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Acute Toxicity of Tetrahydrocannabinol to Mice in Altered Environments

by

Harry L. Froehlich

September 1964

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US Army Edgewood Arsenal
CHEMICAL RESEARCH AND DEVELOPMENT LABORATORIES
Edgewood Arsenal, Maryland 21010

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The chemical name of the compound reported on in CRDLR 3230 is 1-hydroxy-3-(1,2-dimethylheptyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6-dibenzopyran. A short name, tetrahydrocannabinol, has been used throughout the report in referring to this compound.

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September 1964 CRDLR 3230

ACUTE TOXICITY OF TETRAHYDROCANNABINOL TO MICE IN ALTERED ENVIRONMENTS

by

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FOREWORD

This work was conducted under Task 1C522301A07901, Biological Approach to New Agents (U). The data were collected in November 1963.

In conducting the research described in this report, the investigator adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Acknowledgments

The author wishes to acknowledge the assistance of Dr. J. H. Wills, who supplied the agent, and of the crew from the Technical Equipment Branch, who operated the cold room.

DIGEST

A dose range study and an acute intraperitoneal toxicity study were performed with tetrahydrocannabinol.

The purpose was to determine whether any differences in physiological activity or acute intraperitoneal toxicity (LD50) could be observed in mice housed at room temperatures of 70°F and 40°F.

The following conclusions were reached:

1. Mice receiving tetrahydrocannabinol at a room temperature of 40°F exhibited the same physiological effects as mice receiving tetrahydrocannabinol at a room temperature of 70°F, but at about one-tenth the dose.

2. The acute intraperitoneal LD50 of tetrahydrocannabinol in mice at a room temperature of 70°F was 125 ± 37 mg/kg.

3. The acute intraperitoneal LD50 of tetrahydrocannabinol in mice at a room temperature of 40°F was 14 ± 2 mg/kg.

4. Tetrahydrocannabinol is nine times more toxic in mice subjected to the added stress of cold.

ACUTE TOXICITY OF TETRAHYDROCANNABINOL TO MICE IN ALTERED ENVIRONMENTS

I. INTRODUCTION.

Hardman, Domino, and Seevers observed the intravenous lethal dose of tetrahydrocannabinol in the dog to be 100 mg/kg.[1] In the summer of 1955, eight dogs survived a dose of 1.0 mg/kg administered intravenously. When further tests at this dose were carried out in the winter of 1956 with dogs housed in unheated rooms, a 50% mortality was observed (the number of animals was not reported). Hardman, Domino, and Seevers partially resolved the apparent discrepancy between the two experiments when they noticed a correlation between the degree of hypothermia and the incidence of death. They constructed temperature controlled housing facilities for the dogs in which rectal temperatures could be maintained at 38° to 39°C. They then repeated the 1.0 mg/kg intravenous dose. Under these experimental conditions, no deaths were observed.[1]

Because of these findings, an attempt was made to determine whether any difference in toxicity of tetrahydrocannabinol could be observed when mice were subjected to a cold environment.

II. PROCEDURE.

A. Dose Range.

Swiss male albino mice, weighing between 25 and 35 grams, were randomly selected and housed 10 per cage in rooms maintained at 70°F and 40°F for 2 days prior to the dose range study. On the third day, 12 animals at each temperature (3 at each dose) were given intraperitoneal injections of tetrahydrocannabinol in doses ranging from 50 to 400 mg/kg at 70°F and 2.5 to 20.0 mg/kg at 40°F. The animals were observed periodically for 5 hours and after 24 and 48 hours for physiological activity and deaths. One additional animal was given an intraperitoneal injection of the suspending vehicle and served as a control at each dose. Where possible, dilutions were made so that all animals received a volume of 10 ml/kg. At the 200 and 400 mg/kg doses, however, 20 and 40 ml/kg had to be administered because the insolubility of this compound permitted only a 1% stock solution to be made. The 1% stock solution was made up by heating tetrahydrocannabinol in ethyl alcohol, adding equal parts of Span 80 and Tween 80, boiling off the alcohol, and obtaining the desired volume by adding

physiological saline. The control animals at these two doses, therefore, received volumes of 20 and 40 ml/kg of the suspending vehicle.

B. Acute Intraperitoneal LD50.

This phase of the study was initiated after the animals had been in their respective environmental areas for 7 days.

The animals housed at a room temperature of 70°F were given tetrahydrocannabinol in doses ranging from 25 to 400 mg/kg, 10 animals per dose. Ten animals were given intraperitoneal injections of the suspending vehicle at a volume of 40 ml/kg and served as controls. This volume was administered because at the 200 and 400 mg/kg doses the animals received volumes of 20 and 40 ml/kg, respectively.

The animals housed at a room temperature of 40°F were given tetrahydrocannabinol in doses ranging from 1.0 to 40.0 mg/kg. Dilutions were made so that all animals, regardless of dose, received a volume of 10 ml/kg. Ten animals received 10 ml/kg of suspending vehicle intraperitoneally and served as controls.

The LD50 was calculated by the method of Berkson.[2]

III. RESULTS.

A. Dose Range.

Similar physiological effects, such as dyspnea, hypotonia, depression, piloerection and analgesia, were obtained in mice housed at 70°F as in mice housed at 40°F but with approximately one-tenth the dose (tables 1 and 2, appendix).

B. Acute Intraperitoneal LD50.

The acute toxicity of this compound was greater when the animals were subjected to the added stress of cold (tables 3 and 4, appendix).

IV. DISCUSSION.

It has been previously reported[3] that the acute intraperitoneal LD50 in mice is 390 (260-585) mg/kg. There may be many explanations for the discrepancy between the LD50 obtained in this study and the LD50 previously reported. These include differences in the strains of mice used, oxidation of the crude compound, oxidation of the compound in the final

dilution, and the fact that, since the compound is so difficult to suspend in a vehicle, settling may have occurred so that the mice did not receive all of the agent. However, the physiological effects seen here corresponded very well with those observed by other investigators.[4, 5, 6]

A 24-hour LD50 could not be calculated because a greater than 50% mortality did not occur until the third day in the mice housed at 70°F. A 24-hour LD50 for the mice housed at 40°F was calculated to be 24.1 ± 5.0 mg/kg.

An LD50 was also calculated by incorporating the values for the animals used in the dose-range study. There did not appear to be any significant difference in the values obtained utilizing 13 instead of 10 mice per dose (tables 3A and 4A, appendix).

V. CONCLUSIONS.

1. Mice receiving tetrahydrocannabinol at a room temperature of 40°F exhibited the same physiological effects as mice receiving tetrahydrocannabinol at a room temperature of 70°F, but at about one-tenth the dose.

2. The acute intraperitoneal LD50 of tetrahydrocannabinol in mice at a room temperature of 70°F was 125 ± 37 mg/kg.

3. The acute intraperitoneal LD50 of tetrahydrocannabinol in mice at a room temperature of 40°F was 14 ± 2 mg/kg.

4. Tetrahydrocannabinol is nine times more toxic in mice subjected to the added stress of cold.

LITERATURE CITED

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APPENDIX

TABLE 1

EFFECTS OF TETRAHYDROCANNABINOL IN MICE AT A ROOM TEMPERATURE OF 70° TO 72°F

(Three mice at each dose)

Dose	Time after injection in minutes	Observed effects
mg/kg		
50.0	5	No observable effects
	10	Slight depression, dyspnea
	15	Moderate depression, dyspnea, piloerection, hypotonia
	30	Moderate depression, dyspnea, piloerection, hypotonia, slight ataxia, slight analgesia
	45	Moderate depression, dyspnea, piloerection, hypotonia, slight ataxia, slight analgesia
	60	Moderate depression, slight ataxia, dyspnea, hypotonia, slight analgesia
	90	Moderate depression, slight ataxia, dyspnea, hypotonia, slight analgesia
	120	Moderate depression, slight ataxia, hypotonia, slight analgesia, dyspnea, hypothermia
	150	Moderate depression, slight ataxia, hypotonia, slight analgesia, dyspnea, hypothermia
	180	Moderate depression, slight ataxia, hypotonia, slight analgesia, dyspnea, hypothermia
	240	Moderate depression, slight ataxia, hypotonia, slight analgesia, dyspnea, hypothermia
	300	Moderate depression, slight ataxia, hypotonia, slight analgesia, dyspnea, hypothermia
	24 hr	Moderate depression, hypothermia
	48 hr	No observable effects, one animal dead
100.0	5	Ptosis, slight depression
	10	Ptosis, slight depression

TABLE 1 (contd)

Dose	Time after injection in minutes	Observed effects
mg/kg		
100.0	15	Moderate depression, ptosis, slight ataxia, slight hypotonia
	30	Moderate depression, ptosis, slight ataxia, slight hypotonia
	45	Moderate depression, ptosis, moderate ataxia, moderate hypotonia, slight analgesia, loss of myotactic reflex, loss of pinna reflex
	60	Moderate depression, ptosis, moderate ataxia, moderate hypotonia, slight analgesia, loss of myotactic reflex, loss of pinna reflex
	90	Marked depression, marked ataxia, hypotonia, loss of myotactic reflex, loss of pinna reflex, dyspnea, moderate analgesia, hypothermia
	120	Marked depression, marked ataxia, hypotonia, loss of myotactic reflex, loss of pinna reflex, dyspnea, moderate analgesia, hypothermia
	150	Marked depression, marked ataxia, hypotonia, loss of myotactic reflex, loss of pinna reflex, dyspnea, moderate analgesia, hypothermia
	180	Marked depression, marked ataxia, hypotonia, loss of myotactic reflex, loss of pinna reflex, dyspnea, moderate analgesia, hypothermia
	240	Marked depression, marked ataxia, hypotonia, loss of myotactic reflex, loss of pinna reflex, dyspnea, moderate analgesia, hypothermia
	300	Marked depression, marked ataxia, hypotonia, loss of myotactic reflex, loss of pinna reflex, dyspnea, moderate analgesia, hypothermia
	24 hr	One animal dead, one animal no observable effects, one animal depressed
	48 hr	Two animals dead, one animal no observable effects
200.0	5	Ptosis
	10	Ptosis, slight depression, slight analgesia, dyspnea, moderate hypotonia

TABLE 1 (contd)

Dose	Time after injection in minutes	Observed effects
mg/kg		
200.0	15	Ptosis, slight depression, slight analgesia, dyspnea, moderate hypotonia
	30	Ptosis, slight depression, slight analgesia, dyspnea, moderate hypotonia
	45	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, one animal - loss of righting reflex
	60	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, one animal - loss of righting reflex
	90	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, hypothermia, one animal - loss of righting reflex
	120	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, hypothermia, one animal - loss of righting reflex
	150	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, hypothermia, one animal - loss of righting reflex
	180	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, hypothermia, one animal - loss of righting reflex
	240	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, hypothermia, one animal - loss of righting reflex
	300	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, hypothermia, one animal - loss of righting reflex
	24 hr	Moderate depression, ptosis, dyspnea, loss of pinna reflex, loss of myotactic reflex, hypothermia, one animal dead
	48 hr	Three animals dead

TABLE 1 (contd)

Dose	Time after injection in minutes	Observed effects
mg/kg		
400.0	5	Piloerection, slight stimulation
	10	Piloerection, moderate depression, hypersensitivity to sound
	15	Piloerection, moderate depression, hypersensitivity to sound, slight analgesia, loss of myotactic reflex
	30	Piloerection, moderate depression, hypersensitivity to sound, slight analgesia, loss of myotactic reflex
	45	Piloerection, moderate depression, hypersensitivity to sound, slight analgesia, loss of myotactic reflex
	60	Piloerection, moderate depression, dyspnea, moderate analgesia, hypothermia, loss of pinna reflex, loss of myotactic reflex
	90	Piloerection, moderate depression, dyspnea, moderate analgesia, hypothermia, loss of pinna reflex, loss of myotactic reflex
	120	Piloerection, moderate depression, dyspnea, moderate analgesia, hypothermia, loss of pinna reflex, loss of myotactic reflex
	150	Piloerection, moderate depression, dyspnea, moderate analgesia, hypothermia, loss of pinna reflex, loss of myotactic reflex
	180	Piloerection, moderate depression, severe dyspnea, ptosis, hypotonia, moderate analgesia, loss of pinna reflex, loss of myotactic reflex, hypothermia
	240	Piloerection, moderate depression, severe dyspnea, ptosis, hypotonia, moderate analgesia, loss of pinna reflex, loss of myotactic reflex, hypothermia
	300	Piloerection, moderate depression, severe dyspnea, ptosis, hypotonia, moderate analgesia, loss of pinna reflex, loss of myotactic reflex, hypothermia
	24 hr	Piloerection, moderate depression, severe dyspnea, ptosis, hypotonia, moderate analgesia, loss of pinna reflex, loss of myotactic reflex, hypothermia
	48 hr	Three animals dead

TABLE 2

EFFECTS OF TETRAHYDROCANNABINOL IN MICE AT A ROOM TEMPERATURE OF 40° TO 42°F

(Three mice at each dose)

Dose	Time after injection in minutes	Observed effects
mg/kg		
2.5	5	No observable effects
	10	No observable effects
	15	Ptosis
	30	Ptosis, slight depression
	45	Ptosis, slight depression
	60	Ptosis, slight depression
	90	Ptosis, slight depression, piloerection
	120	Ptosis, slight depression, piloerection
	150	Slight depression
	180	Slight depression
	240	Slight depression
	300	No observable effects
	24 hr	No observable effects
	48 hr	No observable effects
5.0	5	No observable effects
	10	Ptosis
	15	Ptosis, slight depression
	30	Ptosis, moderate depression, dyspnea, slight analgesia
	45	Ptosis, moderate depression, dyspnea, slight analgesia, slight hypotonia
	90	Ptosis, moderate depression, slight analgesia, slight hypotonia, piloerection, dyspnea
	120	Ptosis, moderate depression, slight analgesia, slight hypotonia, piloerection, dyspnea
	150	Ptosis, moderate depression, slight analgesia slight hypotonia, piloerection, dyspnea
	180	Ptosis, moderate depression, slight analgesia, slight hypotonia, piloerection, dyspnea

TABLE 2 (contd)

Dose	Time after injection in minutes	Observed effects
mg/kg		
5.0	240	Ptosis, slight depression
	300	Ptosis, slight depression
	24 hr	No observable effects
	48 hr	No observable effects
10.0	5	Ptosis
	10	Ptosis
	15	Ptosis, slight depression, slight analgesia, slight hypotonia
	30	Ptosis, slight depression, slight analgesia, slight hypotonia
	45	Ptosis, moderate depression, moderate analgesia, slight ataxia, moderate hypotonia, dyspnea, piloerection, hypothermia
	60	Ptosis, moderate depression, moderate analgesia, slight ataxia, moderate hypotonia, dyspnea, piloerection, hypothermia
	90	Ptosis, moderate depression, moderate analgesia, slight ataxia, moderate hypotonia, dyspnea, piloerection, hypothermia
	120	Ptosis, moderate depression, moderate analgesia, slight ataxia, moderate hypotonia, dyspnea, piloerection, hypothermia
	150	Ptosis, moderate depression, moderate analgesia, slight ataxia, moderate hypotonia, dyspnea, piloerection, hypothermia
	180	Ptosis, moderate depression, moderate analgesia, slight ataxia, moderate hypotonia, hypothermia, loss of pinna reflex, dyspnea
	240	Ptosis, moderate depression, moderate analgesia, slight ataxia, moderate hypotonia, hypothermia, loss of pinna reflex, dyspnea
	300	Moderate depression, loss of righting reflex, moderate analgesia, severe dyspnea, moderate ataxia, hypothermia, loss of pinna reflex, loss of myotactic reflex

TABLE 2 (contd)

Dose	Time after injection in minutes	Observed effects
mg/kg		
10.0	24 hr	Three animals dead
20.0	5	Ptosis, piloerection, slight depression
	10	Ptosis, piloerection, slight depression
	15	Ptosis, piloerection, moderate depression, dyspnea
	30	Ptosis, piloerection, moderate depression, dyspnea
	45	Ptosis, piloerection, moderate depression, dyspnea
	60	Ptosis, piloerection, moderate depression, slight ataxia, dyspnea, slight analgesia, loss of pinna reflex
	90	Ptosis, piloerection, moderate depression, slight ataxia, dyspnea, slight analgesia, loss of pinna reflex
	120	Ptosis, piloerection, moderate depression, slight ataxia, dyspnea, slight analgesia, loss of pinna reflex
	150	Ptosis, marked depression, loss of pinna reflex, loss of myotactic reflex, loss of righting reflex, severe dyspnea
	180	Ptosis, marked depression, loss of pinna reflex, loss of myotactic reflex, loss of righting reflex, severe dyspnea
	240	Ptosis, marked depression, loss of pinna reflex, loss of myotactic reflex, loss of righting reflex, severe dyspnea
	300	Ptosis, marked depression, loss of pinna reflex, loss of myotactic reflex, loss of righting reflex, severe dyspnea
	24 hr	Three animals dead

TABLE 3

ACUTE INTRAPERITONEAL TOXICITY OF TETRAHYDROCANNABINOL IN MICE AT A ROOM TEMPERATURE OF 70°F TO 72°F

Dose	Number of animals per dose	Volume administered	Mortality			Total number dead	LD50 and standard deviation
			First day	Second day	Third day		
mg/kg		ml/kg					
25.0	10	10.0	0	1	0	1	
50.0	10	10.0	1	1	0	2	
100.0	10	10.0	0	0	0	0	125.4 ± 36.5
200.0	10	20.0	0	4	6	10	
400.0*	10	40.0	0	4	6	10	
Control	10	40.0	0	0	0	0	

* Dose not used in calculation of LD50.

TABLE 3A

ACUTE INTRAPERITONEAL TOXICITY OF TETRAHYDROCANNABINOL IN MICE AT A ROOM TEMPERATURE OF 70°F INCORPORATING DOSE RANGE DATA

Dose	Number of animals per dose	Volume administered	Mortality			Total number dead	LD50 and standard deviation
			First day	Second day	Third day		
mg/kg		ml/kg					
25.0	10	10	0	1	0	1	
50.0	13	10	1	2	0	3	
100.0	13	10	1	1	0	2	130.0 ± 58.3
200.0	13	20	1	6	6	13	
Control	13	20	0	0	0	0	

TABLE 4

ACUTE INTRAPERITONEAL TOXICITY OF TETRAHYDROCANNABINOL IN MICE AT A ROOM TEMPERATURE OF 40° TO 42°F

Dose	Number of animals per dose	Volume administered	Mortality			Total number dead	LD50 and standard deviation
			First day	Second day	Third day		
mg/kg		ml/kg					
1.0*	10	10	0	0	0	0	
2.5*	10	10	0	0	0	0	
5.0*	10	10	0	0	0	0	
10.0	10	10	0	0	0	0	14.1 ± 1.7
20.0	10	10	5	5	-	10	
40.0*	10	10	7	3	-	10	
Control	10	10	0	0	0	0	

* Doses not used in the calculation of LD50.

TABLE 4A

ACUTE INTRAPERITONEAL TOXICITY OF TETRAHYDROCANNABINOL IN MICE AT A ROOM TEMPERATURE OF 40°F INCORPORATING DOSE RANGE DATA

Dose	Number of animals per dose	Volume administered	Mortality			Total number dead	LD50 and standard deviation
			First day	Second day	Third day		
mg/kg		ml/kg					
5.0	13	10	0	0	0	0	
10.0	13	10	3	0	0	3	12.0 ± 1.6
20.0	13	10	8	5	0	13	
Control	13	10	0	0	0	0	

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13 ABSTRACT

A dose range study and an acute intraperitoneal toxicity study were performed with tetrahydrocannabinol. The purpose was to determine whether any differences in physiological activity or acute intraperitoneal toxicity (LD50) could be observed in mice housed at room temperatures of 70° and 40°F. Mice receiving tetrahydrocannabinol at a room temperature of 40°F exhibited the same physiological effects as mice receiving tetrahydrocannabinol at a room temperature of 70°F. The acute intraperitoneal LD50 of tetrahydrocannabinol in mice at 70°F was 125 ±37 mg/kg. The acute intraperitoneal LD50 of tetrahydrocannabinol in mice at 40°F was 14 ±2 mg/kg. Tetrahydrocannabinol is nine times more toxic in mice subjected to the added stress of cold.

14. KEYWORDS

Mice	Intraperitoneal
Toxicity	Chemical agent
Physiology	Stress dose range
Temperature	Tetrahydrocannabinol

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