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# Toxicokinetics, including saturable protein binding, of 4-chloro-2-methyl phenoxyacetic acid (MCPA) in patients with acute poisoning

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ABSTRACT

Human data on protein binding and dose-dependent changes in toxicokinetics for MCPA are very limited. 128 blood samples were obtained in 49 patients with acute MCPA poisoning and total and unbound concentrations of MCPA were determined. The Scatchard plot was biphasic suggesting protein binding to two sites. The free MCPA concentration increased when the total concentration exceeded 239 mg/L (95% confidence interval 198–274 mg/L). Nonlinear regression using a two-site binding hyperbola model estimated saturation of the high affinity binding site at 115 mg/L (95%CI 0–304). Further analyses using global fitting of serial data and adjusting for the concentration of albumin predicted similar concentrations for saturable binding (184 mg/L and 167 mg/L, respectively) without narrowing the 95%CI. In 25 patients, the plasma concentration–time curves for both bound and unbound MCPA were approximately log-linear which may suggest first order elimination, although sampling was infrequent so zero order elimination cannot be excluded. Using a cut-off concentration of 200 mg/L, the half-life of MCPA at higher concentrations was 25.5 h (95%CI 15.0–83.0 h; $n = 16$ patients) compared to 16.8 h (95%CI 13.6–22.2 h; $n = 10$ patients) at lower concentrations. MCPA is subject to saturable protein binding but the influence on half-life appears marginal.



## 1. Introduction

The chlorophenoxy compounds 4-chloro-2-methylphenoxyacetic acid (MCPA) and 2,4-dichlorophenoxyacetic acid (2,4-D) are selective herbicides used in agricultural and household sectors worldwide. 2,4-D is the most commonly used chlorophenoxy herbicide in the US (Kiely et al., 2004) and acute self-poisoning with MCPA is a common reason for presentation to rural hospitals in Sri Lanka where subsistence farming is common (Roberts et al., 2005). Severe poisoning including coma, rhabdomyolysis and renal toxicity may occur and persist for some days. Death occurs in around 5% of patients and is typically 24–48 h post-ingestion (Roberts et al., 2005). The mechanism of fatal toxicity has not been defined (Roberts et al., 2005). Animal studies have also suggested that prolonged elimination of chlorophenoxy herbicides leads to increased toxicity (Timchalk, 2004). Further, saturation of protein binding increases the free (unbound) concentration of the poison, which is then available to distribute from the plasma (central) compartment. In the case of chlorophenoxy compounds, this is important because the mechanism of toxicity is thought to relate to disruption of intracellular processes (Roberts and Buckley, 2007a).

The treatment of acute chlorophenoxy herbicide poisoning consists of decontamination of the gastrointestinal tract, resuscitation and supportive care. Treatments that increase herbicide clearance have been proposed including urinary alkalinisation (which increases renal clearance by 'ion-trapping') and haemodialysis (Bradberry et al., 2004). The toxicokinetics of the chlorophenoxy

*Abbreviations:* MCPA, 4-chloro-2-methylphenoxyacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; $t_{1/2}$, apparent elimination half-life; Cu, free (unbound) plasma concentration; $Kd_i$, affinity constant of binding at the $i$th site; $Bmax_i$, maximum density (concentration of saturation) of binding at the $i$th site; $C_i$, initial concentration; $C_t$, concentration after time $t$; $k$, elimination rate constant; LOD, limit of detection; LOR, limit of reporting; Tmax, time of the maximum plasma concentration; IQR, interquartile range; Koc, octanol solubility coefficient; $pK_a$, acid dissociation constant; CL, clearance; Vd, volume of distribution.

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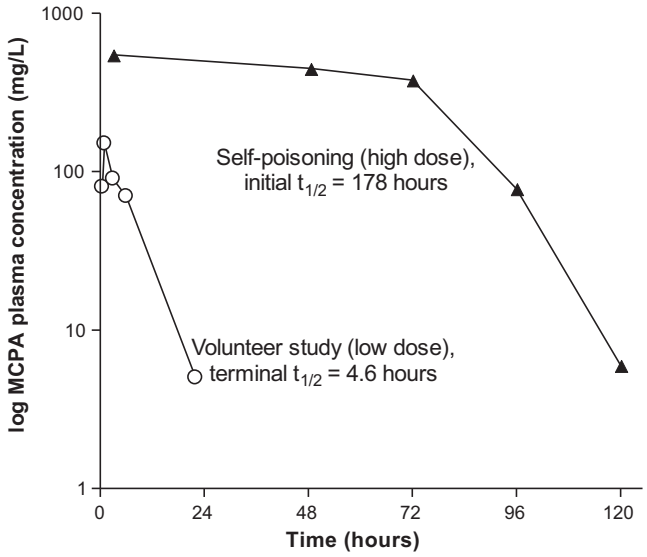


**Fig. 1.** Semi-logarithmic plasma MCPA concentration–time using data from the literature: a case report of acute self-poisoning (Schmoldt et al., 1997) and a low-dose volunteer study (Kolmodin-Hedman et al., 1983).

herbicides must be known to determine or interpret the effect of such interventions.

Animal studies of acute chlorophenoxy exposures demonstrate non-linear kinetics with high exposures due to dose-dependent changes in distribution and clearance for all herbicides within this group (Arnold and Beasley, 1989). MCPA is subject to dose-dependent saturation of protein binding *in vitro* (Roberts and Buckley, 2007a). While there is a prolonged apparent elimination half-life ($t_{1/2}$) in animals with larger exposures it is unclear if this reflects decreased clearance or increased volume of distribution and whether the total and free concentrations are moving in tandem (Arnold and Beasley, 1989; Roberts and Buckley, 2007a; Roberts et al., 2005). It is necessary to better understand the dose-dependent kinetics in order to interpret changes after treatments that aim to increase clearance.

Only two publications have described the kinetics of MCPA in humans, one was a single case of intentional self-poisoning (Schmoldt et al., 1997) and the other was a low-dose volunteer study (Kolmodin-Hedman et al., 1983). Comparison of the apparent elimination $t_{1/2}$ from these reports may indicate that MCPA exhibits dose-dependent elimination (Fig. 1). The authors of this case report attributed the decrease in apparent half-life to treatment with alkaline diuresis (Schmoldt et al., 1997). However, a change in clearance was not directly quantified and dose-dependent changes in kinetics may explain the profile observed.

Details on the kinetics of MCPA are, therefore, of interest to guide research into the clinical management of acute poisoning. In particular, if the elimination of MCPA is confirmed to be prolonged in acute poisoning this will support research into treatments that enhance elimination. If the unbound concentrations are high this would indicate that haemodialysis might be effective. Here, we describe the plasma kinetics of MCPA in patients with acute intentional self-poisoning.

## 2. Materials and methods

### 2.1. Clinical

This is an observational study. Patients were identified by on-site study doctors on presentation to Anuradhapura or Polonnaruwa Hospitals with a history of acute poisoning. These hospitals provide 24-h medical and nursing care to patients. Patients were regularly reviewed and clinical details were recorded prospectively by on-site study doctors until discharge or death. All patients received supportive care which included supplemental oxygen, intravenous fluids, ventilatory and haemodynamic support as required. Antibiotics (usually penicillin and metronidazole) were given when aspiration pneumonitis was suspected clinically. There were no treatments involving urinary alkalinisation or haemodialysis due to resource limitations in the region.

Written informed consent was obtained in all patients who participated in this study. An admission blood sample was provided by patients followed by serial samples at 1, 4, 12, and 24 h, then once daily until discharge or death, as allowed by clinical factors. Blood was collected into an EDTA tube which was promptly centrifuged and the plasma was removed and frozen at −23 °C until the time of analysis.

Ethics approval for this observational study was obtained from Sri Lanka (the Universities of Colombo, Peradeniya and Sri Lankan Medical Association) and the grantholder's universities (Oxfordshire Clinical Research Ethics Committee (UK) and Australian National University).

### 2.2. Laboratory

The total and free (unbound) concentrations of MCPA were quantified in the samples collected above in addition to admission samples collected for a previous study (Roberts et al., 2005). The total MCPA concentration was measured in the above-mentioned plasma samples. 300 μL of plasma was then ultrafiltered using Millipore Centrifree Micropartition Device® (Millipore, Bedford, MA, USA) yielding approximately 100 μg of plasma ultrafiltrate. The concentration of MCPA in the ultrafiltrate is the free (unbound) concentration.

The concentrations of MCPA were determined by Queensland Health Scientific Services (Australia) using a method derived from that of the United States Environmental Protection Agency (EPA, 1980). 100 μg of plasma or ultrafiltrate was hydrolysed in diluted sodium hydroxide and then buffered with acetic acid. The concentration of MCPA was determined by HPLC–MS/MS using an AB/Sciex API4000Q mass spectrometer in the negative ion mode equipped with an electrospray (TurboV) interface. This was coupled to a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan) and a 50 mm × 2 mm C6-phenyl column (Phenomenex, Torrance, CA). The limit of reporting for the LCMSMS method was 1 μg/L for MCPA and the method was linear from at least 1–300 μg/L.

Method recovery was confirmed using MPCA concentrations of 2.5 mg/L to around 300 mg/L with an average recovery of 105% and a standard deviation 0.25. Therefore, the limit of detection (LOD; 3× standard deviation) is 0.75 mg/kg and the limit of reporting (LOR; using 6× LOD) is 4.5 mg/kg. The resulting concentrations (mg/kg plasma) were multiplied by 1.0205 which is the specific gravity of plasma at 37 °C (Trudnowski and Rico, 1974) to allow reporting with the unit mg/L.

To validate the Centrifree® ultrafiltration device, plasma from a patient with MCPA poisoning was ultrafiltered, analysed for MCPA, re-ultrafiltered and then re-analysed for MCPA. There was no change in the concentration of MCPA between the 2 ultrafiltrates so MCPA does not appear to be adsorbed to the ultrafiltration device. Further, control plasma which did not contain MCPA was ultrafiltered and the filtrate was analysed for protein. It was noted that <0.2% of protein remained, suggesting that the Centrifree® ultrafiltration device was efficient for removing protein from plasma samples.

The albumin concentration was determined in all admission samples and the concentration of albumin and creatinine was determined in all serial samples. These analyses were conducted by Queensland Health Forensic and Scientific Services at Princess Alexandra Hospital, Brisbane, Australia. This service is accredited by the National Association of Testing Authorities, Australia and certified to International Standards (ISO 9001).

## 3. Calculations

The MCPA concentration–time profile in patients providing the most serial samples was constructed using the total and free MCPA concentrations. A plot of the free versus total MCPA concentration was then constructed using data from all admission and serial plasma samples to determine whether protein binding was saturable and the approximate concentration at which this occurred.

The bound MCPA concentration was calculated as the difference between the free and total concentration at each time point. A Scatchard plot was constructed using the bound and free MCPA concentrations to estimate the number of apparent protein binding sites. Here, following visual inspection, a one-phase (linear) relationship suggests one-site binding, a two-phase relationship suggests two-site binding, and so on (Kermode, 1989; Molinoff et al., 1981; Motulsky and Christopoulos, 2005). In the case of two-site binding the relationship between free and bound concentrations is quantified by nonlinear regression using a two-site