject	Dose	$\bar{A}$ of three highest scores	$\bar{A}$ of three lowest scores	Difference	$\bar{A}$ difference at each dose level
	µg/kg		%		
Texas Battery Test					
Echols	10	112	95	17	16
Eget	10	103	89	14	
Hardin	20	113	101	12	11
Hallau	20	107	91	16	
Butts	20	113	107	6	
Premus	30	106	92	14	18
Fox	30	106	84	22	
Eget*	40	96	80	16	22
Echols*	40	110	81	29	
Watrous	50	122	93	29	32
Van Ness	50	123	88	35	
McDonald	60	109	63	46	37
Warec	60	115	87	28	
Purdue Pegboard Test					
Echols	10	102	86	16	11
Eget	10	101	95	6	
Hardin	20	97	87	10	10
Hallau	20	102	95	7	
Butts	20	103	91	12	
Premus	30	96	78	18	15
Fox	30	98	86	12	
Eget*	40	102	82	20	22
Echols*	40	103	80	23	
Watrous	50	101	75	26	25
Van Ness	50	108	84	24	
McDonald	60	100	77	23	21
Warec	60	100	81	19	
Stromberg Dexterity Test					
Echols	10	97	90	7	10
Eget	10	102	89	13	
Hardin	20	93	89	4	7
Hallau	20	99	91	8	
Butts	20	101	93	8	
Premus	30	98	84	14	13
Fox	30	98	86	12	
Eget*	40	99	80	19	18
Echols*	40	97	80	17	
Watrous	50	110	80	30	26
Van Ness	50	100	79	21	
McDonald	60	101	79	22	22
Warec	60	109	86	23	

* Same subjects repeated at higher dose.



FIGURE

3. (C) Source of Data.

See Table 8 and preceding Human Data section.

4. (C) Derivation of the Estimates.

If it is assumed that mydriasis does not represent incapacitation, but ptosis in the monkey is a reflection of some central incapacitating effect, the lowest incapacitating intravenous dose of EA 1476 in any animal is not less than 30 μg/kg. This assumption, referring to ptosis, is probably invalid, since, in the VDT, effective doses are between 100 and 316 μg/kg. Also, effective doses in the CAR test on dogs are from 50 to 100 μg/kg, and in the SPE test, effective doses are from 175 to 250 μg/kg.

Earlier studies (Fourteenth Tripartite Conference) showed that no volunteer, given an oral dose of more than 2.5 mg/man (ca. 35 μg/kg), was capable of performing his regular duties. More recent studies with EA 2233, the acetate of EA 1476,[9] indicate that oral doses of 60 μg/kg (4.3 mg/70-kg man) did not cause severe incapacitation. Thus, it is indicated that a dose of 2.5 mg/man or more is required to cause incapacitation in man by oral administration. Doses of 2.5 and 4.2 mg/man are equivalent to the respective ICt50's of 500 and 840-mg min/cu m, assuming a body weight of 70 kg, a minute volume of 10 l/min, and an aerosol respiratory retention of 50%. This also assumes that a respiratory effective dose is the same as an oral effective dose. It must be borne in mind that the inhalation route may be more or less effective than the oral route; however, the human oral doses are in general agreement with the animal data, which indicate an intravenous effective dose of 30 μg/kg or more. Animal experimentation indicates that EA 2233 and EA 1476 are of similar effectiveness.

5. (C) Limitation of the Estimate.

EA 1476, EA 2233, or the isomers have not been studied in man by the inhalation route. The human estimate is based on intravenous doses in animals and oral doses in man. It is not possible to project an aerosol human estimate from present data.

IV. (U) Weapons Systems and Methods of Dissemination.

Not applicable.

(C)

TABLE 8

EFFECTIVE DOSES OF EA 1476, EA 2233, AND ISOMERS 2, 4, AND 6 OF EA 2233 (U)

Species	Route	Criterion	Compound and effective dose				
			EA 1476	EA 2233	Isomer 2	Isomer 4	Isomer 6
			µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
Mouse	iv	Decreased activity, slow, deep respiration	320 a/	-	-	-	-
		Hunched posture	-*	-	-	-	-
		Miosis, increased activity	100 b/	100 c/	-	-	-
Rabbit	iv	Mydriasis, fright	10 b/	10 d/	-	-	-
		Ataxia	100 b/	100 d/	-	-	-
Rat	iv	Mydriasis	100 e/	1,000M-1,036 f/	-	-	-
Cat	iv	Mydriasis	100 e/	100 e/	-	-	-
Dog	iv	Mydriasis	10 e/	-	-	-	-
		Decreased activity	316 g/	-	-	-	-
		Ataxia	50-100 e/	-	-	-	-
		Ataxia	125 i/	-	-	-	-
		Ataxia	316 g/	-	-	-	-
		Condition Avoidance Response	50-100 e/	350 j/	-	-	-
		Condition Avoidance Response	50 e/	-	-	-	-
		Sustained Physical Exercise	175-250 h/	-	-	-	-
Monkey	iv	Mydriasis	-	100-316 h/	100-316 k/	100-316 k/	-
		Ptosis	32-100 g/	-	100-316 k	100-316 k/	-
		Ataxia	1,000 g/	-	-	-	-
		Visual Discrimination Test	100-316 k/	316 k/	100-316 k/	100-316 k/	100-316 l/
Man	Oral	Tachycardia, increased blood pressure, mydriasis, colored visual hallucinations, euphoria	-*	-	-	-	-
		Fatigue, thirst, headache	7-14 m/, n/	-	-	-	-
		Postural hypotension, temperature, blurred or loss of vision; standing weakness; giddiness; slowed motor activity	21-42 m/, n/	-	-	-	-
		Marked psychomotor retardation, sluggishness, inability to concentrate	50-57 m/, n/	-	-	-	-
		Ability to perform regular duties	35 m/, n/	-	-	-	-
		Reduced blood pressure, dizziness; non-incapacitated	-	20-60 n/	-	-	-
		Lethargy, nonincapacitated	-	30-60 n/	-	-	-
		Decreased mental performance	-	60 n/	-	-	-
		Threshold, slight sedation	11 o/	-	-	-	-

* (U) No dose was given

NOTE: (U) Isomers 1, 3, 5, 7, and 8 were ineffective in Visual Discrimination Test at 316 µg/kg.

a/ (U) Toxicity Screening Data Sheet, 16 December 1961.
b/ (U) Toxicity Screening Data Sheet, 29 February 1960.
c/ (U) Toxicity Screening Data Sheet, 18 March 1960.
d/ (U) Toxicity Screening Data Sheet, 24 February 1960.
e/ (U) Hazleton Report, 1 May 1961.
f/ (U) Toxicity Screening Data Sheet, 25 February 1960.
g/ (U) Hazleton Report, 1 October 1961.
h/ (U) DF, Gassing Branch, 5 February 1962 to Chief, Toxicology Division.
i/ (U) Fifth Progress Report, 10 February 1954, Contract DA-18-108-CML-3968, University of Michigan.
j/ (U) Gassing Branch Report, 1 October 1963, to Chief, Toxicology Division.
k/ (U) Hazleton Report, 1 February 1962.
l/ (U) Hazleton Report, 1 October 1962.
m/ (U) Fourteenth Tripartite Conference, U. S. Progress Report, Medical Aspects (1959).
n/ (U) DF, Human Investigation Branch, 26 February 1963, to Chairman, Human Estimates Committee.
o/ (U) CWL Special Publication Report of Symposium IX, vol II, paper II.

V. (U) Protective Measures.

Not applicable.

VI. (U) Specification Data.

Not applicable.

VII. (U) Availability Status.

Not applicable.

VIII. (U) Use Concepts.

Not applicable.

IX. (C) Conclusions.

(C) The actions of EA 1476 and EA 2233 are generally similar to many other psychotropic compounds of military interest; i.e., they yield varying degrees of incapacitation, both physical and mental. Both compounds, however, are unique in eliciting an unequivocal orthostatic hypotension at dose levels far below those required to produce mild mental incapacitation.

(C) No human studies have yet been made on isomers 2 and 4. Primate data do indicate, however, that these specific stereoisomers possess a degree of pharmacologic potency, at least equivalent to that of the racemic mixtures studied in human subjects. Secondly, no human or animal data are available on the effects of the aerosolized agents.

(U) It is believed that data should yet be obtained from the following studies:

1. Exposure of animal and human subjects to the aerosolized racemate.

2. Exposure of human subjects to oral doses of stereoisomers 2 and 4.

(C) LITERATURE CITED

1. Smith, T., and Smith, H. Pharm. J. 6, 675 (1847).
2. Wood, Spivey, and Easterfield. J. Chem Soc. 69, 539 (1896).
3. Wood, Spivey, and Easterfield. ibid. 75, 20 (1899).
4. Cahn. ibid. 1400 (1933).
5. Czerkis. Ann. 351, 467 (1907).
6. Frankel. Arch. Exp. Path. Pharm. 49, 266 (1903).
7. Casparis. Pharm Helv. Acta 1, 210 (1926).
8. Bergel. Ann. 482, 55 (1930).
9. Cahn. J. Chem Soc. 986 (1930).
10. Cahn. ibid. 630 (1931).
11. Cahn. ibid. 1342 (1932).
12. Adams, Baker, and Wearn. J. Am. Chem. Soc. 62, 2194 (1940).
13. Adams, Pease, Clark, and Baker. ibid. 62, 2197 (1940).
14. Adams, Cain, Baker. ibid. 62, 2201 (1940).
15. Adams, Baker, and Wearn. ibid. 62, 2204 (1940).
16. Adams and Baker. ibid. 62, 2208 (1940).
17. Adams and Baker. ibid. 62, 2401 (1940).
18. Todd, Bergel, and Work. Biochem. J. 33, 123 (1939).
19. Todd and Jacob. J. Chem Soc. 649 (1940).
20. Ghosh, Pascall, and Todd. ibid. 1118 (1940).

21. Ghosh, Todd, and Wilkinson. ibid. 1121 (1940).

22. Ghosh, Todd, and Wilkinson. ibid. 1393 (1940).

23. Adams, Hunt, and Clark. J. Am. Chem. Soc. 62, 196 (1940).

24. Adams, Cain, and Wolff. ibid. 732 (1940).

25. Adams, Hunt, and Clark. ibid. 735 (1940).

26. Adams, Wolff, Cain, and Clark. ibid. 62, 1770 (1940).

27. Adams, Wolff, Cain, and Clark. ibid. 62, 2215 (1940).

28. Adams, Pease, Cain, and Clark. ibid. 62, 2402 (1940).

29. Adams, Loewe, Pease, Cain, Wearn, Baker, and Wolff. ibid. 62, 2566 (1940).

30. Adams, Cain, McPhee, and Wearn. ibid. 63, 2209 (1941).

31. Haagen-Smit, Wawra, Koepfli, Alles, Feigen, Prater. Science 91, 602 (1940).

32. Korte and Sieper. Ann. 630, 71 (1960).

33. Todd and Jacob. Nature 145, 350 (1940).

34. Wollner, Marchett, Levine, and Loewe. J. Am. Chem. Soc. 64, 26 (1942).

35. Schultz and Haffner. Arch. Pharmaz. Ber. dtsch. pharmaz. Ges. 291, 391 (1958).

36. Krejci and Santavy. Acta Univ. Palackianae Olomucensis 6, 59 (1955). C. A. 50, 12080 (1956).

37 Krejci, Gorak, and Santavy. Acta Univ. Palackianae Olomucensis 16, 9 (1958). C. A. 54, 12054 (1960).

38. Krejci, Gorak, and Santavy. Pharmazie 14, 349 (1959).

39. Schultz and Haffner. Arch. Pharmaz. Ber. dtsch pharmaz. Ges. 293, 1 (1960)

40. Adams and Baker. J. Am. Chem. Soc. 62, 2405 (1940).

41. Adams, Smith, Jelinck, and Wolff. ibid. 63, 1971 (1941).

42. Adams, Smith, and Loewe. ibid. 64, 2087 (1942).

43. Leaf, Todd, Wilinson. J. Chem. Soc. 185 (1942).

44. Ghosh, Todd, and Wright. ibid. 137 (1941).

45. Adams, Smith, and Loewe. J. Am. Chem. Soc. 63, 1973 (1941).

46. Adams, Smith, Loewe, and McPhee. ibid. 64, 694 (1942).

47. Adams, Loewe, Theobold, and Smith. ibid. 64, 2653 (1942).

48. Adams, Chen, and Loewe. ibid 67, 1534 (1945).

49. Adams, Aycock, and Loewe. ibid. 70, 662 (1948).

50. Adams, MacKenzie, and Loewe. ibid. 664 (1948).

51. Adams, Harfenist, and Loewe. ibid. 71, 1624 (1949).

52. Russell, Todd, Wilkinson, MacDonald, and Woolfe. J. Chem. Soc. 169 (1941).

53. Russell, Todd, Wilkinson, MacDonald, and Woolfe. ibid. 826 (1941).

54. Reeves et al. CWLR 2244. The Preparation of the 1, 2-Dimethylheptyl Analog of Tetrahydrocannabinol (C). August 1958.

55. Monsanto Research Corporation, Everett 49, Massachusetts. Synthesis of 5-Alkylresorcinols. Final Report. Contracts DA-18-108-405-CML-807 and DA-18-108-CML-6632. May 1962.

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

Group 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red cell
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

Group 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

GROUP 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL
1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

CONFIDENTIAL
1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

Group 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

Group 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

GROUP 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

CONFIDENTIAL

1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

CONFIDENTIAL

1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

CONFIDENTIAL

1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

Group 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

Group 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compou	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

AVAILABILITY STATEMENT

Available From DDC
UNRESTRICTED DISTRIBUTION

CONFIDENTIAL

DOWNGRADING GROUP

GROUP 3

SUMMARY REPORT ON EA 1476 AND EA 2233 (U)

Directorate of Medical Research and Directorate of Weapons Systems
U. S. Army Chemical Research and Development Laboratories
Edgewood Arsenal, Maryland

CONFIDENTIAL REPORT - CRDLSP 1-44 August 1963
4C08-02-024-01 and 4C08-03-016-017

Deputy Director of Medical Research and Director of Weapons Systems

Cannabinol compounds	EA 1476
Human experimentation	EA 2233
Animal experimentation	Physical data
Sustained Physical Exercise	Chemical agents
Conditioned Avoidance Response	Red oil
Visual Discrimination Test	Structures
Toxicity screening	Biomedical
Pharmacodynamics	Evaluation
Behavior pattern	Reserpine
Pharmacotoxicity	

CONFIDENTIAL

CONFIDENTIAL

1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

CONFIDENTIAL

1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

CONFIDENTIAL

1-44

(C) The psychophysiologic changes induced in man by extracts of Cannabis sativa have suggested possible military uses for substituted synthetic derivatives of the active moieties present in the extracts. As measured by the dog-ataxia test, the most physiologically active derivative is the 3-(1,2-dimethylheptyl) analog of tetrahydrocannabinol (EA 1476). The stability of EA 1476 is greatly increased by acetylation of the free phenolic hydroxyl function without sacrifice of its pharmacologic properties (EA 2233). In primates, the MED50 was found to be 0.03 mg/kg and the MLD50 was found to be 10.0 mg/kg. A moderately large safety margin was demonstrated. Forty-six volunteers were given the racemate of EA 1476 of EA 2233 in a dose range of 7.0 to 60.0 micrograms/kilograms. Significant orthostatic arterial hypotension was produced at doses as low as 10.0 micrograms/kilograms. Varying degrees of psycophysical impairment were observed at higher dose levels (ca. 40.0 to 60.0 micrograms/kilograms). Although these dose-levels are at the proposed upper limit of logistic feasibility, it is concluded that the data indicate a requirement for studies of the human response to the aerosolized racemate in addition to ingestion of stereoisomers 2 and 4.

CONFIDENTIAL

UNCLASSIFIED