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The comparative toxicology of 4-chloro-2-methylphenoxyacetic acid and its plant metabolite 4-chloro-2-carboxyphenoxyacetic acid in rats

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Abstract

4-Chloro-2-carboxyphenoxyacetic acid (CCPA) residues have occasionally been observed in crops treated with 4-chloro-2-methylphenoxyacetic acid (MCPA). The oral toxicity of MCPA and CCPA was compared in a 4-week rat study at a dietary concentration of 2000 ppm. CCPA was also given at 12,000 ppm (equivalent to 1 g/kg bodyweight/day). MCPA at 2000 ppm caused reduced food consumption and body weight gain and increased water consumption in females only. Changes in clinical chemistry confirmed the liver as a target organ. Increased serum creatinine and urobilinogen, degenerated transitional epithelial cells in the urine showed that the kidney was also affected. Response to CCPA was confined to the 12,000 ppm dose. The target organs were liver and kidney as for MCPA. Microscopic examination revealed an increased severity of basophilic tubules and calcification at the outer/inner medulla transition in the kidneys. The results demonstrate that CCPA is less toxic than MCPA, that CCPA has no different toxicological end points when compared to MCPA, and that any risks associated with consumption of CCPA will not be underestimated if the CCPA residue is treated as if it were parent MCPA. Based on the MCPA–CCPA comparison, criteria for read across and minimal information requirements are proposed.

Keywords: 4-Chloro-2-methylphenoxyacetic acid; 4-Chloro-2-carboxyphenoxyacetic acid; MCPA; CCPA; Rat

1. Introduction

4-Chloro-2-methylphenoxyacetic acid (MCPA, CAS No. 94-74-6) is a systemic foliar herbicide used to control annual and perennial weeds in small grains, grassland, and turf. MCPA is usually formulated as either an aqueous salt (e.g., dimethylamine, DMA or sodium salts) or as an ester (e.g., 2-ethylhexyl, 2-EHE). Formulations of MCPA are registered world-wide for use on agricultural crops such as cereals, grasses and pulses, and in non-crop areas.

The chronic toxicity of MCPA (Fig. 1) has been evaluated in Wistar rats at target doses of 20, 80, and 320 ppm for 2 years (Bellet et al., 1999). Chronic effects, namely elevations in triglycerides and serum glutamic transaminase activity, were noted in male and/or female rats in the 80- and 320-ppm dose groups. Nephrotoxicity was observed in male rats in the 320-ppm dose group. MCPA is not extensively metabolised and urine is the predominant route of excretion (van Ravenzwaay et al., 2004). A hydroxylated metabolite, 4-chloro-2-hydroxymethylphenoxyacetic acid (HMCPA, Fig. 1), was found at low levels, together with its glycine conjugate. These metabolites were more prominent shortly after dosing, suggesting that MCPA is not retained in the liver and that these metabolites may be excreted faster than MCPA itself. Tissue burdens were also consistent with wide distribution of MCPA in body water and subsequent rapid elimination. Following single or multiple oral administration of ^{14}C-MCPA to male and female

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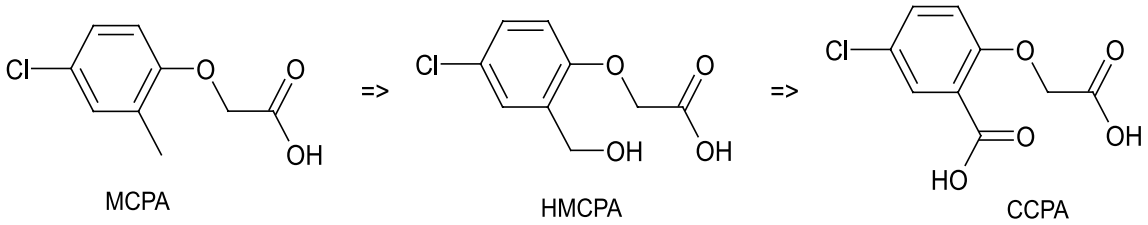

Fig. 1. Chemical structures of MCPA, HMCPA, and CCPA.

rats at 5 mg/kg, quantitative recovery of radioactivity was obtained within 168 h (van Ravenzwaay et al., 2004).

Low levels of 4-chloro-2-carboxyphenoxyacetic acid (CCPA) have occasionally been observed in crops treated with MCPA. CCPA formation has been observed only in plants and most likely results from a further hydroxylation of HMCPA (Fig. 1). Because CCPA is not a mammalian metabolite, at least in the species use for testing, a separate risk assessment must be considered.

CCPA was negative in bacterial reverse mutation tests using *Salmonella typhimurium* and *Escherichia coli* test systems (Mecci, 2001). An acute oral toxicity study in rats with CCPA showed no mortality in rats at the limit dose of 2000 mg/kg bw (Kern, 1999), contrasting with an acute oral LD_{50} in rats of 1000–1500 mg/kg bw for the parent molecule MCPA. The majority of the toxicological changes observed after continuous administration of MCPA were either directly or indirectly related to effects on the kidney (and to a lesser extent to effects on the liver). As CCPA has higher water solubility than MCPA, it is expected to be more rapidly eliminated via the urine and thus, it was anticipated that CCPA would also be less toxic than MCPA in repeated dose studies. In order to investigate this hypothesis a comparative dietary 28-day study in rats with MCPA and CCPA in compliance with the standard OECD 407 guideline was performed. A single high dose level of MCPA (2000 ppm) was chosen to demonstrate target organ toxicity and the effects were compared to the toxicity of CCPA at the same level and at 12,000 ppm in the diet, a dose equivalent to 1 g/kg/day. This latter dose is often considered a limit dose for minor food additives/contaminants and is also a limit dose in regulatory toxicology testing for chemicals.

In addition to the direct comparison of CCPA and MCPA, the results of this study were also considered to provide a potential structure for read-across procedures for similar cases. Within the context of increased information requirements for industrial chemicals such as proposed under the REACH (registration, evaluation, and assessment of chemicals) framework in the EU efficient ways of providing the necessary information for risk assessments, while avoiding excessive use of laboratory animals must be developed. Read across to chemicals with a rich information data basis may provide an efficient means for hazard identification if this procedure is carried out in a transparent and scientifically credible way.

2. Materials and methods

2.1. Test substances

4-Chloro-2-methylphenoxyacetic acid (MCPA, CAS No. 94-74-6), purity 97.0% (HPLC), stored at room temperature, stable until November 2006. 4-Chloro-2-carboxyphenoxyacetic acid (CCPA), purity 99.7% (HPLC), stored in a refrigerator under exclusion of light, stable until May 2005. Test substance analyses were carried out at the Ecology and Environmental Analytics Department of the BASF Agricultural Center Limburgerhof, Germany.

2.2. Animals and maintenance conditions

Male and female Wistar rats (CrlGlxBrlHan:WI) were supplied by Charles River Deutschland GmbH, 97633 Sulzfeld, Germany at the age of 35 ± 1 days (males) and 33 ± 1 days (females). The animals were singly housed in stainless steel wire mesh cages (type DK III, floor area 800 cm^2), supplied by Becker, Castrop-Rauxel, Germany. Waste trays were fixed underneath the cages, containing bedding material (type 3/4 dust free embedding, supplied by SSNIFF, Soest, Germany). Motor activity measurements were conducted in polycarbonate cages (floor area 800 cm^2) with wire covers, supplied by Ehret, Emmendingen, Germany. The animals were maintained in an air-conditioned room at a temperature of 20–24 °C, a relative humidity of 30–70%, and a 12 h light/12 h dark cycle. Before the animals' arrival, the room was completely disinfected using a disinfector ("AUTEX," fully automatic, formalin–ammonia-based terminal disinfector, supplied by Dr. Gruß KG, Neuss, Germany). During the study, the floor and walls were cleaned weekly with a solution of 0.1% Incidin perfect (supplied by Henkel, Düsseldorf, Germany) in water. The animals were maintained on a rat/mouse maintenance diet (GLP meal supplied by Provimi Kliba SA, Kaiseraugst, Switzerland), and tap water, ad libitum. Food was assayed for chemical as well as for microbiological contaminants. Drinking water was regularly