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THE TOXICITY OF ORALLY ADMINISTERED EMETIC PP 796 IN CYNOMOLGUS MONKEYS

Addressee:

Dr. M.S. Rose,
Imperial Chemical Industries Ltd.,
Central Toxicology Laboratory,
Alderley Park,
Nr. MACCLESFIELD,
Cheshire,
SK10 4TJ.

Authors:

David A. Purser,
Patricia Grimshaw,
Leon M. Cobb.

Huntingdon Research Centre,
HUNTINGDON,
Cambridgeshire.

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We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

Leon M. Cobb, Ph.D., M.R.C. Path., M.R.C.V.S.,
Head of Department of Environmental Physiology

David A. Purser, B.Sc., Ph.D.,
Department of Environmental Physiology

Patricia Grimshaw, B.Sc.,
Scientific Officer,
Department of Environmental Physiology

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SUMMARY

Test material: ICI emetic, code number PP 796

Test species: Wild-caught cynomolgus monkeys (Macaca fascicularis). 10 males.

Route of administration: Oral gavage.

Dose level: Each animal received a single oral dose of 100 mg PP 796/kg bodyweight.

Observation period: 14 days after dosing.

Results

Clinical observations:

Dose of PP 796 (mg/kg)	No. of animals vomiting	Mortality
100	9/10	4/10

Four of the 10 animals died, all within 24 hours of dosing.

All except 1 animal showed some degree of collapse after dosing. Three of the animals that subsequently died passed through a state of total collapse before death. The fourth animal that died did so overnight and was therefore not observed to have passed through a state of total collapse.

Increased salivation was observed in all animals within 1 hour of dosing. One of the animals that died had failed to vomit. All other animals vomited within 43 minutes of dosing.

Bodyweight: Treatment had no effect on bodyweight.

Food consumption: Treatment had no effect on food consumption.

Comment: Powerful vomiting usually occurred 2 to 3 times following dosing. No animals suffered extreme retching motions or continuous vomiting.

Vomiting behaviour was comparable with that found at lower dose levels (2 - 30 mg PP 796/kg) in a previous study (HRC Report No. ICI 119/78556*).

* The oral toxicity and mode of action of emetic PP 796 in cynomolgus monkeys, and its effect upon the oral toxicity of several formulations of paraquat.

INTRODUCTION

In a previous study carried out at the Huntingdon Research Centre (HRC Report No. IC1 119/78556) an emetic substance, code number PP 796, was found to be a potent emetic in cynomolgus monkeys when administered orally at a dose level of 2 mg PP 796/kg bodyweight. Doses of up to 30 mg PP 796/kg were used and all animals subsequently made a full recovery.

The present study was designed to investigate the toxicity and vomiting behaviour induced by PP 796 when administered orally at a high dose level to cynomolgus monkeys. The maximum concentration of PP 796 providing a stable aqueous solution was 5 mg/ml. Since we did not wish to exceed a total volume of 100 ml dosing solution, the maximum practicable dose that could be administered to 4 - 5 kg monkeys was 100 mg PP 796/kg. All animals were dosed at a level of 100 mg PP 796/kg. The animals were dosed once only.

The study was carried out at the Huntingdon Research Centre from April to July 1977.

MATERIALS AND METHODS

Animals

Ten wild-caught male cynomolgus monkeys (Macaca fascicularis) were obtained from a commercial supplier (Shamrock Farms Limited, Henfield, Sussex). The animals, 2 of which were supplied on 7 March and 8 on 26 April 1977, weighed between 2.3 and 4.4 kg.

All animals were held in England for at least 12 weeks before delivery to the Huntingdon Research Centre. On arrival they were examined by a veterinary surgeon, and their general health was found to be satisfactory. Examination included an intrapalpebral tuberculin test (10000 i.u. mammalian P.P.D.) and a chest X-ray.

A period of at least 2 weeks was allowed for the animals to adjust to their new environment prior to any investigations.

Accommodation

The animals were housed individually in primate cages measuring 70 cm x 68 cm x 98 cm. The cages were constructed of aluminium and stainless steel, incorporating a squeeze-back system, food hopper, bottle holder and floor grid. The cages were mounted on racks in a well-ventilated holding area maintained at a temperature of 22 $\pm$ 3 °C.

Diet

The animals were fed daily with 200 g of dry diet consisting of a 1 : 1 ratio of "F.P.1" (Dixon and Sons Limited, Ware, Hertfordshire) and "Mazuri Primate Diet" (BP Nutrition (U.K.) Limited, Stepfield, Witham, Essex) and 2 x 25 g "Bonio" biscuits (Spratt's Patent Limited, Barking, Essex). This was supplemented daily by a slice of brown bread and on 5 days out of 7 by a weighed quantity of fresh fruit or vegetable produce.

Water was available at all times. In addition each animal had access to a solution of Blackcurrant Syrup B.P.C. (Boots Drug Company, Nottingham) and Cytacon* vitamin B_{12} liquid (Glaxo Laboratories Limited, Greenford, Middlesex), 20 ml of each in a litre of tap water.

Test material and dosing solution

A 5 g sample of PP 796, a white crystalline solid, was received from ICI Central Toxicology Laboratory, Alderley Park, Nr. Macclesfield, Cheshire on 24 November 1976. The sample was supplied in a brown glass bottle labelled as follows:

5 grams PP 796
From bottle of ICI 63, 197
ADM 52124/74
22/11/77

The material was stored at room temperature and protected from the light. Aqueous dosing solutions containing 5 mg PP 796/ml were freshly prepared shortly before use.

* Contains Cyanocobalamin BP (7 µg/ml)

Administration of the test material

Prior to dosing, each animal was starved overnight for a period of at least 16 hours and then weighed in order to calculate the dose corresponding to a dose level of 100 mg PP 796/kg bodyweight.

Care was taken to ensure that the stomach was moderately full and full to the same extent in all animals. To this end, all doses were made up to a total volume of 100 ml with tap water.

Details of the dosing schedule are presented in Table 1.

Clinical observations

Vomiting 'behaviour'

The timing and frequency of vomiting was recorded for each animal during the first 6 hours after dosing.

Clinical signs

Each animal was observed for signs of reaction to treatment continuously for the first 6 hours after dosing and then twice daily for the rest of the 14-day observation period.

Bodyweight

Individual bodyweights were recorded weekly throughout the study.

Week 0 refers to the bodyweight recorded on the day of dosing.

Food consumption

Food consumption of individual animals was recorded daily throughout the study and expressed as g/monkey.day^{-1}.

Day 1 refers to the food eaten on the day of dosing.

MORTALITY

Four of the 10 animals died during the study, all within 24 hours of dosing.

CLINICAL OBSERVATIONS

Vomiting 'behaviour'

The occurrence of vomiting in individual animals is presented in Table 2.

A synopsis of the results is as follows:

Animal 645♂ failed to vomit, although increased salivation was observed 30 minutes after dosing. All other animals first vomited at between 4 and 43 minutes after dosing.

In all but 3 animals, vomiting was observed only during the first hour after dosing.

The volume of vomit produced by animals on each occasion was not measured, but observations suggested that animals vomiting most frequently did not always appear to produce the largest total volumes of vomit. There did not appear to be any clear relationship between the vomiting 'behaviour' and the survival or otherwise of the animals. However, the 1 animal which failed to vomit (645♂) subsequently died 6 hours after dosing.

Clinical signs

The development of signs in individual animals may be described by the following stages.

I Lethargy.

II Some loss of co-ordination.

III Salivation.

IV Semi-collapse:- some weakness, animal unable to sit up without support.

V Total collapse:- animal extremely weak and lying on the floor of the cage, little movement detectable.

VI Slight recovery:- signs of improvement in the condition of the animal.

VII Total recovery:- no abnormalities detectable.

VIII Death.

Observations of individual animals are presented in Table 3.

A synopsis of the results is as follows:

Increased salivation was observed in all animals within 1 hour of dosing.

Animal 647♂ showed no signs other than lethargy and began to recover within 1 hour of dosing. All other animals reached a state of semi-collapse within 1 hour 20 minutes of dosing.

All 3 animals progressing to a state of total collapse subsequently died. One of these animals (649♂) began to recover approximately 5 hours after dosing but was found dead the following morning. Animals 645♂ and 663♂ remained in a collapsed state and died at 6 and 24 hours after dosing, respectively.

Animal 657♂ reached only a state of semi-collapse, began to recover approximately 5 hours after dosing but was found dead the following morning.

All surviving animals began to recover between 4 and 6 hours after dosing and appeared to be completely recovered within 24 hours of dosing.

Bodyweight

Individual weekly bodyweights are presented in Appendix 1.

Treatment had no effect on bodyweight and all values were considered to be within normal limits.

Food consumption

The daily food consumption of individual animals is presented in Appendix 2.

Treatment had no effect on food consumption and all values were considered to be within normal limits.

COMMENTS

1. Powerful vomiting usually occurred 2 to 3 times following dosing. No animals suffered extreme retching motions or continuous vomiting. Vomiting behaviour was comparable with that found at lower levels (2 - 30 mg PP 796/kg) in a previous study (HRC Report No. ICI 119/78556).

2. The main difference between the effects of high and low dose levels of PP 796 was the greater degree of lethargy and collapse observed at high dose levels.

3. Although the investigations used in this study were not sufficiently extensive to ascertain the cause of death, the combination of emetic activity and a state of collapse is an extremely dangerous one. The chances of a collapsed or semi-collapsed animal inhaling its own vomit are high. The increase in salivation could also present a hazard.

   One animal, 645♂, which suffered dyspnoea prior to death, was subjected to a post mortem examination. Copious amounts of grey/green fluid emanated from the trachea when cut. The lungs appeared to be slightly oedematous and on compression more grey/green fluid exuded from the trachea. The stomach was found to be filled with a similar grey/green fluid. The oesophagus was not ruptured. The indication was that this animal had inhaled vomit.

TABLE 1

Dosing schedule

Animal No.	Bodyweight (kg)	Dose of PP 796 (mg/kg)	Volume of dosing solution (5 mg PP 796/ml)	Date dosed
595♂	2.90	100	58	28 April 1977
597♂	2.45		49	
633♂	3.30	100	66	1 June 1977
635♂	2.40		48	
657♂	2.70	100	54	9 June 1977
663♂	2.55		51	
645♂	2.30	100	46	29 July 1977
647♂	4.35		87	
649♂	2.50		50	
637♂	3.70		74	

TABLE 2

Vomiting 'behaviour' - individual observations

Animal No.	Occasion	Time after dosing	Deaths
595♂	1st vomit 2nd vomit 3rd vomit 4th vomit 5th vomit	25 minutes 37 minutes 1 hour 25 minutes 2 hours 30 minutes 3 hours 23 minutes	S
597♂	1st vomit 2nd vomit 3rd vomit 4th vomit	42 minutes 50 minutes 2 hours 12 minutes 3 hours 17 minutes	S
633♂	1st vomit 2nd vomit 3rd vomit	31 minutes 40 minutes 1 hour 0 minute	S
635♂	1st vomit 2nd vomit	5 minutes 45 minutes	S
657♂	1st vomit 2nd vomit	43 minutes 50 minutes	D
663♂	1st vomit 2nd vomit 3rd vomit	35 minutes 50 minutes 5 hours 10 minutes	D
645♂	*		D
647♂	1st vomit	5 minutes	S
649♂	1st vomit	4 minutes	D
637♂	1st vomit 2nd vomit	5 minutes 15 minutes	S

* Animal failed to vomit

S = survived
D = died

TABLE 3

Clinical signs - individual observations

Animal No.	Time to development of stages*							
	I	II	III	IV	V	VI	VII	VIII
595♂	-	-	15 minutes	15 minutes	-	6 hours	<24 hours	-
597♂	15 minutes	-	15 minutes	60 minutes	-	4 hours	<24 hours	-
633♂	35 minutes	60 minutes	35 minutes	80 minutes	-	6 hours	<24 hours	-
635♂	25 minutes	-	30 minutes	30 minutes	-	4 hours	<24 hours	-
657♂	15 minutes	25 minutes	45 minutes	40 minutes	-	5 hours	-	8 - 24 hours
663♂	-	-	35 minutes	15 minutes	1 hour	-	-	24 hours
645♂	-	-	30 minutes	10 minutes	4 hours	-	-	6 hours
647♂	10 minutes	-	16 minutes	-		1 hour	<24 hours	-
649♂	7 minutes	8 minutes	24 minutes	14 minutes	24 minutes	5 hours	-	8 - 24 hours
637♂	4 minutes	10 minutes	10 minutes	20 minutes	-	5 hours	<24 hours	-

* I Lethargy
II Some loss of co-ordination
III Salivation
IV Semi-collapse
V Total collapse
VI Slight recovery
VII Total recovery
VIII Death

APPENDIX 1

Individual weekly bodyweights (kg)

Animal No.	Week of study				
	-2	-1	0	1	2
595♂	2.85	2.85	2.90	2.80	2.60
597♂	2.60	2.60	2.45	2.40	2.40
633♂	*	3.35	3.30	3.30	3.30
635♂	*	2.37	2.40	2.35	2.35
657♂	*	*	2.70	**	
663♂	*	*	2.55	**	
645♂	2.35	2.30	2.30	**	
647♂	4.37	4.30	4.35	4.25	4.42
649♂	2.55	2.45	2.50	**	
637♂	3.60	3.50	3.70	3.55	3.57

Week 0 indicates the bodyweight recorded on the day of dosing.

* The bodyweights for week -2 were recorded on Day -14 of the study. Animals 633♂ and 635♂ were held in a communal primate run until Day -13 of the study; individual bodyweights were therefore not recorded for week -2. Animals 657♂ and 663♂ were held in the run throughout the pre-dosing period.

** Animal dead

APPENDIX 2

Individual daily food consumption (g/monkey.day^{-1})

Animal No.	Day of study																											
	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
595♂	145	140	120	175	250	150	245	230	215	185	180	155	135	115	95	175	155	100	170	230	135	135	145	85	115	90	155	60
597♂	170	185	145	160	245	255	250	240	280	150	165	205	200	230	140	215	145	185	275	265	235	245	265	170	160	235	270	240
633♂	*	270	300	175	170	255	270	290	185	235	170	165	260	255	110	170	215	170	180	115	165	210	185	180	170	170	230	230
635♂	*	210	215	160	155	210	225	300	250	235	175	170	320	250	130	125	140	160	165	185	185	260	210	215	175	170	230	230
657♂	*	*	*	*	*	*	*	*	*	*	*	*	*	*	**													
663♂	*	*	*	*	*	*	*	*	*	*	*	*	*	*	**													
645♂	115	90	80	105	115	140	130	135	100	90	125	160	115	65	**													
647♂	250	210	205	260	265	255	265	275	210	200	265	280	240	245	115	160	270	285	275	230	265	245	170	220	275	295	240	270
649♂	245	215	215	260	250	255	230	250	215	195	220	280	255	245	85	**												
637♂	140	120	165	205	135	90	185	180	210	175	185	215	185	120	140	15	135	170	165	135	195	225	60	160	140	165	200	260

All animals were starved overnight prior to dosing. Dosing took place on Day 1 of the study.

* These values are not available as the animals were held in a communal primate run during this period.

** Animal dead.