

Plasma Phencyclidine Pharmacokinetics in Dog and Monkey Using A Gas Chromatography Selected Ion Monitoring Assay^{†‡}

A. E. Wilson and E. F. Domino

Department of Pharmacology, The University of Michigan, Ann Arbor, Michigan 48109, USA and Lafayette Clinic, Detroit, Michigan 48207, USA

Phencyclidine was determined by gas chromatography selected ion monitoring in six dogs and seven monkeys. Aliquots of venous blood were taken over 4 h in the monkey after 1.1 mg kg⁻¹ and over 24 h in the dog after 1.0 mg kg⁻¹ of phencyclidine i.v. Pentadeuterated phencyclidine was used as the internal standard. In the electron impact mode the most abundant fragments in the mass spectrum of phencyclidine were *m/e* 91 and 200, and 96 and 205 in the [²H₅]phencyclidine spectrum. These fragments were used to quantitate the amount of phencyclidine present. In both species, a complex exponential decline of plasma phencyclidine was found in most animals that fit a two compartment open model. In monkeys, the mean half-life (β phase) was 2.36 h and in the dog it was 2.86 h. Compared with the monkey, the dog exhibited considerable emergence delirium. The two species had rather different pharmacokinetics which may be relevant to the observed differences in degree of anesthesia and recovery.

INTRODUCTION

In recent years the need to identify phencyclidine [PCP, 1-(1-phenylcyclohexyl) piperidine] in biological fluids and tissues has become important. This drug is increasingly abused. A number of clinical reports on human overdose cases have appeared.¹⁻⁵ Previous means of detection of PCP by chromatography and fluorometry^{6,7} are not specific. Gas chromatography mass spectrometry has done much to advance our knowledge in this field.⁸⁻¹⁴ Since PCP undergoes thermal decomposition above temperatures of 150 °C to form 1-phenyl-cyclohexene,^{11,12} temperatures in all phases of the experiment must be carefully controlled. A number of investigators¹¹⁻¹⁴ have reported quantitative GCMS assays for PCP. We have adapted the GCMS chemical ionization (methane) assay of Lin *et al.*¹² for electron impact (EI). We have used GC selected ion monitoring to assay PCP in the plasma of the dog and monkey and have determined its plasma pharmacokinetics as described in this report.

EXPERIMENTAL

Animals

Six mongrel dogs of either sex were fasted 12 h prior to being given 1.0 mg kg⁻¹ PCP HCl i.v. calculated as

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[‡] PCP = phencyclidine; [²H₅]PCP = pentadeuterated phencyclidine

base. Control bloods were taken prior to the injection of PCP. Blood was collected from an indwelling venous cannula for 24 h during which time the dogs were allowed complete freedom of movement. PCP was given over a 1 min period.

Seven former morphine dependent *Macaca mulatta* monkeys of either sex were used in these experiments. At the time they were given PCP they had completely recovered from their narcotic dependence for 3-6 months. These animals were not on any medication. The animals were given 1.1 mg kg⁻¹ of PCP HCl i.v. over 1 min with the dose calculated as base. Venous blood samples were collected over a 4 h period from an indwelling venous catheter. In both groups of animals all blood removed was replaced by an equal volume of 0.9% NaCl. Whole blood was collected in heparinized tubes, spun at 1000 g for 15 min and the plasma removed for PCP extraction.

Assay

The method of Lin *et al.*¹² was used to extract PCP. Briefly, this involves adding 1 μ g of pentadeuterated PCP ([²H₅] PCP) to the sample, buffering to pH 9.5 and extracting twice with hexane. A back extraction was done into 0.2 N H₂SO₄. After removal of the organic layer, the aqueous phase was buffered to pH 9.5 for a final extraction into hexane. The hexane layer was removed and evaporated to dryness. One to 2 μ l of the reconstituted sample in 25 μ l of hexane was used for GCMS analysis. In this study a Finnigan 3200 quadrupole gas chromatograph mass spectrometer with a 6102 data system was used in the EI mode. The GC conditions were with a column temperature of 190 °C and injection port of 200 °C to minimize thermal decomposition of PCP. Helium flow was 20 ml min⁻¹.

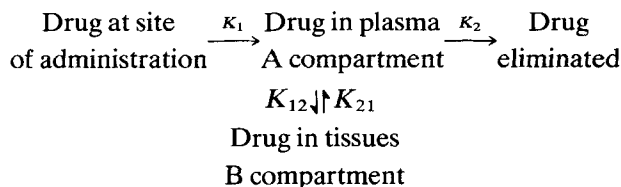
A 5 ft × 2 mm glass column was packed with 2.5% SE-30 on Chromasorb G or 3% OV-17 on Gas Chrom Q. Two different columns were used since the OV-17 column separated a small interfering peak in recording m/e 200 in the selected ion profile of the monkey plasma. The glass jet separator was at 210 °C to prevent sample condensation in the jet tip. All other lines were glass lined. The fragments were produced by EI at 70 eV.

Pharmacokinetic analysis

The number of compartments in an open compartment model was determined by an Amdahl 470 V/C computer using the method of Wagner *et al.*¹⁵ The exponential equation to describe a multicompartment model was

$$C = Ae^{-\alpha t} + Be^{-\beta t} + \dots$$

where C is the concentration at any time, t , A and B are constants for each compartment of which α and β are exponents. A two compartment open model is shown below in which the phencyclidine was introduced directly into the plasma compartment:



The parameters A , B and α, β were determined by a curve stripping program and C_0 , K_2 , K_{21} and K_{12} were found by a nonlinear regression program [Wagner, personal communication (1977)]. The rate constants of the model were calculated as $K_2 = 1/(A'/\alpha + B'/\beta)$, $K_{21} = A'\beta + B'\alpha$, $K_{12} = A'B'(\beta - \alpha)^2/K_{21}$ where $A' = A/A + B$ and $B' = B/A + B$.

RESULTS

The combination of GCMS in the selected ion monitoring mode gives a very sensitive and quantitative means for determining PCP. With the use of [²H₅]PCP as the internal standard, other possible drugs and materials found in biological samples can be avoided. In Fig. 1 is illustrated a total ion chromatogram (TIC) of 1 μg of PCP and [²H₅]PCP each added to monkey plasma. Since the final amount is concentrated in 20–25 μl of hexane, these peaks represent 40–50 ng when 1 μl is injected. The mass spectra of PCP and [²H₅]PCP are illustrated in Fig. 2. Note the molecular ions m/e 243 and m/e 248 are present but are of relatively low intensity as if often true of EI produced spectra. The base peak of PCP is m/e 200 and 205 for [²H₅]PCP. In view of the fact that m/e 91, 96, 200 and 205 represent the major fragments of both PCP and [²H₅]PCP, these were monitored to obtain a standard calibration curve. The m/e 91, 96 peak area ratios were monitored as a qualitative check for PCP while m/e 200, 205 peak area ratios were used to determine the amount of PCP present. With this method of ex-

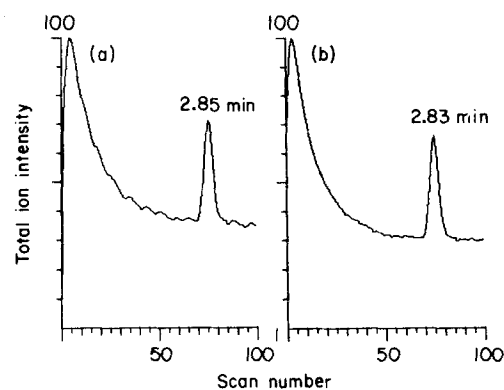


Figure 1. Total ion chromatogram of (a) phencyclidine and (b) [²H₅]phencyclidine. PCP and [²H₅]PCP (1 μg ml⁻¹) were run separately under the conditions listed. [²H₅]PCP has a slightly shorter retention time, indicating an isotope effect. Conditions were as follows: Col. = 3% OV-17; Sol. = hexane; Col. temp. = 190 °C; Sep. temp. = 210 °C; He flow = 20 cc min⁻¹.

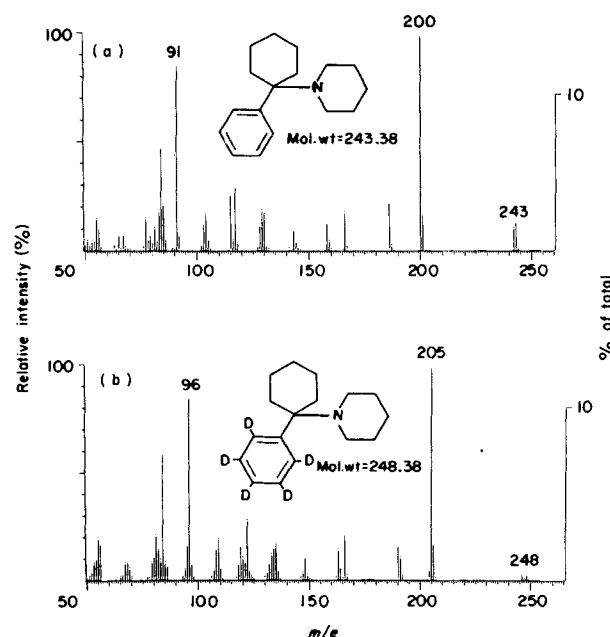


Figure 2. Mass spectra of (a) phencyclidine and (b) [²H₅]phencyclidine. Note that the major ions of PCP, m/e 91, 200 and 243, are 5 m/e higher for [²H₅]PCP.

traction, PCP metabolites are not extracted. Their retention times are also known to be different from that of PCP.¹²

A typical mass fragmentogram is illustrated in Fig. 3. It can be noted that there are no interfering constituents in the plasma sample. With a TIC 100 ng was the limit of detectability; in selected ion monitoring 5 ng could be easily detected. A standard curve for PCP in dog and monkey plasma is shown in Fig. 4. Determinations were done to 0.1 ng; however, they are not indicated on the graph. The peak area ratio of 200/205 was used to quantitate the amount of PCP present. A recovery check was done by splitting six samples with an unknown endogenous amount of PCP and adding a known amount of PCP to one-half of each sample in addition to 1 μg of [²H₅]PCP. The amount of exogenous PCP added to each half was calculated as

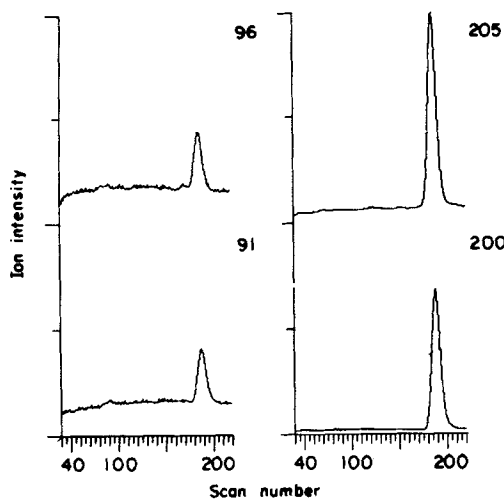


Figure 3. Selected ion profile of phencyclidine from monkey plasma using [$^2\text{H}_5$]phencyclidine as the internal standard. This is a typical run on a monkey plasma sample in which slightly less PCP is present than the [$^2\text{H}_5$] internal standard.

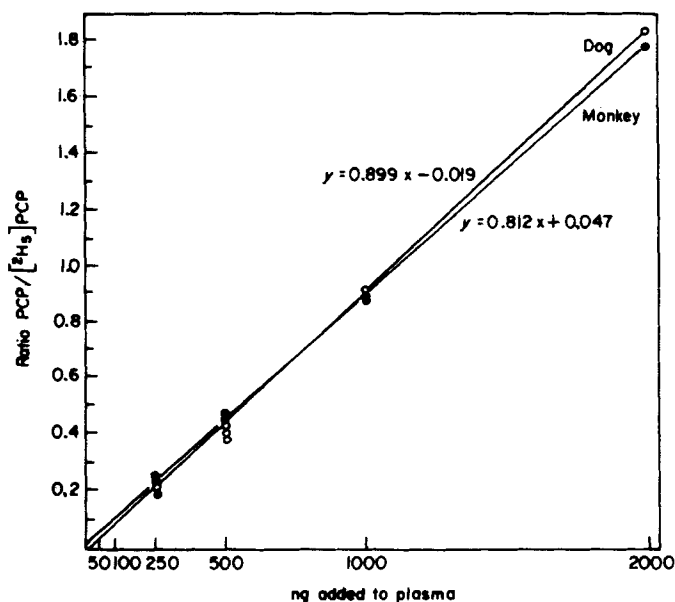


Figure 4. Standard calibration for phencyclidine in dog and monkey plasma using EI selected ion monitoring. Note linearity of increasing amounts of PCP added to the plasma samples with [$^2\text{H}_5$]PCP constant at 1000 ng. Approximately 1/20 (1 μl) of the final volume was injected into the gas chromatograph mass spectrometer. The x-axis represents added standards in 1 ml.

recovery and found to be 96.6%. All samples were run two to three times and never varied more than $\pm 6\%$ from the mean. Plasma samples determined from the calibration curve had a $\text{SE} \pm 5\%$ or less.

The plasma concentration of six dogs given 1.0 mg kg^{-1} of PCP i.v. is illustrated in Fig. 5. Mean $\pm \text{SE}$, plasma levels of $85.90 \text{ ng ml}^{-1} \pm 10.8$ were found 30–45 min later when the dogs tried to lift their heads as a sign of partial recovery from anesthesia. While anesthetized, the dogs exhibited nystagmus, salivation, analgesia and pupillary dilatation. An emergence delirium of varying intensity was noted subsequently. This began approximately 45 min after injection and lasted up to 3 h with corresponding mean $\pm \text{SE}$ plasma

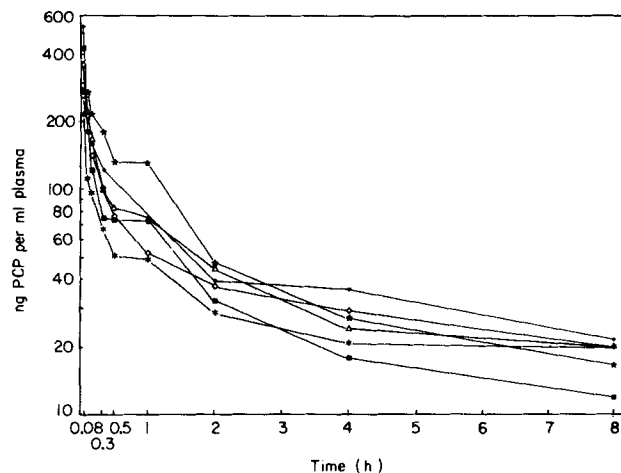


Figure 5. Dog plasma phencyclidine concentration—time course. PCP was given in a dose of 1.0 mg kg^{-1} i.v. to six dogs. Each symbol represents the plasma PCP level of a different animal over an 8 h period.

PCP levels of 61.6 ± 7.2 and $35.1 \pm 2.7 \text{ ng ml}^{-1}$ respectively.

The plasma levels of PCP in individual monkeys given 1.1 mg kg^{-1} of PCP i.v. are shown in Fig. 6. At approximately 1 h, monkeys became conscious with mean $\pm \text{SE}$ plasma levels of $110.0 \pm 6.3 \text{ ng ml}^{-1}$. The monkeys recovered over the next 3 h but did not show any emergence delirium in contrast to the dogs. The dogs were fully recovered by 8 h at which time levels were $18.8 \pm 2.8 \text{ ng ml}^{-1}$. No PCP was detectable in dog plasma 24 h after injection.

Figure 7 and 8 represent the computer printouts for a two compartment model of the mean data for the six dogs and monkeys, respectively. Note that the predicted and observed points are very similar. The equations for each are given on the figures. The plasma PCP pharmacokinetic analysis of the individual animals and the group means are given in Table 1 for the dog (8 h data) and Table 2 for the monkey (4 h data). The SD and 95% confidence limits are indicated.

Although the monkeys received 10% more PCP, their plasma levels are much higher than expected. Part of this may be explained by the following. The mean body weight of the seven monkeys was 4.87 kg. The

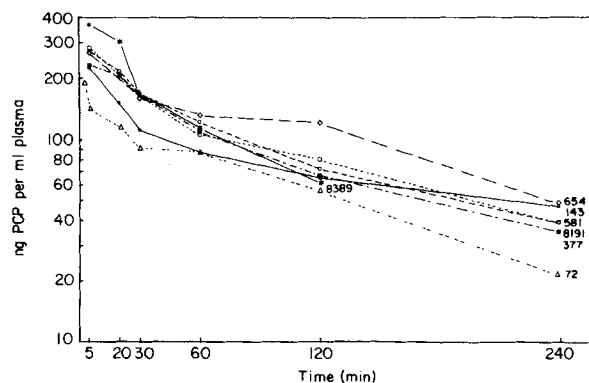


Figure 6. Monkey plasma phencyclidine concentration—time course. PCP was given in a dose of 1.1 mg kg^{-1} i.v. to seven monkeys whose numbers are given. Each symbol represents the plasma PCP levels of a different animal over a 4 h period.

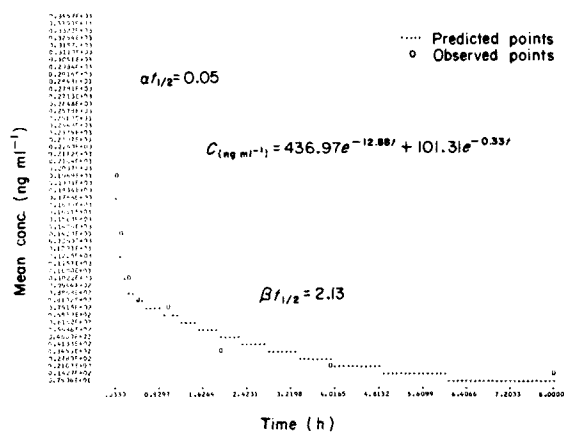


Figure 7. Computer plot of observed and predicted mean plasma levels of phencyclidine in the dog. The plasma concentration is plotted over an 8 h interval following a dose of 1.0 mg kg⁻¹, i.v.

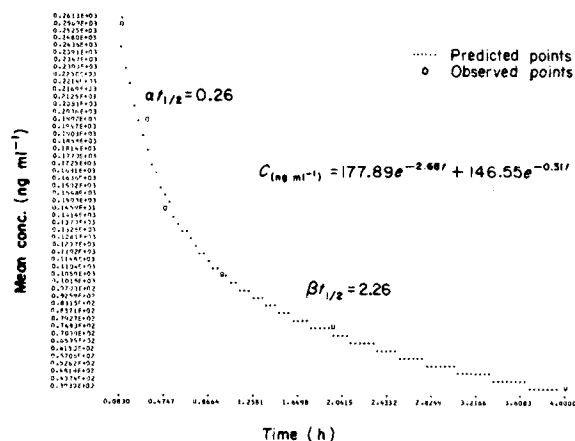


Figure 8. Computer plot of observed and predicted mean plasma levels of phencyclidine in the monkey. The plasma concentration is plotted over a 4 h interval following a dose of 1.1 mg kg⁻¹, i.v.

mean body weight of the five dogs with complete pharmacokinetic data was 18.96 kg. The apparent volume of distribution was calculated as

$$V_{\text{central}} = \frac{\text{Dose}}{A+B} \text{ and } V_{\text{peripheral}} = V_c \times \frac{K_{12}}{K_{21}}$$

If we use the computer estimated mean for each compartment then the monkey has a mean central volume of distribution 16.5 l compared with 35.2 l in the dog. The mean volume of the peripheral compartment in the monkey was 12.1 l compared with 99.2 l in the dog. In the monkey K_{21} and K_{12} are very similar,

Table 1. Pharmacokinetic parameters of phencyclidine in the dog^a

Dog	A ^b	B	α ^c	β	C ₀	K ₂₁	K ₂	K ₁₂	t _{1/2} (h)		r ^d
									α	β	
1	332.62	84.20	9.630	0.270	442.38	2.156	1.207	6.569	0.072	2.57	0.994
2	226.62	63.15	4.957	0.136	292.69	1.037	0.651	3.405	0.140	5.10	0.997
3	338.46	74.15	17.622	0.355	319.38	4.100	1.524	12.352	0.039	1.95	0.985
5	178.77	123.24	1.895	0.234	281.79	0.706	0.628	0.795	0.366	2.96	0.976
6	775.91	85.29	23.830	0.402	807.10	3.137	3.052	18.050	0.029	1.72	0.997
Mean	370.42	86.01	11.588	0.279	428.67	2.227	1.412	8.234	0.129	2.86	0.990
±SD	236.83	22.65	9.059	0.104	221.02	1.420	0.992	6.977	0.139	1.35	0.009
Computer estimated mean	436.97	101.31	12.880	0.326	458.09	3.104	1.350	8.748	0.054	2.13	0.994
± Confidence limits	—	—	—	—	201.52	2.306	0.826	7.737	—	—	—

^a The data of dog #4 are partially missing and were excluded from analysis.

^b A, B and C₀ are ng ml⁻¹.

^c All rate constants h⁻¹.

^d r = correlation coefficient.

Table 2. Pharmacokinetic parameters of phencyclidine in the monkey^a

Monkey	A ^b	B	α ^c	β	C ₀	K ₂₁	K ₂	K ₁₂	t _{1/2} (h)		r ^d
									α	β	
1	96.34	204.80	2.753	0.330	296.16	1.962	0.463	0.658	0.252	2.10	0.980
2	153.28	127.78	23.844	0.408	276.62	11.075	0.878	12.299	0.029	1.70	0.995
3	342.58	—	1.108	—	373.50	—	1.108	—	0.625	—	0.957
4	110.61	149.57	1.638	0.358	259.30	1.070	0.547	0.378	0.423	1.94	0.992
5	116.08	133.88	1.764	0.274	302.02	0.856	0.563	0.618	0.393	2.53	0.998
6	246.95	104.73	4.270	0.184	325.12	1.429	0.550	2.475	0.162	3.77	0.999
7	172.55	157.58	3.257	0.326	338.06	1.613	0.657	1.312	0.213	2.13	0.999
Mean	184.05	146.37	5.519	0.313	310.11	3.001	0.681	2.957	0.300	2.36	0.989
±SD	85.22	34.01	8.152	0.077	38.70	3.970	0.231	4.640	0.201	0.74	0.015
Computer estimated mean	177.89	146.55	2.677	0.307	297.12	1.379	0.591	1.013	0.259	2.26	0.995
± Confidence limits	—	—	—	—	104.70	0.381	0.382	1.789	—	—	—

^a The data of monkey #3 are partially missing and hence not included.

^b A, B and C₀ are ng ml⁻¹.

^c All rate constants h⁻¹.

^d r = correlation coefficient.

while in the dog K_{12} appears larger than K_{21} . The dog has a higher excretion rate constant. Both species have similar plasma half-lives in the β phase.

It should be pointed out that volumes of distribution are mathematical artifacts that usually are referred to as 'apparent' or 'effective' volumes to indicate this fact and to emphasize they have no anatomical significance. Furthermore, a comparison of clearances of phencyclidine in the two species would be meaningful. Obviously, further analysis of the plasma pharmacokinetics of PCP are indicated with earlier and more frequent time points in both species. The reliability of the first order multicompartmental model

should also be tested by varying the dose administered. The methods employed herein do indicate the remarkable utility of GCMS techniques in analyzing this problem further.

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