MCPA

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Explanation

MCPA is the International Organization for Standardization (ISO)–approved common name for 4-chloro-*o*-tolyloxyacetic acid (International Union of Pure and Applied Chemistry) (Chemical Abstracts Service [CAS] No. 94-76-6). MCPA is a selective, systemic, hormone-type herbicide belonging to the phenoxyacetic acid family. It is used to control annual and perennial weeds in cereals, grassland and turf.

MCPA has not previously been evaluated by the Joint FAO/WHO Meeting on Pesticide Residues and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

There are several different formulations of MCPA, including the 2-ethylhexyl ester (2-EHE) (CAS No. 29450-45-1) and dimethylamine (DMA) (CAS No. 2039-46-5) salt of MCPA. Data on these formulations and, where appropriate, data on the sodium and potassium salts are included in this monograph, because both convert to MCPA ion in the mammalian digestive tract prior to absorption.

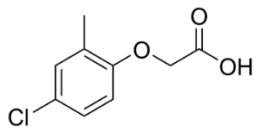
On this basis, doses of MCPA 2-EHE and MCPA DMA administered to laboratory animals are expressed as MCPA acid equivalents, calculated using their molecular weights (312.5 and 245.7, respectively) relative to the parent MCPA (200.6).

Unless otherwise stated, the contemporary unpublished studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and that complied, where appropriate, with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the European Union or United States Environmental Protection Agency. As these guidelines specify the clinical pathology tests normally performed and the tissues normally examined, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters. The studies in humans were conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki, or equivalent statements prepared for use by national and/or multinational authorities.

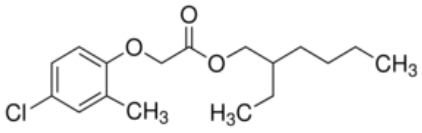
The chemical structures of MCPA, MCPA 2-EHE and MCPA DMA are given in Figure 1.

Figure 1. Chemical structures of MCPA, MCPA 2-EHA and MCPA DMA

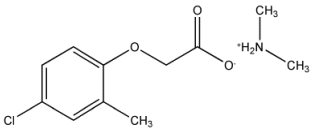
MCPA



MCPA 2-EHE



MCPA DMA



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

Rats

In a study predating GLP principles and contemporary test guidelines, phenyl-labelled [^{14}C]MCPA (radiochemical purity 97%) in 1% weight per volume (w/v) carboxymethylcellulose was administered by gavage to groups of fasted male and female Sprague-Dawley (SD) rats (CD strain). Following a single gavage [^{14}C]MCPA dose of 100 mg/kg body weight (bw) (three rats of each sex), radioactivity was excreted predominantly in urine (approximately 95% and 86% of the administered dose in males and females, respectively), with relatively low levels eliminated in faeces (6.2% and 5.6% in males and females, respectively). The majority of this radioactivity was eliminated within 48 hours of dosing (approximately 78% in urine and 5% in faeces), with the remainder excreted from 48 to 192 hours. The urine of two rats of each sex that contained the highest levels of radioactivity was subjected to metabolite analysis using thin-layer chromatography (TLC). The main metabolites were MCPA (approximately 71%) and one other undefined fraction (approximately 14%). Three minor fractions were also detected (approximately 5%, 4% and 3%, respectively).

The excretion of radioactivity in bile, urine and faeces was analysed in three male bile duct–cannulated rats following a single gavage [^{14}C]MCPA dose of 100 mg/kg bw. Recovery of radioactivity was low (mean of 34.5%), which the authors attributed to the poor condition of the animals. The mean levels of radioactivity in 0- to 24-hour bile, urine and faeces were 3.7%, 20.3% and 10.5%, respectively.

In a tissue distribution experiment, 14 rats of each sex received a single gavage dose of [^{14}C]MCPA at 100 mg/kg bw, with two rats of each sex sacrificed at various times to 192 hours for the analysis of radioactivity in plasma and tissues. The highest plasma and tissue concentrations of radioactivity were detected at the earliest sampling time (3 hours) and declined thereafter. At 3 and 6 hours after dosing, plasma contained the highest concentration of radioactivity. At later sampling times, the carcass, skin, fat and adrenals had higher levels of radioactivity compared with plasma. In a

separate experiment, whole-body autoradiography of three rats of each sex that received a single gavage dose of [^{14}C]MCPA at 100 mg/kg bw and were analysed at 6, 24 and 48 hours (one rat of each sex) confirmed this pattern of tissue distribution of radioactivity. In a further tissue distribution experiment, 10 rats of each sex received up to 14 consecutive daily gavage doses of [^{14}C]MCPA at 1 mg/kg bw. Two rats of each sex were sacrificed on days 1, 5, 10, 14 and 18. The pattern of uptake, tissue distribution and elimination of radioactivity was similar to that observed in the preceding single-dose studies (Gilbert & Hopkins, 1978).

In a published study by Tynnelä, Elo & Ylatilo (1990), MCPA (purity > 98%) in propylene glycol was administered to three or four fasted male SD rats as a single gavage dose of 475 mg/kg bw. Separate groups of rats were dosed concurrently with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) at 238 or 475 mg/kg bw or 2,4-dichlorophenoxyacetic acid (2,4-D) at 300 mg/kg bw. Three hours after dosing, rats received a single intravenous injection of phenyl-labelled [^{14}C]MCPA. One hour later, blood and cerebrospinal fluid were collected, and the brain was dissected for the analysis of radioactivity by liquid scintillation counting (LSC). Plasma protein binding was also analysed. Lethargy and myotonia were observed following dosing with MCPA. Radioactivity was detected in blood, cerebrospinal fluid and all analysed brain regions (cerebral cortex, striatum, medulla oblongata, cerebellum and midbrain). The tissue to plasma ratio of radioactivity was approximately 1:8. Binding of MCPA to plasma proteins was observed (4–43% protein-unbound fraction).

Phenyl-labelled [^{14}C]MCPA (radiochemical purity 99.3%) was administered to male and female Wistar rats (Crl:(WI)BR strain) (five of each sex per group) as a single gavage dose of 5 or 100 mg/kg bw in 1% w/v carboxymethylcellulose. A separate group of rats (five of each sex per group) received 14 daily gavage doses of unlabelled MCPA (purity 99.7%) at 5 mg/kg bw followed by a single gavage dose of [^{14}C]MCPA at 5 mg/kg bw. Rats were fasted for 4 hours prior to and after dosing. Urine and faeces were collected at various times to 168 hours after administration of the radiolabelled dose. Rats were sacrificed at 96 hours (5 mg/kg bw dose) or 168 hours (100 mg/kg bw dose) after dosing, and various tissues were sampled. Radioactivity was analysed in excreta and tissues by LSC. Metabolites of MCPA were analysed in urine and faeces by TLC, high-performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC-MS) using appropriate standards; samples were analysed with and without acid and alkaline hydrolysis and deconjugation with β-glucuronidase and arylsulfatase. In a pharmacokinetics experiment, rats received a single gavage dose of [^{14}C]MCPA at 5 or 100 mg/kg bw, and blood was collected at various times to 168 hours and then analysed for radioactivity; standard pharmacokinetic parameters were calculated for total radioactivity. In an additional tissue distribution experiment, groups of 12 rats of each sex received a single gavage dose of [^{14}C]MCPA at 5 mg/kg bw; at 1, 3 and 6 hours after dosing, four rats of each sex were sacrificed, and various tissues were analysed for radioactivity.

No signs of toxicity were reported.

The results of the excretion/mass balance experiment are summarized in Table 1. Recovery of radioactivity was greater than 95%. Irrespective of the dose or dosing regimen, the kidneys were the main route of excretion and accounted for greater than 93% of the administered dose in males and greater than 88% of the administered dose in females (urine plus cage wash); the majority of excretion occurred with 24 hours of dosing. Based on the level of radioactivity in urine and the cage wash, gastrointestinal absorption is estimated to be at least 95%. The faeces were a minor route of elimination, becoming more prominent in high-dose females, for which faecal excretion attained 20% of the administered dose in two of five rats. During a pilot study involving two male rats from the repeated-dose group, no radioactivity was detected in expired air.

The distribution of radioactivity in selected tissues of rats sacrificed at the end of the excretion/mass balance study (96 or 168 hours) is summarized in Table 2. The highest level of radioactivity was detected in fat, followed by the skin and carcass; the level of residual radioactivity in the carcass was less than 0.8% of the administered dose. The slight retention of radioactivity in the

ovaries and uterus of high-dose females was assumed by the study authors to be associated with visceral fat. Radioactivity was not detected in bone, brain, heart, lung, spleen, stomach contents or the thyroid.

Table 1. Mass balance in rats following oral dosing with [^{14}C]MCPA

Sample	Mean % of administered radioactive dose					
	5 mg/kg bw (single dose)		100 mg/kg bw (single dose)		5 mg/kg bw (repeated dose)	
	Males	Females	Males	Females	Males	Females
Urine						
- 6 h	32.9	24.8	16.9	8.1	27.9	36.1
- 12 h	39.5	39.9	43.6	37.1	35.7	31.2
- 24 h	3.2	8.9	19.6	16.8	13.6	9.7
- 48 h	1.0	1.8	3.9	7.4	1.9	1.7
- 72 h	0.2	0.3	1.2	1.9	0.3	0.3
- 96 h	0.1	0.2	0.6	1.2	0.1	0.1
- 120 h	—	—	0.3	0.5	—	—
- 144 h	—	—	0.2	0.3	—	—
- 168 h	—	—	0.2	0.3	—	—
Urine total	76.9	75.8	86.4	73.5	79.5	79.8
Cage wash	16.2	19.3	10.5	13.8	17.5	24.5
Urine + cage wash	93.3	95.4	97.2	88.3	97.4	104.6
Faeces	2.0	2.3	4.0	11.9	4.9	3.8
Total	95.1	97.7	101.1	100.1	102.3	109.0

From Jahanshahi & Stow (1995)

—, not analysed

Table 2. Tissue distribution of radioactivity in rats following oral dosing with [^{14}C]MCPA

Tissue	Mean concentration (µg [^{14}C]MCPA Eq/g tissue)					
	5 mg/kg bw (single dose)		100 mg/kg bw (single dose)		5 mg/kg bw (repeat dose)	
	Males	Females	Males	Females	Males	Females
Carcass	0.037	0.042	0.839	1.931	0.069	0.056
Skin	0.074	0.091	2.374	3.647	0.070	0.055
Plasma	0.002	0.005	0.345	0.923	0.005	ND
Blood	ND	ND	0.129	0.477	0.226	0.014
Fat	0.074	0.107	7.108	16.32	0.025	0.033
Skeletal muscle	ND	ND	0.083	0.059	ND	ND
Liver	0.026	ND	0.596	0.385	0.048	ND
Kidney	0.036	0.034	0.637	1.718	0.049	0.032
Stomach	ND	ND	0.109	0.506	0.021	ND
Gonads	ND	0.031	0.099	8.198	ND	ND
Uterus	NA	0.008	NA	6.164	NA	ND
Adrenals	ND	ND	ND	0.732	ND	ND

From Jahanshahi & Stow (1995)

Eq, equivalent; NA, not applicable; ND, not detected

Toxicokinetic parameters are summarized in Table 3. Maximum plasma concentrations (C_{max}) of radioactivity were achieved by about 2.5 hours (T_{max}), taking somewhat longer in high-dose females (approximately 4 hours). Plasma elimination half-lives were about 10–20 hours in males and about 20–30 hours in females; these values are discordant with the mass balance data indicating that the majority of radioactivity was excreted in the urine by 12 hours (Table 1). At both doses, the majority of plasma radioactivity showed an initial "rapid" decline to 24 or 48 hours (5 and 100 mg/kg bw, respectively), with subsequent levels declining more slowly to 168 hours. At both doses, a small number of rats still had detectable levels of radioactivity in plasma at the end of the experiment.

Table 3. Toxicokinetic parameters in rats following a single oral dose of [^{14}C]MCPA

Parameter	Mean values[a]			
	5 mg/kg bw		100 mg/kg bw	
	Males	Females	Males	Females
C_{max} (µg Eq/g)	26.8	33.2	373.1 (14)	357.8 (11)
T_{max} (h)	2.7	2.4	2.4	4.2
$t_{½}$ (h)	19.2	34.1	10.6	20.9
$AUC_{(0–\infty)}$ (µg Eq·h/g)	210	250	5180 (25)	5628 (23)

From Jahanshahi & Stow (1995)

AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}

[a] Values in parentheses represent the overall fold increase relative to the low dose.

In the additional tissue distribution experiment, radioactivity was detected in all analysed tissues at 1, 3 and 6 hours after a single dose of [^{14}C]MCPA at 5 mg/kg bw. The concentration of radioactivity in all tissues declined over time. With the exception of the stomach, stomach contents and kidneys, the concentration of radioactivity in all tissues was lower than that in plasma and/or blood. Other than the ovaries and testes, there were no apparent sex-related differences in the distribution pattern of radioactivity.

Analysis of pooled urine (0–6, 6–12 and 12–24 hours) from the excretion/mass balance experiment by TLC detected parent MCPA and 4-chloro-2-hydroxymethyl-phenoxyacetic acid (HMCPA). At the low single or repeated dose, urinary MCPA accounted for about 20–31%, 28–37% and 3–12% (51–80% in total) of the administered dose at 0–6, 6–12 and 12–24 hours, respectively. At this same dose, HMCPA accounted for approximately 4–7%, 2–7% and 0.2–2% (6–16% in total) of the administered dose at 0–6, 6–12 and 12–24 hours, respectively. At the high single [^{14}C]MCPA dose of 100 mg/kg bw, a somewhat larger proportion of urinary radioactivity was attributable to HMCPA at 0–6 hours, with a concomitant reduction in the proportion of MCPA. However, at 6–12 and 12–24 hours, the proportion of MCPA/HMCPA was consistent with that seen at 5 mg/kg bw. HPLC confirmed the proportions of MCPA and HMCPA in urine.

Some urine samples contained low levels of two minor unidentified metabolites (< 0.5% of the administered dose). Pretreatment of urine samples with β-glucuronidase or arylsulfatase prior to TLC or HPLC did not lead to the detection of glucuronide or sulfate conjugates of MCPA or its metabolites. Acid pretreatment of urine samples yielded a non-polar region coincident with anisole and reduced the level of HMCPA while increasing that of MCPA. Pretreatment of urine samples with alkali did not lead to the detection of any alkali-sensitive conjugates.

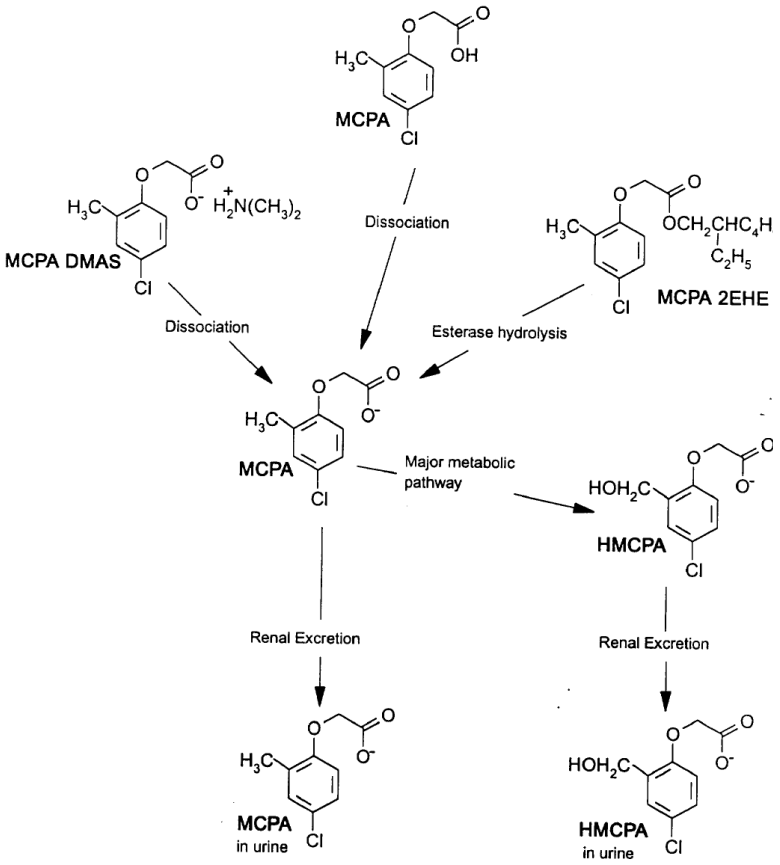
TLC of pooled (0- to 24-hour) faecal samples collected from rats that received a single 100 mg/kg bw dose of [^{14}C]MCPA identified MCPA and HMCPA (approximately 2% and 0.5% of the administered dose, respectively, in males; approximately 7% and 1.3% of the administered dose, respectively, in females). Three other regions representing unknown species were also reported. HPLC of these same samples did not confirm the presence of HMCPA; the most abundant region was

an unknown species (approximately 2% of the administered dose in males and 8.3% in females), followed by MCPA (approximately 1.1% of the administered dose in males and 2.2% in females). Some changes in the distribution of radioactivity were noted following pretreatment of faecal samples with β-glucuronidase or arylsulfatase, acid or alkali, but no definitive conclusions could be made by the study authors on the nature of these species.

Analysis of urine and faeces by LC-MS confirmed the presence of MCPA and HMCPA. In urine, a glycine conjugate of MCPA was identified, whereas two minor unidentified regions were also detected in faeces (Jahanshahi & Stow, 1995).

The proposed metabolic pathway of MCPA in rats is presented in Figure 2. MCPA is metabolically relatively stable but can be metabolized to HMCPA (via the C-oxidation of the 2-methyl moiety of MCPA) or by glycine conjugation.

Figure 2. Proposed metabolic pathways of MCPA, MCPA 2-EHE and MCPA DMA in rats



MCPA (purity 97.0%) was admixed in the diet at a concentration of 125, 250, 500, 1000, 1500 or 2200 parts per million (ppm) and offered to groups of four male Wistar rats (CrlGlxBrlHan:WI strain) for 14 days (equivalent to nominal doses of 10, 20, 40, 80, 120 and 180 mg/kg bw per day, respectively). On day 15, the diet was replaced with untreated diet, and rats were administered a single gavage dose of phenyl-labelled [^{14}C]MCPA (radiochemical purity 98.3%) in aqueous 0.5% w/v carboxymethylcellulose at an equivalent dose. Blood was sampled at 1, 2, 4, 8, 12, 24 and 48 hours after dosing and analysed for radioactivity by LSC; standard toxicokinetic parameters were determined. Rats were offered diets containing unlabelled MCPA from 24 to 48 hours.

There were no treatment-related clinical signs. The body weight gain of high-dose rats was about 45–75% lower than that of all other groups from day 0 to day 14. With the exception of the

high-dose diet, achieved doses were higher than the nominal doses during weeks 1 and 2 (17–54% and 14–24% higher, respectively).

Toxicokinetic parameters are summarized in Table 4. At 120 and 180 mg/kg bw, the time to C_{max} (T_{max}) was 2–8 times higher than at lower doses, which, in association with the under-proportional increase in C_{max} from 120 to 180 mg/kg bw, led the authors to suggest that gastrointestinal absorption might be delayed at these higher doses. Alternatively, the higher T_{max} at 120 and 180 mg/kg bw might reflect the achievement of equilibrium between absorption and excretion. Following attainment of C_{max}, radioactivity showed a continuous decline over time, with three different phases identified by the authors at every dose: phase 1—mixed absorption/excretion phase (approximately 0–12 hours); phase 2—excretory phase (12–24 hours); and phase 3—mixed absorption/excretion, where rats were redosed with non-labelled MCPA (24–48 hours). Up to 80 mg/kg bw, there was a generally linear increase in the area under the plasma concentration–time curve (AUC). From 120 to 180 mg/kg bw, the AUC increased by a greater proportion than the dose increment, particularly at 12–24 hours (the excretory phase), suggesting saturation of excretion. Initial plasma half-lives calculated from plasma concentration–time curves during phase 2 were consistent from 10 to 80 mg/kg bw, but thereafter increased markedly. While not a reliable indicator of the saturation of excretion, these initial half-lives support the AUC results—namely, that excretion is saturated at doses greater than 80 mg/kg bw. Plasma concentrations of radioactivity at 24 hours after dosing (which can be taken to represent the carry-over to the next dosing day) increased by a greater proportion than the dose increment at and above 80 mg/kg bw. The carry-over at 120 and 180 mg/kg bw was relatively large and would likely lead to an increased body burden on subsequent treatment days. The kinetics suggested that this phenomenon would be progressive over repeated dosing regimens at high oral doses. On the basis of these findings, the authors concluded that doses greater than 80 mg/kg bw per day may result in progressive toxicity in rats (Beimborn & Leibold, 2003a).

Table 4. Toxicokinetic parameters in rats following repeated dietary exposure to MCPA followed by a single dose of [^{14}C]MCPA

Parameter	Mean values[a]					
	10 mg/kg bw	20 mg/kg bw	40 mg/kg bw	80 mg/kg bw	120 mg/kg bw	180 mg/kg bw
T_{max} (h)[b]	1	2	4	2	8	8
C_{max} (µg Eq/g)	39	70 (1.8)	130 (4.3)	201 (5.2)	250 (6.4)	324 (8.3)
$AUC_{0-\infty}$ (µg Eq·h/g)	367	634 (1.7)	1243 (3.4)	2394 (6.5)	5743 (16)	11 185 (30.5)
$AUC_{12-14\ h}$ (µg Eq·h/g)	66	75 (1.1)	145 (2.2)	543 (8.2)	2138 (32.4)	3226 (48.9)
Plasma concentration after 24 h (µg Eq/g)	0.56	0.96 (1.7)	1.76 (3.1)	4.93 (8.8)	120.08 (214)	237.28 (424)
$t_{½}$ (h)						
- phase 1	10.55	13.47	18.46	34.64	—	—
- phase 2	2.89	2.93	2.87	3.08	14.43	35.48
- phase 3	16.63	12.74	15.30	14.95	5.91	13.63

From Beimborn & Leibold (2003a)

AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}

[a] Values in parentheses represent the overall fold increase relative to the low dose.

[b] Observed value.

Groups of 12 time-mated female Wistar rats (CrlGlxBrlHan:WI) were administered daily gavage doses of unlabelled MCPA (purity 97.0%) at 20, 40, 60, 80 or 120 mg/kg bw per day in 0.5% w/v aqueous carboxymethylcellulose from days 6 to 18 post-coitum. On day 19, rats were administered a single gavage dose of phenyl-labelled [^{14}C]MCPA (radiochemical purity 98.3%) in aqueous 0.5% w/v carboxymethylcellulose at an equivalent dose. Three dams per group were sacrificed at 4, 8, 12 and 24 hours after this dose, and blood was collected. Samples of fetal blood (pooled by dam) and amniotic fluid were also taken at these same times. Total radioactivity was analysed by LSC, and the results were used to calculate AUC values.

The concentration of radioactivity in plasma (dams and fetuses) and amniotic fluid was maximal at 4 hours and rapidly declined thereafter. At 80 and 120 mg/kg bw, the maximum concentration of radioactivity was attained at about 8 hours, and the concentration also showed a rapid decline thereafter. In maternal and fetal plasma and amniotic fluid, AUCs indicated two phases of decline: phase 1—mixed absorption/excretion phase (0–12 hours after dosing); and phase 2—excretory phase (12–24 hours after dosing). The concentration of radioactivity in fetal plasma and amniotic fluid was approximately 3- to 7-fold and 4- to 9-fold lower, respectively, than that in maternal plasma. In both fetal plasma and amniotic fluid, $AUC_{4–24\ h}$ increased by greater than the dose increment at and above 40 mg/kg bw, whereas in maternal plasma, the increase was proportional (Table 5) (Beimborn & Leibold, 2003b).

Table 5. $AUC_{4–24\ h}$ values in plasma and amniotic fluid

Parameter	Mean $AUC_{4–24\ h}$ (µg Eq·h/g)[a]				
	20 mg/kg bw	40 mg/kg bw	60 mg/kg bw	80 mg/kg bw	120 mg/kg bw
Maternal plasma	612	1057	1660	2556	3653
		(1.7)	(2.7)	(4.2)	(6)
Fetal plasma	89	202	421	813	1315
		(2.3)	(4.8)	(9.2)	(14.8)
Amniotic fluid	65	162	288	419	820
		(2.5)	(4.4)	(6.4)	(12.6)

From Beimborn & Leibold (2003b)
AUC, area under the plasma concentration–time curve; Eq, equivalent
[a] Values in parentheses represent the overall fold increase relative to the low dose.

In a pharmacokinetics study, phenyl-labelled [^{14}C]MCPA 2-EHE (radiochemical purity 99.6%) or phenyl-labelled [^{14}C]MCPA DMA (radiochemical purity 98.5%) was administered to male Wistar rats (Crl:(WI)BR strain) (five per group) as a single gavage dose of 5 mg/kg bw in 1% w/v carboxymethylcellulose. Blood was sampled at various times to 168 hours after dosing. In an absorption, distribution, metabolism and excretion study conducted under the same conditions, urine and faeces were collected at various times to 168 hours after dosing. Expired air was collected to 48 or 72 hours (MCPA 2-EHE and MCPA DMA, respectively). Rats were sacrificed at 168 hours after dosing, and various tissues were sampled. Radioactivity was quantified in plasma, excreta and tissues by LSC. Metabolites were analysed in urine and faeces by TLC and HPLC, and their identity was confirmed by LC-MS. Samples were also subjected to glucuronidase or aryl sulfatase digestion or to acid hydrolysis.

There were no signs of toxicity, and rats gained body weight normally over the duration of the study. Pharmacokinetic parameters for MCPA 2-EHE and MCPA DMA were comparable (Table 6), with radioactivity detected up to 72 and 48 hours after dosing, respectively. These parameters are consistent with those determined for MCPA in the study by Jahanshahi & Stow (1995).

Table 6. Plasma pharmacokinetic parameters in male rats following a single oral dose of [^{14}C]MCPA 2-EHE or [^{14}C]MCPA DMA

Parameter	Mean value	
	5 mg/kg bw MCPA 2-EHE	5 mg/kg bw MCPA DMA
C_{max} (µg Eq/g)	26	24.1
T_{max} (h)	3.4	2.4
$t_{½}$ (h)	7.7	6.3
$AUC_{0–\infty}$ (µg Eq·h/g)	247.3	215.5

From Burke (1995)
AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}

The results of the excretion/mass balance study are summarized in Table 7. Mean recovery of radioactivity was approximately 102% following dosing with [^{14}C]MCPA 2-EHE and approximately 104% following dosing with [^{14}C]MCPA DMA. Following dosing with either compound, about 80% of the administered radioactivity was detected in urine within 12 hours, and 89% over 168 hours. Based on the levels of radioactivity in urine and the cage wash, gastrointestinal absorption was greater than 99%. The faeces were a minor route of elimination, accounting for approximately 2% and 4% of the administered dose of MCPA 2-EHE and MCPA DMA, respectively. Radioactivity was detected in the faeces of rats for up to 72 hours after dosing with MCPA 2-EHE and for up to 72–168 hours after dosing with MCPA DMA. No radioactivity was detected in expired air.

Table 7. Mass balance in rats following oral dosing with [^{14}C]MCPA 2-EHE or [^{14}C]MCPA DMA

Parameter	Mean % of the administered radioactive dose	
	5 mg/kg bw MCPA 2-EHE	5 mg/kg bw MCPA DMA
Urine		
- 0–6 h	15.5	13.5
- 6–12 h	63.1	67.9
- 12–24 h	7.4	6.3
- 24–48 h	2.2	0.93
- 48–72 h	0.26	0.20
- 72–96 h	0.11	0.10
- 96–120 h	0.050	0.073
- 120–144 h	0.046	0.046
- 144–168 h	0.028	0.038
Urine total	88.72	88.99
Cage wash	11.43	10.85
Urine + cage wash	100.15	99.84
Faeces	1.71	4.101
Carcass	0.33	ND
Total	102.2	104.0

From Burke (1995)
ND, not detected

At 168 hours after dosing, radioactivity was detected in the skin (mean of 0.067 and 0.056 µg Eq/g for MCPA 2-EHE and MCPA DMA, respectively), fat (mean of 0.007 and 0.033 µg Eq/g for MCPA 2-EHE and MCPA DMA, respectively) and kidney (0.010 µg Eq/g for MCPA DMA), but was

below the limit of detection for all other analysed tissues (adrenals, blood, brain, spleen, stomach, muscle, bone, plasma, stomach contents, gonads and heart).

No parent compounds were detected in urine or faeces. The major urinary metabolite was MCPA (approximately 70–79% of the administered dose), followed by HMCPA (approximately 13–15% of the administered dose) and the glycine conjugate of MCPA, which was not quantifiable due to its similar chromatographic properties to MCPA. No glucuronide or sulfate conjugates of MCPA or its metabolites were detected, whereas acid pretreatment did not lead to the detection of any acid-sensitive conjugates. In faeces, HMCPA and MCPA were detected (0.3–3.6% and 0.1–1.5% of the administered dose, respectively). The proposed metabolic pathways for MCPA 2-EHE and MCPA DMA are presented in Figure 2. In the digestive tract, MCPA 2-EHE undergoes ester hydrolysis, whereas MCPA DMA dissociates to yield MCPA ion. Following absorption, MCPA may undergo C-oxidation to yield HMCPA or may be glycine conjugated (Burke, 1995).

In a published study, the renal handling of MCPA was investigated in Wistar rats (Uje:WIST strain) under conditions of forced diuresis (with frusemide, 5 mg/kg bw, intraperitoneal), an oral water load (50 ml/kg bw), sodium bicarbonate administration (50 ml/kg bw, intraperitoneal administration of a 2% solution), when renal tubular transport was blocked (with probenecid, 200 mg/kg bw, intraperitoneal; or *p*-aminohippurate, 2.5 g/kg bw, intraperitoneal) or in the presence of triiodothyronine (T_3) (200 µg/kg bw intraperitoneally for 3 days). In the in vivo phase of the study, [^{14}C]MCPA (unspecified position of label) was mixed with unlabelled MCPA (purity > 99%) at 0.1–0.25% of the total dose in 0.9% sodium chloride and administered as a single intraperitoneal dose of 0.5, 5, 10 or 20 mg/kg bw to six rats per group. Urine was sampled at 1 and 2 hours after dosing for the analysis of MCPA by LSC. Plasma concentrations of MCPA were also analysed from 0.5 to 24 hours following a single intraperitoneal injection of MCPA at 5 or 10 mg/kg bw.

Renal excretion of MCPA was about 50% of the administered dose 5 hours after dosing and about 60% 10 hours after dosing at 0.5–10 mg/kg bw. At the highest dose of MCPA (i.e. 20 mg/kg bw), the level of renal excretion was decreased (approximately 20% of the administered dose). Maximum plasma concentrations of MCPA were 28 and 54 µg/ml at 5 and 10 mg/kg bw, respectively. The plasma half-life was approximately 8–10 hours, whereas a low volume of distribution (17% or 18%) was calculated. At plasma concentrations of MCPA up to 80 µg/ml, binding to plasma albumin was 85%; this high degree of binding would limit the tissue distribution of MCPA. Renal excretion of MCPA was increased 3-fold following the inhibition of tubular reabsorption by an oral water load or bicarbonate administration. An approximately 25% reduction in renal excretion was achieved following simultaneous administration of probenecid or *p*-aminohippurate and MCPA. Repeated administration of T_3 significantly ($P < 0.05$) increased the excretion of MCPA.

In the in vitro phase of the study, the accumulation of MCPA in renal cortical slices was investigated at concentrations ranging from 10^{-8} to 10^{-3} mol/l under aerobic (oxygen) or anaerobic (nitrogen) conditions. The accumulation of MCPA was reduced under anaerobic conditions.

The authors concluded that the observations suggested active tubular transport of MCPA, with renal excretion increased by inhibiting such tubular reabsorption (Bräunlich et al., 1989).

Dogs

Phenyl-ring labelled [^{14}C]MCPA (radiochemical purity 93.8%) was administered to four male Beagle dogs as a single oral dose of 5 mg/kg bw in gelatine capsules. Blood, urine and faeces were sampled at various times to 120 hours after dosing. Radioactivity was quantified by LSC. Urinary and faecal metabolites were analysed by HPLC and LC-MS. The same four dogs were then redosed (after an unspecified washout period) with [^{14}C]MCPA at 100 mg/kg bw under the same experimental conditions.

No toxicological signs were observed during the course of the study.

Excretion/mass balance results are summarized in Table 8. Recovery of radioactivity was approximately 79% and 85% following dosing at 5 and 100 mg/kg bw, respectively. At 5 mg/kg bw,

radioactivity was excreted mainly in the urine, accounting for about 58% of the administered dose, with approximately 17% of the administered radioactivity eliminated in faeces. At 100 mg/kg bw, a greater proportion of radioactivity was detected in faeces (approximately 49%) than in urine (34%). At both doses, elimination was incomplete by 120 hours, with about 4% and 2% of the dose still detectable in urine and faeces, respectively.

Table 8. Mass balance in dogs following a single oral dose of [^{14}C]MCPA

Sample	Mean % of the administered radioactive dose	
	5 mg/kg bw	100 mg/kg bw
Urine	58.4	34.0
Faeces	16.8	49.1
Cage wash	3.5	2.1
Cage debris	0.03	0.06
Total	78.7	85.2

From Hardwick (1999, 2000)

Pharmacokinetic data are summarized in Table 9. Radioactivity was detected in plasma within 1 hour of dosing and was maximal at 4.5 and 7.0 hours at 5 and 100 mg/kg bw, respectively. At 100 mg/kg bw, C_{max} and $AUC_{0-\infty}$ were about 10-fold higher than at 5 mg/kg bw. Between 19% and 32% of the AUC was extrapolated to be after the last time point, indicating that clearance of radioactivity was not complete at the final measurement, 120 hours after dosing. Concentrations of radioactivity in blood paralleled those in plasma but were approximately 2-fold lower, indicating that MCPA was substantially extracellular. The plasma half-life values were approximately 45 and 47 hours at 5 and 100 mg/kg bw, respectively.

Table 9. Plasma pharmacokinetic parameters in dogs following a single oral dose of [^{14}C]MCPA

Parameter	Mean value[a]	
	5 mg/kg bw	100 mg/kg bw
C_{max} (µg Eq/g)	35.95	341.2 (9.5)
T_{max} (h)	4.5	7.0
$t_{½}$ (h)	45.27	46.70
AUC_{0-t} (µg Eq·h/g)	2030	17 195 (8.5)
$AUC_{0-\infty}$ (µg Eq·h/g)	2539	20 454 (8.1)

From Hardwick (1999, 2000)
AUC, area under the plasma concentration–time curve; C_{max}, maximum concentration in plasma; Eq, equivalent; $t_{½}$, half-life; T_{max}, time to reach C_{max}
[a] Values in parentheses represent the overall fold increase relative to the low dose.

At both doses, four major radioactive regions were detected in urine by HPLC, the author noting the high level of inter-animal variation. At 5 mg/kg bw, MCPA and HMCPA accounted for 4.0–29.3% and 1.1–6.5% of the administered dose, respectively. At 100 mg/kg bw, MCPA and HMCPA accounted for 1.7–4.0% and 2.1–15.4% of the administered dose, respectively. The two other (unidentified) major regions of radioactivity respectively accounted for 12.1–37.6% and 5.1–10.6% of the administered dose at 5 mg/kg bw and 6.5–13.6% and 3.1–7.6% of the administered dose at 100 mg/kg bw. Urine samples taken at 12 and 28 hours after dosing with [^{14}C]MCPA at 100 mg/kg bw were subjected to LC-MS. The presence of MCPA (29.3% of the administered dose) and HMCPA (1.1–6.5% of the administered dose) was confirmed. Glycine and taurine conjugates of MCPA were

also detected (12.1–37.6% and about 10% of the administered dose, respectively). An additional unidentified peak was also detected, which the author suggested was probably another conjugate of MCPA, but further characterization was not possible.

In faeces, three main radioactive peaks were detected by HPLC. One peak was identified as MCPA (5.8–9.6% of the administered dose at 5 mg/kg bw and 11.5–27.6% of the administered dose at 100 mg/kg bw), whereas the two other peaks were tentatively identified as the glycine and taurine conjugates of MCPA (0.5–1.5% and 1.8–4.2% of the administered dose, respectively, at 5 mg/kg bw and 0.7–4.4% and 0.9–18.8%, respectively, at 100 mg/kg bw). HMCPA was not identified in faeces. The presence of conjugated MCPA in the faeces of dogs indicated biliary excretion to be significant in this species (Hardwick, 1999, 2000).

Timchalk (1998, 2004) compared published toxicokinetic parameters (volume of distribution, renal clearance and half-life) for MCPA (and 2,4-D) in the rat, dog and human using allometric scaling on the basis of body weight (Table 10). Volume of distribution scaled as a function of body weight and was only marginally larger than blood volume. In the dog, half-life and renal clearance of MCPA did not scale as a function of body weight, whereas for rats and humans, these parameters were in reasonable agreement with allometric scaling. Longer half-lives and slower elimination in the dog result in substantially higher body burden at comparable nominal doses relative to other species. Proposed mechanisms for reduced clearance in the dog include saturation of renal clearance and increased renal tubule reabsorption. On the basis of these comparisons, the author suggested that it is inappropriate to extrapolate observations of toxicity induced by MCPA (or other phenoxyacetic acid herbicides) in the dog to humans because of the limited capacity of the dog to excrete organic acids relative to other species.

Table 10. Interspecies comparison of toxicokinetic parameters for MCPA

Parameter	Rat	Dog	Human
Body weight (kg)	0.25	13	70
Dose of MCPA (mg/kg bw)	5	5	0.015
V_d (l)	0.04	1.81	5.25
Renal clearance (ml/h)	3.7	12	132
$t_{½}$ (h)	5.8	46	11

From Timchalk (1998, 2004)
$t_{½}$, half-life; V_d, volume of distribution

Humans

In a non-guideline study predating the most recent Declaration of Helsinki, Fjeldstat & Wannag (1977) investigated the urinary excretion of MCPA to ascertain whether it could be used to estimate MCPA exposure. A single 5 mg dose of MCPA (unspecified purity and vehicle) was ingested by four healthy volunteers (32–36 years of age). Urine was collected prior to ingestion and then at various times to 5 days after dosing. Samples were hydrolysed with sulfuric acid prior to HPLC analysis. Blood was sampled at 2, 7, 12 and 24 hours and at 2, 3, 4 and 10 days after dosing for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, alkaline phosphatase (ALP), creatine kinase and gamma-glutamyl transpeptidase (GGT). Recovery was approximately 60% of the administered dose by 96 hours after dosing. The mean level of MCPA in urine was about 41%, 52%, 54% and 55% of the administered dose at 24, 48, 72 and 96 hours after dosing, respectively. Graphically presented data illustrated that urinary excretion was nearly completed by 48 hours. After 5 days, the concentration of MCPA in urine was below the limit of detection (0.2 µg/ml). There was no treatment-related effect on any of the analysed serum enzymes.

In a second non-guideline study also predating the most recent Declaration of Helsinki, five healthy volunteers (three males and two females) ingested a single oral dose of MCPA (as the potassium salt) (unspecified purity) in gelatine capsules at 15 µg/kg bw. Blood was sampled at 30 minutes and 1, 3, 6 and 24 hours; urine was sampled over 72 hours. The C_{max} of 0.15 µg/ml was measured at 1 hour after dosing, declining to 0.09, 0.07 and less than 0.04 µg/ml at 3, 6 and 22 hours, respectively. Based on these concentrations, the elimination half-life from plasma is approximately 6 hours. Urinary excretion was the highest over the first 6 hours after dosing (approximately 0.45 µg/minute), declining to 0.08 µg/minute by 48 hours. Approximately 40% of the administered dose was excreted within 24 hours. Urine collected from one subject was reported to contain 56–73% conjugated MCPA, although the identity of the conjugated species was not described (Kolmodin-Hedman et al., 1983).

Kolmodin-Hedman et al. (1983) also examined the dermal absorption and urinary excretion of MCPA in the same volunteers (presumably after a washout period). In the first experiment, the volunteers dipped their hands and forearms into a 2% aqueous solution of MCPA, allowed them to dry and then washed them with soap and water. Blood was sampled at 30 minutes and 1, 3, 6 and 24 hours; urine was sampled over 72 hours. No MCPA was detectable in blood or urine.

In a second experiment, about 1 g of MCPA (prepared from a 75% liquid formulation) was applied to 10 cm^2 of the thigh via an occlusive dressing. After 2 hours, the dressing was removed and the application site washed with soap and water. Blood was sampled at 30 minutes and 1, 3, 6 and 24 hours; urine was sampled over 72 hours. The experiment was repeated, with blood and urine sampled up to 55 hours and 7 days, respectively. No clinical signs were observed. The plasma concentration of MCPA was variable across the five volunteers. In two volunteers, MCPA was not detectable after 3 hours. In the other three volunteers, plasma concentrations of MCPA increased slowly, reaching a maximum at about 24 hours after application and thereafter declining slowly; MCPA was still detectable after 55 hours. In urine, the concentration of MCPA was detected within 3 hours of dermal application, reaching a maximum at 24–48 hours and remaining detectable to 144–168 hours. The urinary half-life in two volunteers was 25 and 52 hours. The mean cumulative urinary excretion of MCPA was 1.73 mg after 6 days, accounting for approximately 0.002% of the applied amount of 1 g.

Roberts et al. (2011) examined the toxicokinetics and saturable protein binding of MCPA in patients with acute MCPA poisoning. The time to hospital admission was 2.5–6 hours (mean 3.3 hours) after poisoning, which was the T_{max} for 22 of 25 patients. C_{max} values were 3.7 hours in one of the remaining patients and 7 hours in the other two, suggesting prolonged absorption. At plasma concentrations above 200 mg/l, the apparent half-life was estimated to be 25.5 hours, whereas at lower concentrations, it was approximately 17 hours. Dose-dependent protein binding occurred, which was saturated when plasma concentrations of MCPA exceeded about 240 mg/l. Scatchard analysis suggested protein binding at two sites, with saturation of the high-affinity site occurring at 115 mg/l.

1.2 In vitro studies

The mechanism of intestinal uptake of MCPA was investigated in monolayers of the human colorectal adenocarcinoma epithelial cell line, Caco-2, in the presence of various metabolic inhibitors. At pH 6, uptake occurred within 5 minutes, with about a 5-fold lower uptake at pH 7.4. Uptake was biphasic, with an initial saturable carrier-mediated process at low concentrations and a non-saturable process (simple diffusion) at higher concentrations. Uptake was significantly ($P < 0.05$) inhibited at low temperature and in the presence of sodium azine, 2,4-dinitrophenol and carbonyl cyanide *p*-(trifluoromethoxy) phenylhydrazone. A number of monocarboxylic acids significantly ($P < 0.05$) inhibited uptake, with the most potent including benzoic acid, ferulic acid, salicylic acid and *p*-coumaric acid (30–40% of the control). The results suggested that MCPA uptake is via an H^+-linked monocarboxylic acid transporter (Kimura, Tsukagoshi & Endo, 2008).

In a subsequent study by Kimura et al. (2012), the uptake from apical membranes and apical to basolateral transport (i.e. secretory transport) of MCPA were investigated in monolayers of Caco-2 cells. Absorptive transport via an H^+-linked monocarboxylic acid transporter on the apical membranes of the monolayer was confirmed. Secretory transport was found to be mediated by a probenecid-sensitive transporter present on the basolateral side of the monolayer.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity tests of MCPA, MCPA 2-EHE and MCPA DMA administered to different animal species are summarized in Table 11. Clinical signs generally occurred within hours of dosing and included piloerection, apathy, hunched posture, abnormal gait, decreased respiration, ptosis, pallor and occasionally ataxia, twitching and tonic convulsions; survivors recovered within about 2 days.

(b) Dermal and ocular irritation

The results of skin and eye irritation tests on MCPA, MCPA 2-EHE and MCPA DMA conducted in rabbits are summarized in Table 12.

(c) Dermal sensitization

In a non-guideline study, MCPA (purity 94.8%) was analysed for its skin sensitization potential in the guinea-pig maximization test. Twenty female guinea-pigs were included in the test group, and 10 in the control group. Following the first and second challenges with 10% MCPA, two guinea-pigs showed very slight erythema or slight erythema and very slight oedema. On the basis of these findings, MCPA was classifiable as a non-skin sensitizer (Kirsch & Hildebrand, 1984).

In a non-guideline study, Grundler & Kirsch (1984) examined the skin sensitization potential of MCPA (purity 94.8%) in the guinea-pig open epicutaneous test. Groups of eight female guinea-pigs were induced with 1.5%, 5%, 15% or 50% MCPA and challenged twice thereafter with these same four concentrations across the four treated groups. In the 1.5% and 50% induction groups, very slight (barely perceptible) erythema was recorded in one of eight animals in each of the 1.5% and 50% induction groups following the first challenge. Following the second challenge, very slight (barely perceptible) erythema was recorded in the 1.5% induction group (4/8, 3/8 and 1/8 animals at challenge concentrations of 50%, 15% and 5%, respectively) and the 5% induction group (1/8 and 2/8 animals, respectively, at challenge concentrations of 50% and 15%, respectively). No oedema was observed in any animal following either challenge. MCPA was not classified as a skin sensitizer.

Using a modification of the Buehler method, Denton (1993a,b) examined the potential of MCPA 2-EHE (purity 93.9%) or MCPA DMA (purity 77.67%) to cause delayed contact hypersensitivity in the guinea-pig. There were 10 animals in both the control and test groups. No evidence of skin sensitization was recorded for either MCPA 2-EHE (Denton, 1993a) or MCPA DMA (Denton, 1993b).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 4-week range-finding study, MCPA (purity 94.8%) was admixed in the diet and fed ad libitum to groups of B6C3F1 mice (five of each sex per group) at a concentration of 0, 100, 300, 900 or 2700 ppm. The 300 ppm group consisted of four males and six females. The respective mean intakes of MCPA were 0, 20, 59, 177 and 696 mg/kg bw per day for males and 0, 24, 72, 208 and 765 mg/kg bw per day for females at 0, 100, 300, 900 and 2700 ppm. Blood was sampled at the end of the study for the analysis of haematology parameters and a limited number of clinical chemistry

parameters (creatinine, urea, ALT and ALP). Organ weights were recorded, and histopathology was undertaken on selected tissues. Only body weight data were statistically analysed.

Table 11. Results of studies of acute toxicity of MCPA and its salts

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD_{50} or LC_{50} (mg/kg bw or mg/l)	Reference
MCPA acid							
Rat	Wistar	Male and female	Oral	Not specified	0.5% w/v aqueous CMC	1160	Kirsch & Hildebrand (1983a)
Rat	SD	Female	Oral	97.5	Arachis oil	~500 (ATC method)	Sanders (2006)[a]
Rat	Wistar	Male and female	Dermal	Not specified	0.5% w/v aqueous CMC	> 4000	Kirsch & Hildebrand (1983b)
Rat	Wistar	Male and female	Inhalation (4 h, head only)	94.8 MMAD = 5.2 µm Dust aerosol mixture	1% fumed silica	> 6.36	Klimisch et al. (1986)[a]
MCPA 2-EHE							
Rat	SD	Male and female	Oral	93.9	Water	1152 ae (male) 832 ae (female)	Allan (1992a)[a]
Rabbit	NZW	Male and female	Dermal	93.9	Water	> 2000	Denton (1992)[a]
Rat	SD	Male and female	Inhalation (4 h, whole body)	93.9 MMAD = 2.9 µm	Water	> 4.5	Jackson, Molloy & Hardy (1993a)[a]
MCPA DMA							
Rat	SD	Male and female	Oral	77.7	Water	820 ae	Allan (1992b)[a]
Rabbit	NZW	Male and female	Dermal	77.7	Water	> 2000	Allan (1992c)[a]
Rat	SD	Male and female	Inhalation (4 h, whole body)	77.7 MMAD = 2.2 µm	Water	> 4.72	Jackson, Molloy & Hardy (1993b)[a]

ae, acid equivalents; ATC, acute toxic class; CMC, carboxymethylcellulose; LC_{50}, median lethal concentration; LD_{50}, median lethal dose; MMAD, mass median aerodynamic diameter; NZW, New Zealand White; SD, Sprague-Dawley; w/v, weight per volume

[a] Statement of compliance with principles of good laboratory practice; guideline study.

There were no deaths. Clinical signs were confined to all high-dose mice and consisted of motor disturbances (drawing and convulsive lateral stretching of the hindlimbs; jerky motor activities), which commenced in the majority of mice on day 3 and persisted for the duration of the study. These disturbances were accompanied by other signs (squatting posture, closed eyelids and staggering gait), which had resolved in the majority of mice by day 10. In a swimming test designed to examine neuromuscular coordination and grip reflex, there was a delay in the beginning of swimming in all high-dose mice, but this had normalized after a few swimming movements.

Table 12. Results of studies of dermal and ocular irritation of MCPA and its salts in rabbits

Strain	Sex	Test material	Application site	Exposure period	Result	Reference
MCPA acid						
White Vienna	Male and female	50% w/w aqueous formulation	Abraded and intact skin	24 h, occluded	Primary irritation index = 2.5	Kirsch & Hildebrand (1983c)
NZW	Female	94.22% purity	Intact skin	4 h, semi-occluded	No reaction to treatment	Liggett (1992a)[a]
NZW	Male	97.5% purity	Intact skin	4 h, semi-occluded	No reaction to treatment	Pooles (2007)[a]
White Vienna	Male and female	~64 mg MCPA powder	Eye	—	Severe eye irritation (primary irritation index = 76)	Kirsch & Hildebrand (1983d)
MCPA 2-EHE						
NZW	Female	93.9% purity	Intact skin	4 h, semi-occluded	Slight skin irritation	Liggett (1992b)[a]
NZW	Male and female	93.9% purity	Eye	Eye unwashed for 24 h	Slight eye irritation	Liggett (1992e)[a]
MCPA DMA						
NZW	Male and female	77.67% purity	Intact skin	4 h, semi-occluded	Very slight skin irritation	Liggett (1992c)[a]
NZW	Female (*n* = 1)	77.67% purity	Eye	Eye unwashed for 24 h	Severe eye irritation[b]	Liggett (1992d)[a]
White Vienna	Male and female	1.6% aqueous solution	Eye	—	No irritation	Kieczka (1984)

NZW, New Zealand White; w/w, weight per weight

[a] Statement of compliance with principles of good laboratory practice; guideline study.

[b] Animal killed for humane reasons on day 7.

At 2700 ppm, body weight gain was significantly lower ($P < 0.01$) than the control values throughout the dosing period; all high-dose mice lost approximately 10–20% of their body weight during the study. At this same dietary concentration, feed consumption was lower than the control value from day 1 to day 7 (2.9 g versus 4.4 g in males and 2.7 g versus 3.9 g in females).

Perturbations in haematology and clinical chemistry parameters occurred at the highest dose. Relative to the control group, there was a 10–15% reduction in haemoglobin, haematocrit and erythrocyte counts in both sexes, a 45% (males) and 26% (females) reduction in platelets, an approximately 40% reduction in lymphocytes and an 8-fold (male) and 4-fold (female) increase in monocytes. ALT activity was increased 14-fold (males) and 39-fold (females), whereas ALP activity was increased 6-fold (males) and 8-fold (females). Reduced serum urea and creatinine concentrations were noted in high-dose females (but not males), which may have been a consequence of the body weight loss in this group rather than a direct effect of treatment. While there were no apparent differences in mean values between the control and 900 ppm groups, the authors reported isolated cases of increased ALT activity (one female) and decreased creatinine (one female), haemoglobin (two males and one female), haematocrit and erythrocytes (one male and one female) at 900 ppm. There were no treatment-related effects on any haematology or clinical chemistry parameter at 100 or 300 ppm.

Macroscopic examination revealed emaciation in all high-dose mice, with a loss of adipose tissue consistent with a peroxisome proliferating effect. The decrease in the weight or size of a

number of organs (adrenals, kidney, spleen, testes, ovaries and uterus) was attributable to this emaciation. Histopathology revealed lymphocyte depletion in the spleen, atrophy of the germinal epithelium of the testes and the absence of corpora lutea and glandular atrophy of the uterus consistent with cachexia. In contrast to other organs, liver weights were increased relative to the control at the highest dose (mean increase of 37% in males and 72% in females) in association with cloudy swelling of hepatocytes (all mice) and necrosis (4/5 males and 4/5 females). At 900 ppm, cloudy swelling of the hepatocytes was observed in one female. No treatment-related pathology was noted at lower doses.

The no-observed-adverse-effect level (NOAEL) was 300 ppm (equal to 59 and 72 mg/kg bw per day in males and females, respectively), based on reduced body weight gain and liver pathology at the next higher dose of 900 ppm (equal to 177 and 208 mg/kg bw per day in males and females, respectively) (Kirsch et al., 1985a).

Rats

In a non-guideline study, MCPA (purity 94.8%) was admixed in the diet and fed ad libitum to Wistar rats (15 of each sex per group) at a dietary concentration of 0, 50, 150 or 450 ppm for 3 months. The mean intake of MCPA was 0, 3.9, 11.8 and 35.4 mg/kg bw per day for males and 0, 4.4, 13.1 and 38.7 mg/kg bw per day for females at 0, 50, 150 and 450 ppm, respectively. Blood and urine were sampled from 10 rats of each sex per group on days 43 and 85 for the analysis of haematology, clinical chemistry or urine analysis parameters. At the end of treatment, all rats were assessed for gross pathological abnormalities, organs were weighed and histopathology was performed.

There were no deaths or clinical signs and no treatment-related effects on body weight gain or feed consumption. Ophthalmoscopy was unremarkable. Treatment-related effects occurred mainly in high-dose males, consisting of increased absolute ($P < 0.01$) and relative kidney weights (both about 14% higher than the control values), reduced serum calcium (day 43: 2.66 mmol/l versus 2.79 mmol/l in the controls, $P < 0.01$; day 85: 2.58 mmol/l versus 2.66 mmol/l in the controls, $P < 0.05$) and cholesterol (1.11 mmol/l versus 1.52 mmol/l in the controls at day 85, $P < 0.01$). In high-dose females, there was a significant increase in creatinine at day 85 (59.44 µmol/l versus 52.21 µmol/l in the controls; $P < 0.01$). There were no treatment-related macroscopic or histopathological abnormalities. The significantly increased ($P < 0.05$) absolute kidney weight in 150 ppm males was not considered treatment related, as it was not corroborated by a higher relative kidney weight, any histopathology or evidence of impaired renal function.

The NOAEL was 150 ppm (equal to 11.8 mg/kg bw per day), based on increased kidney weights, increased serum creatinine and reduced plasma calcium and cholesterol at the next higher dose of 450 ppm (equal to 35.4 mg/kg bw per day) (Kirsch et al., 1985b).

In a published study, Strauss et al. (2009) examined the influence of rat strain on the profile of endogenous metabolites (e.g. carbohydrates, amino acids) in blood following 28 days of dietary exposure to MCPA (unspecified purity) at a nominal concentration of 2500 ppm (five rats of each sex per strain). Control rats had access to untreated diet. The four rat strains examined were Crl:WI(Han), Han:RCC:WIST(SPF), Crl:CD® and F-344/Crl; the achieved doses were approximately 144, 151, 175 and 138 mg/kg bw per day for males and 169, 187, 189 and 137 mg/kg bw per day for females, respectively. Blood was sampled on days 7, 14 and 28 for the analysis of limited haematology and clinical chemistry parameters and endogenous metabolites.

There were no marked differences in endogenous metabolite profiles across the four strains following dietary exposure to MCPA. These profiles were consistent with reference patterns for peroxisome proliferation or renal organic anionic transporter inhibition. The adverse effects of MCPA were discernible across all four rat strains and included increased urea and creatinine (renal effects) and liver changes consistent with enzyme induction (decreased bilirubin and increased triglyceride) and slight hepatocyte membrane damage (increased ALT activity).

Dogs

In a 4-week range-finding study that predated principles of GLP, MCPA (purity 98.4%) was administered to one Beagle dog of each sex per group in the diet or in capsules (soya bean oil vehicle) at a nominal dose of 0, 8, 20 or 32 mg/kg bw per day. Standard gross toxicological end-points were recorded, with blood and urine sampled on days 25–27 for the analysis of haematology, clinical chemistry or urine analysis parameters. At the end of the study, survivors were killed and necropsied; organs were weighed and examined histopathologically.

Data were combined for each dose group, as there were no apparent sex differences. There were no deaths and no treatment-related clinical signs or ophthalmic abnormalities. Reduced body weight gain occurred in males at and above 20 mg/kg bw per day, whereas one high-dose female failed to gain body weight. Haematology and urine analysis parameters were unremarkable, whereas there was a dose-related increase in mean blood urea nitrogen and ALT (combined values for males and females: 10.1, 16.2, 17.7 and 19.6 mg% and 9.5, 15.9, 31.9 and 34.0 Reiman-Frankel units at 0, 8, 20 and 32 mg/kg bw per day, respectively). At the highest dose, mean absolute and relative thymus weights were about half those of the control group, with slight cortical atrophy of the thymus observed in one male and one female (Reuzel et al., 1978).

In a non-guideline study that predated principles of GLP, MCPA (purity 94.6%) was admixed in the diet and fed to groups of four Beagle dogs of each sex per group at a nominal dose of 0, 3, 12 or 48 mg/kg bw per day for 13 weeks. Satellite groups of four dogs of each sex per group were offered diets containing MCPA technical (purity 94.6%) at a nominal dose of 0, 0.3, 1 or 12 mg/kg bw per day or purified MCPA (purity 99.25%) at a single dose of 12 mg/kg bw per day. Dogs were examined daily for clinical signs. Ophthalmoscopy was performed prior to the commencement of dosing and during weeks 7 and 13. Body weight and feed consumption were recorded at various times throughout the study. Blood and urine were sampled pretreatment and during weeks 7 and 12 for the analysis of haematology and clinical chemistry or urine analysis parameters. In the satellite study, haematology was performed only on dogs in the 12 mg/kg bw per day groups. Faeces were analysed during weeks 1, 4, 8 and 12 for occult blood. Liver and kidney function tests were performed during week 13 using the bromosulfophthalein and phenolsulfonphthalein methods, respectively (no liver function test was performed in the satellite study). At the end of the main and satellite studies, survivors were sacrificed, gross pathological abnormalities were recorded, organs were weighed and histopathology was performed.

At 48 mg/kg bw per day, clinical signs occurred in seven of the eight dogs and included pustules, papules, necrotic skin lesions, focal stomatitis, conjunctivitis, diarrhoea, anorexia, dehydration, lethargy and signs of icterus. In addition, jaundice, bloody diarrhoea or urine, and bile vomiting were noted in two or three dogs. One of the seven dogs was found dead on day 48, with the remaining six sacrificed in a moribund condition on days 56–63. The remaining male dog, which exhibited no treatment-related clinical signs, was withdrawn from treatment during week 7. There were no deaths or treatment-related clinical signs at any of the lower doses, including in the satellite groups.

Ophthalmoscopy revealed slight conjunctivitis progressing to muco-purulent conjunctivitis in three males and three females at 48 mg/kg bw per day, with the three males also displaying corneal damage possibly resulting from the conjunctivitis. Two males and two females had jaundice of the schlera. No treatment-related ophthalmic abnormalities occurred at lower doses. Slight to moderate muco-purulent conjunctivitis was detected in the 12 mg/kg bw per day satellite group (one dog dosed with the MCPA technical and two dogs receiving purified MCPA).

At 48 mg/kg bw per day, the seven dogs that exhibited overt signs of toxicity failed to gain body weight, whereas reduced body weight gain was noted in the survivor. Reduced body weight gain occurred at 12 mg/kg bw per day in the main study (approximately 20% compared with the control group), but not in the satellite group, where body weight gain was unremarkable. At 48 mg/kg bw per

day, feed consumption was reduced during week 5 to the point where supplementary feeding with basal diet was necessary for 1 week. When feeding with the test diet was resumed, dogs ceased eating.

In the main study, perturbations in haematology parameters were observed most obviously in blood collected during week 6, with limited opportunity to analyse week 12 samples because of the high number of deaths at 48 mg/kg bw per day. At 48 mg/kg bw per day, reduced haemoglobin (6.6 mmol/l versus 8.6 mmol/l in the controls, $P < 0.05$), haematocrit (0.34 l/l versus 0.42 1/1 in the controls), erythrocytes (4.3×10^{12}/l versus 5.8×10^{12}/l in the controls) and thrombocytes (278×10^{9}/l versus 327×10^{9}/l in the controls) occurred in females during week 6. Similar evidence of anaemia was not observed at lower doses. In the main study, serum urea and creatinine were significantly elevated ($P < 0.05$) at and above 3 mg/kg bw per day during week 6 (approximately 1.4- to 2.8-fold and 1.2- to 1.4-fold higher than the control values, respectively). This was corroborated by significantly elevated ($P < 0.05$) urea and creatinine in the 12 mg/kg bw per day satellite groups during weeks 6 and 12 (up to about 1.5- and 1.3-fold higher than the control values, respectively). At 48 mg/kg bw per day, liver enzymes were elevated most obviously during week 6 because of the high number of deaths by week 12; relative to the control, AST was increased 3.7-fold in females, ALT by 2.9- or 11.7-fold (males and females, respectively) and ornithine transcarbamoylase by 40-fold in females. Additionally at 48 mg/kg bw per day, elevated bilirubin (53-fold in females) and an approximately 20% decrease in glucose ($P < 0.05$ in both sexes) were noted. In the satellite study, ornithine transcarbamoylase was elevated at 12 mg/kg bw per day (up to about 1.5- to 2.5-fold higher than the control value). Treatment-related urine analysis findings were confined to the presence of bilirubin (one male, three females) and increased epithelial cells (two females) at 48 mg/kg bw per day.

There was a significant ($P < 0.05$) dose-related increase in the concentration of urinary phenolsulfonphthalein at and above 3 mg/kg bw per day in the main study and at 12 mg/kg bw per day in the satellite study, which indicated that kidney function was adversely affected by treatment. Liver function was impaired in the surviving dog at 48 mg/kg bw per day. Faecal blood was detected in several dogs at 48 mg/kg bw per day and in two of the dogs fed purified MCPA at 12 mg/kg bw per day.

At 48 mg/kg bw per day, the relative kidney weights of dogs that were sacrificed in extremis were higher than those of the controls (approximately 20% in males). In the satellite groups, absolute and relative prostate weights were significantly lower ($P < 0.05$) than those of the control group at 12 mg/kg bw per day with technical MCPA (absolute: 1.72 g versus 4.37 g in the controls; relative: 0.15 versus 0.38 in the controls). However, given the large variation in prostate weights and the absence of similar findings with purified MCPA at the equivalent dose, these significant differences were not considered treatment related. In the main study, treatment-related macroscopic abnormalities included jaundice (three males and three females at 48 mg/kg bw per day), distended gall bladder (two males and two females at 12 mg/kg bw per day; all dogs at 48 mg/kg bw per day), gastrointestinal haemorrhages (two males and two females at 48 mg/kg bw per day) and pale or yellow liver (two males and two females at 48 mg/kg bw per day). No treatment-related macroscopic abnormalities were observed at lower doses or in the satellite groups. Histopathology revealed degenerative and/or regenerative effects in the liver (necrosis, inflammatory infiltrates, cytomegaly and karyomegaly, centrilobular liver cell degeneration, bile duct proliferation and the accumulation of brown bile pigment), kidneys (regeneration of the tubular epithelium in the intercorticomedullary layer) and gastrointestinal tract (mucosal haemorrhage, focal necrosis and polymorphonuclear cell infiltrates, cystic dilatation of the intestinal crypts) of dogs fed 48 mg/kg bw per day. There were no treatment-related microscopic abnormalities at the lower doses tested in the main study. In the satellite study at a dose of 12 mg/kg bw per day, slight to moderate bile duct proliferation was noted in some dogs fed technical MCPA (three males) or purified MCPA (two males).

The NOAEL was 1 mg/kg bw per day, based on increased serum creatinine and urea and reduced kidney function at 3 mg/kg bw per day (Reuzel et al., 1980).

In a published study conducted according to OECD Test Guideline No. 409, groups of four Beagle dogs of each sex per group received MCPA (unspecified purity) in gelatine capsules at a dose of 0, 1, 5 or 15 mg/kg bw per day for 90 days. An additional four dogs of each sex were allocated to the control and high-dose groups to study reversibility after 4 weeks of recovery. Blood was sampled at 30, 60 and 90 days for the analysis of clinical chemistry parameters. At necropsy, an extensive selection of tissues was weighed, and histological examinations were conducted.

There were no deaths, treatment-related clinical signs, ophthalmic abnormalities or effects on body weight or haematology parameters. Selected clinical chemistry parameters are presented in Table 13. Relative to the control group and pretreatment values, AST was significantly increased ($P < 0.05$) at and above 5 mg/kg bw per day in males and at 15 mg/kg bw per day in females. Serum urea was significantly elevated ($P < 0.05$) at 5 and 15 mg/kg bw per day in both sexes, whereas serum creatinine was significantly elevated ($P < 0.05$) at every dose in both sexes (noting that there was a significant [$P < 0.05$] time-related increase in serum creatinine in the controls). All of these clinical chemistry parameters recovered 28 days after the cessation of treatment. Mean testes weights were significantly decreased ($P < 0.05$) in high-dose males (right testis: 0.78 g versus 1.06 g in the controls; left testis: 0.73 g versus 1.05 g in the controls), but not in dogs allowed to recover for 4 weeks. Testicular atrophy was observed in one high-dose male, with a low occurrence of multinucleate giant cells, and a second male of this group showed an absence of spermatogenic cells in several tubules. Focal hepatocellular necrosis together with inflammatory change and mononuclear cell nodules was reported in treated animals, predominantly in high-dose males, but the incidence of this finding was unreported.

Table 13. Clinical chemistry findings in dogs following 90 days of capsular administration of MCPA

Parameter	Mean value							
	0 mg/kg bw per day		1 mg/kg bw per day		5 mg/kg bw per day		15 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
ALT (µkat/l)								
Pretreatment	0.59	0.84	0.60	0.62	0.71	0.79	0.78	0.81
Day 30	0.67	1.04	0.60	1.15	1.11*	0.94	1.37#	1.76+
Day 60	0.52	0.65	0.42	0.78	0.73	0.72	0.72	1.33#
Day 90	0.51	0.66	0.48	0.78	0.71	0.65	1.06#	1.28+
Recovery	0.53	0.73	—	—	—	—	0.48	0.61+
Urea (mmol/l)								
Pretreatment	3.34	4.27	3.60	5.04	3.70	3.91	4.32	4.53
Day 30	3.49	4.83	4.63	5.25	6.89#	6.38	7.35#	7.30#
Day 60	3.60	4.17	3.70	5.25	6.69	6.70*	7.10#	7.05#
Day 90	3.34	4.06	4.42	4.53	5.76*	6.38*	5.81*	6.43#
Recovery	3.67	4.43	—	—	—	—	3.24	5.51
Creatinine (µmol/l)								
Pretreatment	58.7	66.8	60.3	65.7	66.5	57.9	63.08	67.6
Day 30	61.0	81.8	66.5	99.0	79.6*	104.8#	81.1#	125.2#
Day 60	64.7	81.8+	71.2#	99.0#	90.9#	104.8*	96.6#	125.2#
Day 90	70.0	75.8+	77.6+	84.2#	99.4#	88.5#	94.0#	98.3#
Recovery	70.2	79.0+	—	—	—	—	67.3	78.3

From Sadloňová, Hózová & Flaškárová (2006)
— not analysed; * $P < 0.05$ compared with the control group; # $P < 0.05$ compared with the control and pretreatment value; + $P < 0.05$ compared with the pretreatment value

No NOAEL was identified. The lowest-observed-adverse-effect level (LOAEL) was 1 mg/kg bw per day, the lowest dose tested, based on elevated serum creatinine (Sadloňová, Hózová & Flaškárová, 2006).

MCPA 2-EHE (purity 93.5%) was admixed in the diet at a concentration of 0, 20, 80 or 360 ppm and fed to groups of four Beagle dogs of each sex per group for 110–118 days. Mean achieved doses of MCPA 2-EHE were, respectively, 0, 0.6, 2.5 and 11.1 mg/kg bw per day for males and 0, 0.7, 2.8 and 12.7 mg/kg bw per day for females (equal to 0, 0.4, 1.6 and 7.1 mg/kg bw per day for males and 0, 0.4, 1.6 and 8.1 mg/kg bw per day for females, respectively, expressed as acid equivalents). The study was conducted concurrently with and shared the control group for an identical study with MCPA DMA, reported below. Dogs were examined daily for clinical signs. Body weight was recorded weekly, and feed consumption daily. Ophthalmoscopy was performed pretreatment and at the end of the study (day 105 or 106). Blood and urine were collected pretreatment and during weeks 6 and 14 for the analysis of standard haematology, clinical chemistry or urine analysis parameters. At the end of the study, all dogs were assessed for gross pathological abnormalities, organs were weighed and histology was performed.

There were no deaths and no treatment-related clinical signs, ophthalmic abnormalities or effects on body weight or feed consumption. Haematology and urine analysis parameters were generally unremarkable, with the exception of partial thromboplastin time (PTT), which was significantly increased ($P < 0.05$) at the highest dose (Table 14). Significantly increased urea ($P < 0.01$; mid- and high-dose females) and creatinine ($P < 0.01$; mid- and high-dose males and females) were noted (Table 14). The significant increase ($P < 0.05$) in creatinine in low-dose males was considered an incidental finding, as there was no effect at the next higher dose (i.e. mid-dose). At the highest dose only, elevated ALT, serum magnesium and cholesterol (females only) and decreased blood glucose and ALP (females only) were noted (Table 14). There were no treatment-related macroscopic abnormalities or effects on organ weights. Histopathology revealed chronic interstitial hepatitis in four males and three females at the highest dose.

The NOAEL was 20 ppm (approximately equal to 0.4 mg/kg bw per day expressed as acid equivalents), based on increased serum urea and creatinine at the next higher dose of 80 ppm (approximately equal to 1.6 mg/kg bw per day expressed as acid equivalents) (Hellwig et al., 1995a).

In a study conducted concurrently and consistently with the preceding study, MCPA DMA salt (purity 99.9%; 63.4% acid equivalents) was admixed in the diet at a concentration of 0, 20, 80 or 360 ppm and fed to four Beagle dogs of each sex per group for 110–118 days. The achieved doses of MCPA DMA were, respectively, 0, 0.6, 2.4 and 10.9 mg/kg bw per day for males and 0, 0.7, 2.9 and 12.8 mg/kg bw per day for females. These doses are equal to approximately 0, 0.5, 2 and 9 mg/kg bw per day for males and approximately 0, 0.6, 2.4 and 10.5 mg/kg bw per day for females expressed as acid equivalents.

There were no deaths and no treatment-related clinical signs, ophthalmic abnormalities or effects on body weight or feed consumption. Consistent with the preceding study was the significant increase in PTT (high-dose females), ALT (high-dose males), serum creatinine (mid- and high-dose dogs of both sexes) and urea (high-dose dogs of both sexes, mid-dose females) and decreased ALP (high-dose males) (Table 15). There was no treatment-related effect on mean absolute or relative organ weights, and there were no treatment-related gross abnormalities. Subacute to chronic interstitial hepatitis was detected in one male and three females of the high-dose group. There were no microscopic findings at 20 or 80 ppm.

The NOAEL was 20 ppm (approximately equal to 0.5 and 0.6 mg/kg bw per day for males and females, respectively, expressed as acid equivalents), based on increased serum creatinine and urea at the next higher dietary concentration of 80 ppm (approximately equal to 2 and 2.4 mg/kg bw per day for males and females, respectively, expressed as acid equivalents) (Hellwig et al., 1995b).

Table 14. Clinical pathology findings in dogs exposed to MCPA 2-EHE in the diet

Parameter	Mean value							
	0 ppm		20 ppm		80 ppm		360 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
PTT (s)								
Pretreatment	11.3	11.8	11.4	11.2	11.6	12.0	11.4	11.7
Day 40 or 41	12.0	12.2	12.3	12.1	12.8	13.3	13.5	14.0*
Day 103 or 104	11.8	11.6	12.1	11.9	12.2	12.0	13.7*	12.9
ALT (µkat/l)								
Pretreatment	1.00	0.61	0.79	0.72	0.69	0.77	0.44*	0.64
Day 40 or 41	0.83	0.77	1.01	0.81	0.77	1.07	3.40	2.49
Day 103 or 104	0.81	0.72	0.87	0.8	0.80	0.93	3.05	1.71
ALP (µkat/l)								
Pretreatment	3.66	3.57	3.53	4.15	4.29	3.92	4.50	4.03
Day 40 or 41	2.61	3.21	2.83	3.00	2.43	2.74	2.56	1.93*
Day 103 or 104	2.56	2.68	2.57	3.03	2.12	2.61	2.12	1.79
Urea (µmol/l)								
Pretreatment	4.98	4.06	4.47	4.44	4.10	4.03	4.36	3.85
Day 40 or 41	4.28	5.05	4.89	5.61	5.14	5.35	6.18	7.55
Day 103 or 104	5.38	5.32	5.55	6.06	6.43	6.46*	7.55	7.3*
Creatinine (µmol/l)								
Pretreatment	75.3	69.4	79.1	69.9	70.3	69.5	69.0	66.5
Day 40 or 41	76.6	75.4	92.1*	77.1	86.3	84.6*	94.7*	97.9*
Day 103 or 104	80.8	80.0	87.9	85.8	95.1*	93.0	105.2*	102.7*
Glucose (µmol/l)								
Pretreatment	6.35	6.19	6.43	6.43	6.00	6.90	6.33	6.37
Day 40 or 41	6.17	6.47	6.57	6.19	6.18	6.49	5.98	6.22
Day 103 or 104	6.23	6.65	6.43	6.53	6.14	6.97	5.77*	5.89
Cholesterol (mmol/l)								
Pretreatment	6.05	5.10	5.02	4.44	4.54	4.61	5.04	5.05
Day 40 or 41	5.10	4.99	4.46	4.06	4.59	4.47	5.99	6.11
Day 103 or 104	5.47	4.66	4.57	4.10	4.67	6.51	5.44	7.30*
Magnesium (mmol/l)								
Pretreatment	0.71	0.82	0.79	0.81	0.71	0.82	0.78	0.83
Day 40 or 41	0.82	0.85	0.80	0.76	0.82	0.83	0.93	0.92
Day 103 or 104	0.68	0.85	0.67	0.82	0.75	0.88	0.88*	0.94*

From Hellwig et al. (1995a)
* $P < 0.05$

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 6, 30 or 150 ppm and fed to six Beagle dogs of each sex per group for 52 weeks. Mean intakes of MCPA over 52 weeks were 0, 0.22, 1.1 and 5.7 mg/kg bw per day for males and 0, 0.22, 1.1 and 5.5 mg/kg bw per day for females at 0, 6, 30 and 150 ppm, respectively. Clinical observations were made, and feed consumption was recorded daily. Body weight was recorded weekly. Haematology, clinical chemistry and urine analysis parameters were analysed in blood or urine collected pretreatment and at weeks 13, 26 and 52. Ophthalmoscopy was performed pretreatment and at 6 months and 1 year. At the end of

the study, all survivors were killed and necropsied; organs were weighed and examined histopathologically. There were no deaths, treatment-related clinical signs or ophthalmic abnormalities. At 150 ppm, mean absolute body weight and body weight gain of males were lower than the control values over most of the study, reaching statistical significance ($P < 0.05$) from day 126 to day 161 and from day 42 to day 175, respectively; overall, these dogs gained about 1 kg less than the other groups. Body weight gain also tended to be lower in males at 30 ppm, but the decrease did not achieve statistical significance. There was no treatment-related effect on body weight or body weight gain in females. Two females at both 30 and 150 ppm had reduced feed consumption, with the mean feed intake of both groups up to about 10% lower than the control values from week 8.

Table 15. Clinical pathology findings in dogs exposed to MCPA DMA in the diet

Parameter	Mean value							
	0 ppm		20 ppm		80 ppm		360 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
PTT (s)								
Pretreatment	11.3	11.8	11.7	11.2	11.7	11.9	10.9	12.1
Day 40 or 41	12.0	12.2	12.1	12.0	12.8	12.8	12.9	14.6*
Day 103 or 104	11.8	11.6	12.1	11.4	13.1	12.6	12.7	13.1*
ALT (µkat/l)								
Pretreatment	1.00	0.61	0.60	0.86	0.65	1.00	1.00	0.51
Day 40 or 41	0.83	0.77	0.69	0.82	0.78	0.84	2.44*	1.39
Day 103 or 104	0.81	0.72	0.75	0.82	0.75	1.09	2.33*	1.28
ALP (µkat/l)								
Pretreatment	3.66	3.57	3.95	3.91	4.50	2.95	4.38	3.62
Day 40 or 41	2.61	3.21	2.98	3.21	3.12	2.91	2.13	1.98
Day 103 or 104	2.56	2.69	2.53	3.03	2.54	2.63	1.69*	1.87
Urea (µmol/l)								
Pretreatment	4.98	4.06	4.98	4.23	4.05	4.26	4.40	3.87
Day 40 or 41	4.28	6.05	4.53	6.03	4.28	6.70	6.67*	7.38
Day 103 or 104	5.38	5.32	5.68	6.14	5.48	6.75*	6.64	7.53*
Creatinine (µmol/l)								
Pretreatment	75.3	69.4	75.3	70.3	71.5	72.8	73.7	75.3
Day 40 or 41	76.6	75.4	82.9	80.4	88.8*	89.6	102.3*	102.9*
Day 103 or 104	80.8	80.0	85.9	83.7	93.0	100.6*	104.4*	106.9*

From Hellwig et al. (1995b)
* $P < 0.05$

There was no treatment-related effect on any haematology or urine analysis parameter. Selected clinical chemistry parameters are presented in Table 16. Plasma bilirubin was significantly elevated ($P < 0.01$ or 0.05) in males at 30 ppm (week 13) and 150 ppm (weeks 13 and 26). Serum creatinine was significantly elevated at 30 and 150 ppm in both sexes (weeks 13, 26 and 52). At 150 ppm, serum urea was significantly elevated in both sexes (weeks 13 and 26 in males; weeks 13, 26 and 52 in females). At 30 ppm, serum urea was also significantly elevated at some time points (week 13 in males; weeks 13, 26 and 52 in females). On occasion, serum creatinine and urea were significantly lower ($P < 0.05$) than the control values at 6 ppm, but this was discordant with the significant and consistent elevation in these parameters at the middle and high doses. In females, significantly elevated plasma potassium occurred at 30 ppm (week 13) and 150 ppm (weeks 26 and 52).

Table 16. Clinical chemistry parameters in dogs exposed to MCPA for up to 52 weeks in the diet

Parameter	Mean value							
	0 ppm		6 ppm		30 ppm		150 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Bilirubin (µmol/l)								
Week 0	3.17	2.89	2.14	2.98	2.58	2.23	2.26	2.67
Week 13	2.60	3.42	3.14	3.70	3.68**	3.55	3.28*	3.88
Week 26	3.57	3.71	3.22	3.63	3.39	3.60	4.09**	5.21
Week 52	1.97	2.96	1.90	3.02	2.00	2.89	2.35	3.73
Creatinine (µmol/l)								
Week 0	67.19	71.91	71.16	71.40	77.03	75.82	76.38	68.18
Week 13	78.72	81.65	79.74	80.31	91.19*	91.58**	103.98**	96.45**
Week 26	84.40	77.29	79.49	78.77	94.42*	87.15**	112.14**	94.03**
Week 52	86.17	81.37	78.62*	86.51	93.18*	92.04**	107.61**	102.95**
Urea (mmol/l)								
Week 0	4.83	4.16	3.91	4.19	5.28	3.74	5.00	4.04
Week 13	4.95	6.36	5.28	5.95*	6.18*	6.60**	7.39**	7.40**
Week 26	6.12	5.03	5.52*	4.67	6.66	4.83**	7.80**	5.72**
Week 52	4.64	4.69	4.36	4.89	5.14	5.49**	5.83	5.15**
Potassium (mmol/l)								
Week 0	4.45	3.99	4.32	4.17	4.29	3.93	4.26	4.11
Week 13	4.19	4.11	4.26	4.18	4.43	4.35*	4.33	4.30
Week 26	3.94	3.84	4.12	4.05	4.20	4.04	4.19	4.17*
Week 52	4.12	3.88	3.99	4.20	4.10	4.16	4.18	4.23**

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$

The mean absolute and relative thyroid weights of 150 ppm males were significantly higher ($P < 0.05$) than the control group values (absolute: 0.94 g versus 0.67 g in the controls; relative: 0.0074 versus 0.006 in the controls). Gross pathology revealed whitish-yellowish dots in the thyroid parenchyma of two males from this group, with focal hyperplasia of the thyroid follicles observed in one of these animals.

At necropsy, a dark brown coloration of the kidneys was detected in four females of the 30 ppm group, all females of the 150 ppm group and four males of the 150 ppm group. Histopathology revealed pigment storage (probably lipofuscin, based on an examination of unstained sections, which revealed a yellowish-brown pigment) in the epithelia of the proximal portions of the renal tubules of all dogs. At 30 and 150 ppm, dogs appeared to have stored more pigment in comparison with the controls (Table 17).

The NOAEL was 6 ppm (equal to 0.22 mg/kg bw per day), based on elevated serum creatinine and urea and increased pigment storage in the proximal portions of the renal tubules at and above 30 ppm (equal to 1.1 mg/kg bw per day) (Hellwig et al., 1986).

Table 17. Incidence of pigment storage in the proximal portions of the renal tubules of dogs exposed to MCPA for 52 weeks

Degree of pigment storage	Incidence (n = 6)							
	0 ppm		6 ppm		30 ppm		150 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Minimal	2	4	2	1	0	0	0	0
Slight	2	1	2	4	3	2	0	0
Moderate	2	1	2	1	3	4	1	4
Pronounced	0	0	0	0	0	0	3	0

From Hellwig et al. (1986)

(b) Dermal application

Rats

In a dermal toxicity study, MCPA 2-EHE (purity 93.5%) in olive oil was applied to the dorsal skin (10% of the body surface) of groups of five rats (Chbb=THOM (SPF) strain) of each sex under semi-occluded conditions at a dose of 0, 16, 160 or 1000 mg/kg bw per day for 6 hours/day, 5 days/week, for 4 weeks. The application site was examined daily for signs of irritation. Feed consumption and body weight were recorded weekly. Blood was sampled at the end of the study for the analysis of haematology and clinical chemistry parameters. Following sacrifice, all rats were necropsied, organs were weighed and tissues were histopathologically examined.

Treatment-related findings were confined to high-dose females, consisting of significantly reduced ($P < 0.01$) erythrocyte counts, haemoglobin and haematocrit, indicative of mild anaemia (7.38×10^{12}/l versus 7.85×10^{12}/l for the control, 8.8 mmol/l versus 9.5 mmol/l for the control and 0.4 l/l versus 0.43 l/l for the control, respectively). The NOAEL was therefore 160 mg/kg bw per day, based on reduced red cell parameters in females at 1000 mg/kg bw per day (Kirsch et al., 1995a).

The dermal toxicity of MCPA DMA (purity 91.7%) in distilled water was assessed under similar conditions to those described in Kirsch et al. (1995a) at a dose of 0, 12, 120 or 1000 mg/kg bw per day.

There were no findings suggestive of systemic toxicity, and therefore the NOAEL was 1000 mg/kg bw per day, the highest dose tested. All high-dose rats had skin reactions at the application site throughout most of the exposure period consisting of slight to well-defined erythema, scaling and superficial scabbing. Minimal to moderate acanthosis (three males, four females) and slight hyperkeratosis (one male, two females) were observed histologically at the highest dose (Kirsch et al., 1995b).

Rabbits

The dermal toxicity of MCPA (purity 94.22%) in distilled water was assessed in groups of five New Zealand White rabbits of each sex per dose at a dose of 0, 10, 100 or 1000 mg/kg bw per day. The test substance was applied to the dorsal skin (approximately 10% of the body surface) under semi-occluded conditions for 6 hours/day, 5 days/week, for 4 weeks. Dermal responses were recorded before the first application and thereafter on a daily basis. Blood and urine were sampled at the end of the study for the analysis of haematology, clinical chemistry or urine analysis parameters. Following sacrifice, all rabbits were necropsied, organs weighed and tissues examined histopathologically.

There were no findings suggestive of systemic toxicity, and therefore the NOAEL was 1000 mg/kg bw per day, the highest dose tested. Skin reactions at the application site were observed at 100 and 1000 mg/kg bw per day. At 100 mg/kg bw per day, slight erythema and oedema were noted, with minimal diffuse acanthosis also observed in some animals. At 1000 mg/kg bw per day, erythema

and oedema progressing from slight to well defined were recorded in 5 of the 10 animals by day 10. Varying levels of discoloration, dryness and desquamation also occurred. Minimal diffuse acanthosis with or without hyperkeratosis of the treated skin was observed histologically in all high-dose animals (Baldrick et al., 1992).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 20, 100 or 500 ppm and fed ad libitum to groups of male and female B6C3F1 mice for 104 weeks. The mean intakes of MCPA were 0, 3.4, 16 and 83 mg/kg bw per day for males and 0, 4.2, 21 and 103 mg/kg bw per day for females at 0, 20, 100 and 500 ppm, respectively. Each dose group consisted of one main cohort of 50 mice of each sex and a satellite group of 10 mice of each sex. Selection of the highest dose was based on a 4-month pilot study in which clinical chemistry changes suggestive of nephrotoxicity occurred at 900 ppm. Mice were observed daily for mortalities and clinical signs. Body weight and feed consumption were recorded weekly to 3 months and every 4 weeks thereafter. Blood was sampled at approximately 52 (satellite groups) and 104 weeks (10 mice from each of the main groups) for the analysis of haematology parameters; no clinical chemistry parameters were analysed. Following sacrifice (week 52 for the satellite groups and week 104 for the main groups), mice were examined for gross pathological abnormalities, and organs were weighed and examined histologically. There were no treatment-related mortalities, clinical signs or effects on body weight or feed consumption. It was noted that while the mean body weight of 500 ppm males from the main group was approximately 5% lower than the control value over most of the study (reaching statistical significance at some time points), there was no such difference over the same period of time in the satellite group or in females. On this basis, these small differences in body weight were not considered treatment related.

Haematology was generally unremarkable. In females at 500 ppm at 104 weeks, there was an increase in the number of mice with atypical lymphocytes (2/10, 3/10, 0/10 and 7/10 at 0, 20, 100 and 500 ppm, respectively) and Howell-Jolly bodies in erythrocytes (5/10 versus 2/9 in the controls); with no similar findings at 52 weeks or in males, the poor dose–response relationship and the "not very strongly pronounced" nature of the finding, this increase is unlikely to be treatment related.

In 100 and 500 ppm females, mean absolute and relative heart weights were approximately 8% lower than the control values ($P < 0.05$), but in the absence of a dose–response relationship or any histopathological abnormalities, these findings were not attributable to treatment. Mean absolute and relative kidney weights in 500 ppm females were approximately 10% higher ($P < 0.01$ or 0.05) than the control values. There were no treatment-related gross abnormalities. In both sexes at 500 ppm, there was an increased incidence of intratubular calcification (9/50, 11/50, 11/50 and 27/50 in males and 0/50, 1/50, 2/50 and 9/50 in females at 0, 50, 100 and 500 ppm, respectively) and hyaline casts (26/50, 32/50, 25/50 and 36/50 in males and 15/50, 14/50, 26/50 and 33/50 in females at 0, 50, 100 and 500 ppm, respectively). Tubular epithelial hyperplasia was increased in 500 ppm males (9/50, 12/50, 10/50 and 32/50 at 0, 20, 100 and 500 ppm, respectively).

There were no treatment-related neoplastic or non-neoplastic lesions. Under the conditions of the study, MCPA showed no carcinogenic potential in B6C3F1 mice.

The NOAEL was 100 ppm (equal to 16 mg/kg bw per day), based on nephrotoxicity (increased kidney weight, intratubular calcification, hyaline casts and tubular epithelial hyperplasia) at 500 ppm (equal to 83 mg/kg bw per day) (Kühborth et al., 1988).

Rats

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 20, 80 or 320 ppm and fed ad libitum to groups of 50 Wistar rats of each sex for 24 months. Two satellite groups of 10 and 15 rats of each sex per dose (satellite groups I and II, respectively) were also included in the study. The mean achieved doses of MCPA (determined for the main cohort and satellite group I) were 0, 1.2,

4.8 and 19 mg/kg bw per day for males and 0, 1.5, 6.1 and 24.3 mg/kg bw per day for females at 0, 20, 80 and 320 ppm, respectively. Rats were observed daily for mortalities and clinical signs. Body weight and feed consumption were recorded weekly to week 14, then monthly thereafter. Ophthalmology was conducted at the beginning of the study and subsequently every 6 months in the control and high-dose groups from the main cohort. Blood was collected from the main cohort and satellite group II pretreatment and at about weeks 26, 52, 78 and 104 for the analysis of standard haematology and clinical chemistry parameters. Blood was collected during week 52 from satellite group I for the analysis of T_3 and thyroxine (T_4). Urine was collected from satellite group I at 26 and 52 weeks and from 10 rats of each sex per dose in the main cohort at 104 weeks for the analysis of urine analysis parameters. Survivors in satellite group I, in satellite group II and in the main cohort were killed after 12, 24 and 24 months, respectively. Gross pathological and histopathological examinations were performed on all rats. There were no treatment-related mortalities or clinical signs. At 320 ppm in the main cohort, the mean body weight of males was up to 9% lower than the control value, occasionally reaching statistical significance ($P < 0.05$). A similar difference in absolute body weight did not occur in the satellite groups over comparable time frames or in females at the same dose, where in fact significantly higher ($P < 0.05$) absolute body weights were determined at some time points. Feed consumption was unaffected by treatment. There were no treatment-related ophthalmic abnormalities.

Significant increases in plasma ALT activity showed no dose-, time- or sex-related consistency and were not corroborated by effects on other liver enzymes or histological effects consistent with liver toxicity (Table 18). In addition, the statistically significant increases were relatively modest (< 20%) and were not corroborated by the results of two subchronic studies. At 320 ppm, plasma triglycerides were significantly lower ($P < 0.01$ or 0.05) than the control values in males (weeks 78 and 104) and females (weeks 52 and 78). At 80 ppm, significantly lower ($P < 0.05$) triglycerides were determined in the blood collected from females during week 52 and males at week 104 (Table 18). These results might reasonably be expected, as MCPA is a known peroxisome proliferator. On this basis, the effects on triglycerides are considered to reflect a pharmacological rather than a toxicological effect.

Table 18. Selected clinical chemistry findings in rats exposed to MCPA in the diet for up to 2 years

Parameter	Mean value							
	0 ppm		20 ppm		80 ppm		320 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
ALT (µkat/l)								
Week 0	1.24	1.04	1.31	1.13	1.32	1.04	1.44**	1.06
Week 26	1.09	0.90	1.05	0.88	1.37	0.85	1.31*	0.94
Week 52	0.85	0.87	0.97**	0.91	1.05**	0.97**	0.95	1.04**
Week 78	0.81	0.81	0.84	0.88	0.95	0.86	0.87	0.93*
Week 104	0.81	0.79	0.79	0.80	0.84	0.80	0.91	0.92*
Triglycerides (mmol/l)								
Week 0	1.27	1.65	1.22	1.68	1.28	1.69	1.35	1.87
Week 26	3.35	4.62	3.92	3.31	4.55	3.81	3.39	3.82
Week 52	5.10	5.62	4.02	4.35	4.53	3.86*	3.58	3.38**
Week 78	5.60	6.55	4.21	5.78	5.19	5.45	3.81*	4.93*
Week 104	6.08	6.43	4.67	6.68	4.02*	6.11	3.94**	6.23

From Kirsch et al. (1988)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic abnormalities. The mean absolute and relative kidney weights of 320 ppm males (satellite group I) were approximately 13% higher than the control

values at the 12-month sacrifice, but only the difference in absolute kidney weight was statistically significant ($P < 0.05$). There was no similar finding in females or in both sexes at 24 months. The increased kidney weight in 320 ppm males at 52 weeks was coincident with an increase in the severity of chronic-progressive nephropathy (Table 19). Also at the highest dose, only in rats sacrificed at 52 weeks, there was an increase in the severity of pigment storage in the spleen. However, this finding occurred without any haematological correlates or pigment storage in other tissues. The mean relative (but not absolute) liver weight of 320 ppm females was significantly lower ($P < 0.01$) than the control value at both the 12-month (satellite group I) and 24-month sacrifice (main cohort) (13% and 8% lower than the control values, respectively). In the absence of any treatment-related histopathological abnormalities of the liver, these lower weights are attributable to the lower terminal body weight of the group. There were no treatment-related neoplastic lesions.

Table 19. Grading of chronic progressive nephropathy and pigment storage in the spleen in rats sacrificed after 12 months

Finding	Incidence (n = 10)							
	Males				Females			
	0 ppm	20 ppm	80 ppm	320 ppm	0 ppm	20 ppm	80 ppm	320 ppm
Chronic progressive nephropathy								
Total	10	10	10	10	10	10	10	10
Slight	9	5	5	2	3	9	6	4
Minimal	1	5	5	2	1	1	2	1
Moderate	0	0	0	6	0	0	0	0
Pigment storage in the spleen								
Total	10	9	9	10	10	10	10	10
Slight	7	6	7	3	2	6	3	1
Minimal	3	3	2	5	8	4	6	6
Moderate	0	0	0	2	0	0	1	3

From Kirsch et al. (1988)

The NOAEL was 80 ppm (equal to 4.8 mg/kg bw per day), based on increased kidney weight and slight increases in the severity of chronic progressive nephropathy in males and haemosiderin deposition at 320 ppm (equal to 19 mg/kg bw per day). MCPA had no carcinogenic potential under the conditions of the study (Kirsch et al., 1988).

2.4 Genotoxicity

The results of genotoxicity studies on MCPA, MCPA 2-EHE and MCPA DMA are summarized in Tables 20, 21 and 22, respectively. All unpublished studies contained statements of compliance with principles of GLP or international/national test guidelines.

Table 20. Results of genotoxicity assays on MCPA

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference
In vitro studies					
Gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	5–1000 µg/plate ±S9; ethanol vehicle	94.2	Negative	Jones et al. (1993a)[a]

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference
Gene mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Up to 5000 µg/plate ±S9; DMSO vehicle	NR	Negative	Moriya et al. (1983)
Gene mutation	*S. typhimurium* TA97a, TA98, TA100, TA1535, TA1537 and TA1538	10–1000 µg/plate ±S9; ethanol or DMSO vehicle	NR	Negative[b]	Kappas (1988)
Gene mutation	*S. typhimurium* TA98 and TA100	1 µmol/plate ±S9; DMSO vehicle	NR	Negative	Nishimura et al. (1982)
Gene mutation	*Escherichia coli* WP2 (hcr)	Up to 5000 µg/plate ±S9; DMSO vehicle	NR	Negative	Moriya et al. (1983)
Gene mutation	*Saccharomyces cerevisiae*	Up to 6 µmol/l; ethanol vehicle	NR	Negative[c]	Zetterberg (1979)
Gene mutation	CHO cells	50–1200 µg/ml; ethanol vehicle	94.2	Negative[d]	Adams et al. (1993a)[a]
Gene mutation	Chinese hamster V79 cells	0.05–2 µg/ml; culture medium vehicle	NR	Negative	Fiskesjö (1988)
Chromosomal aberration	Human lymphocytes	50–2000 µg/ml ±S9	94.2	Negative −S9 Positive[e] +S9	Akhurst et al. (1993a)[a]
DNA damage and repair, SCE	CHO cells	10^{-5}, 10^{-4} and 10^{-3} mol/l ±S9; culture medium vehicle	NR	Negative	Linnainmaa (1984)
DNA damage and repair, SCE	Chick embryos	0.35–2.6 mg/embryo or 0.1–4.2 mg/embryo	95	Positive[f]	Arias (1992, 1996)
DNA damage and repair, mitotic segregation	*Aspergillus nidulans*	750–3000 µmol/l; ethanol or DMSO vehicle	NR	Positive −S9[g] Negative +S9	Kappas (1988)
In vivo studies					
Micronucleus	Swiss CD-1 mice, bone marrow	0, 96, 192 or 383 mg/kg bw; 1% CMC	94.2	Negative	Proudlock et al. (1993a)[a]
Cytogenicity	Chinese hamsters, bone marrow	0, 33, 200 or 1200 mg/kg bw; 0.5% CMC	94.8	Negative	Engelhardt & Gelbke (1985c, 1986)[a]
Sex-linked recessive	*Drosophila melanogaster*	5 mmol/l in the diet for 3 days	NR	Negative	Vogel & Chandler (1973)
SCE	Chinese hamsters, bone marrow	0, 33, 200 or 1200 mg/kg bw; 0.5% CMC	94.8	Negative	Engelhardt & Gelbke (1985a,b)[a]
SCE	Chinese hamsters and Wistar rats, bone marrow	100 mg/kg bw per day for 2 weeks by gavage; saline vehicle	NR	Negative	Linnainmaa (1984)

Table 20 (continued)

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference
SCE	Wistar rats, lymphocytes	100 mg/kg bw per day for 2 weeks by gavage; DMSO vehicle	99	Negative	Mustonen et al. (1989)
DNA binding	SD male rats, liver	50 or 500 mg/kg bw; distilled water vehicle	97	Negative	McGregor (1986)

CHO, Chinese hamster ovary; CMC, carboxymethylcellulose; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; NR, not reported; S9, 9000 × *g* supernatant fraction of rat liver homogenate; SCE, sister chromatid exchange; SD, Sprague-Dawley

[a] Conducted in compliance with GLP.

[b] Significant increase in revertants at 250 ($P < 0.05$), 500 ($P < 0.01$) and 750 ($P < 0.05$) µg/plate in the presence of S9 in strain TA97a, but all increases were less than 1.3-fold above the control; no increases observed in the absence of S9 or in any of the other tested strains.

[c] Approximately 1.3-fold increase in mutants compared with the control at highly cytotoxic concentrations, where cell survival was less than 5%.

[d] Cytotoxicity at 1000 µg/ml and above.

[e] Significant increase ($P < 0.001$) in aberrant cells at 1750 µg/ml and at 1250 and 1500 µg/ml in a repeat test in the presence of S9 only. These positive responses occurred only in the presence of cytotoxicity.

[f] There was a 1.3-fold increase ($P = 0.048$) in SCEs per cell at 2.8 mg/embryo; significant increase ($P < 0.05$; < 2-fold higher than the control) in SCEs per cell at 2.8 and 4.2 mg/embryo concomitant with mortality (21–42% at 1.4–4.2 mg/embryo).

[g] Up to a 2-fold, non-significant increase in mitotic segregation compared with the vehicle control at 2250 and 3000 µmol/l concomitant with toxicity of approximately 20–30%.

Table 21. Results of genotoxicity assays on MCPA 2-EHE

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference[a]
In vitro studies					
Gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	50–5000 µg/plate ±S9; water vehicle	93.9	Negative	Jones et al. (1993b)
Gene mutation	CHO cells (HGPRT locus)	10–200 µg/plate ±S9; ethanol vehicle	93.9	Negative	Adams et al. (1993c)
Chromosomal aberration	Human lymphocytes	10–320 µg/ml ±S9; ethanol vehicle	93.9	Negative[b]	Akhurst et al. (1993c)
In vivo studies					
Micronucleus	Swiss CD-1 mice, bone marrow	0, 450, 900 or 1800 mg/kg bw in 1% CMC	93.9	Negative	Proudlock et al. (1993c)

CHO, Chinese hamster ovary; CMC, carboxymethylcellulose; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] All studies conducted in compliance with GLP.

[b] Toxicity and cell cycle delay at and above 80 g/ml.

Table 22. Results of genotoxicity assays on MCPA DMA

End-point	Test object	Concentration; vehicle	Purity (%)	Results	Reference[a]
In vitro studies					
Gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	50–5000 µg/plate ±S9; water vehicle	77.3	Negative	Jones et al. (1992)
Gene mutation	CHO cells (HGPRT locus)	250–3000 µg/plate ±S9; water vehicle	77.3	Negative[b]	Adams et al. (1993b)
Chromosomal aberration	Human lymphocytes	100–5000 µg/ml ±S9; water vehicle	77.3	Negative −S9 Positive +S9[c]	Akhurst et al. (1993b)
In vivo studies					
Micronucleus	Swiss CD-1 mice, bone marrow	0, 144, 288 or 576 mg/kg bw; water vehicle	77.3	Negative[d]	Proudlock et al. (1993b)

CHO, Chinese hamster ovary; S9, 9000 × *g* supernatant fraction of rat liver homogenate

[a] All studies conducted in compliance with GLP.

[b] Cytotoxicity at 1250 µg/ml and above.

[c] Significant increase ($P < 0.05$) in aberrant cells (2.5% versus 0.5% in the controls) at 1000 µg/ml in the absence of S9; significant increases in aberrant cells at 250 ($P < 0.01$), 1000 ($P < 0.001$) and 2000 µg/ml ($P < 0.001$) (3.5%, 5% and 13.5% versus 0.5% in the controls, respectively); cytotoxicity at 2000 µg/ml and above.

[d] Decrease in polychromatic erythrocytes at 48 hours, indicative of a transient depression in bone marrow.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

MCPA (purity 94.8%) was admixed in the diet at a concentration of 0, 50, 150 or 450 ppm and fed ad libitum to two parental generations of Crl:CD®(SD)BR rats (25 of each sex per group) and their offspring. Rats were observed for mortalities and clinical signs at least twice daily, with a more detailed clinical assessment made weekly. Body weight and feed consumption were recorded prior to treatment and weekly during the premating period; dams were weighed on days 0, 4, 7, 14 and 20 of gestation. Standard reproduction and litter parameters were recorded or calculated. Necropsies were performed on all parental rats and 10 pups of each sex per group. The following tissues were examined histopathologically in all control and high-dose F_0 and F_{1b} adult rats: gross lesions, epididymides, kidneys, ovaries, prostate, seminal vesicles, testes, uterus and vagina.

Estimated doses received by rats throughout all phases of the study were calculated by the sponsor based on weekly feed consumption and mean body weight and are presented in Table 23.

There were no treatment-related deaths or clinical signs. Body weight gain was unremarkable in F_0 and F_1 parental rats, noting that absolute body weight was significantly lower ($P < 0.05$) than the control value (by about 10%) at 450 ppm in F_1 rats of both sexes early in the premating period. Feed consumption by F_0 parental rats was unremarkable, whereas significantly higher ($P < 0.05$) feed consumption was determined at 450 ppm in F_{1b} females during the premating and rest periods (up to 13% higher than the control value) and in F_{1b} males during the rest period (approximately 5% higher than the control value); these increases in feed consumption are not considered toxicologically significant.

Table 23. Doses of MCPA received by parental rats

Phase	Dose (mg/kg bw per day)					
	50 ppm		150 ppm		450 ppm	
	Males	Females	Males	Females	Males	Females
F_0 premating						
Week 1	5.3	5.5	15.7	17.5	47.4	52.0
Week 5	3.5	4.4	10.4	13.4	32.3	41.5
Week 10	2.8	3.5	8.3	11.4	25.2	32.4
Mean	3.6	4.4	10.7	12.7	39.6	41.0
F_1 premating						
Week 1	7.7	7.9	22.8	23.9	72.5	78.6
Week 5	4.0	5.0	12.2	14.4	37.1	47.4
Week 10	3.1	3.9	8.9	11.7	28.2	37.7
Mean	4.5	5.2	13.4	15.5	41.5	45.8

From MacKenzie (1986); Bellet et al. (2000)

At 450 ppm, body weight gain between days 14 and 21 for F_{1a} pups (males and females; approximately 12% lower than the control value) and F_{1b} pups (females; approximately 9% lower than the control value) was significantly lower ($P < 0.05$) than the control value. The absolute body weight of F_{2a} pups was significantly lower ($P < 0.05$) than the control value on day 14 (males; 8% lower) and day 21 (both sexes; approximately 10% lower). Body weight gain by F_{2a} male pups was significantly lower than the control value ($P < 0.05$) at 150 ppm (days 14–21, 9% lower) and 450 ppm (days 4–14, 9% lower; and days 14–21; 14% lower). In the F_{2b} litters, female body weight (day 4) and body weight gain (days 0–4) were significantly lower ($P < 0.05$; approximately 15% lower) than the control value at 50 ppm, but not at higher doses or other times. At 450 ppm, absolute body weight of F_{2b} pups at day 14 (males; approximately 7% lower) and day 21 (males and females; approximately 8% lower) and female body weight gain between days 14 and 21 (approximately 12%) were significantly lower ($P < 0.05$) than the control values. The consistency of the lower absolute body weight and body weight gain in pups at 450 ppm is clearly attributable to treatment. Significant differences in either absolute body weight or body weight gain at 50 or 150 ppm showed no consistency between generations, litters or sexes and on this basis are unlikely to be treatment related.

There was no treatment-related effect on reproduction or litter parameters or the incidence of gross or histopathological abnormalities.

The NOAEL for reproductive toxicity was 450 ppm (approximately equal to 40 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 150 ppm (approximately equal to 12 mg/kg bw per day), based on lower absolute body weight at 450 ppm. The NOAEL for pup toxicity was also 150 ppm, based on reduced body weight gain at 450 ppm (MacKenzie, 1986; Bellet et al., 2000).

A one-generation reproduction study was conducted in groups of Alpk:AP_fSD (Wistar-derived) rats (12 of each sex per group) that were fed diets containing MCPA (purity 97.4%) at a concentration of 0, 450, 750 or 1000 ppm. As the purpose of the study was to clarify the dose–response relationship in rat pups compared with adults, feed consumption was measured throughout all phases of the study to allow an accurate determination of the achieved dose. From day 1 postpartum, the concentrations of MCPA in the diet were reduced to 300, 500 and 667 ppm, respectively, to avoid administering particularly high doses to lactating dams and pups. Litters were raised to weaning (day 29 postpartum), then 10 F_1 pups of each sex were selected for a further 2 weeks of exposure to the premating dietary concentrations (450, 750 and 1000 ppm, respectively). Body weight was recorded at least weekly. Reproductive performance was determined by examining

mating success, gestation length and precoital interval. Pup survival and body weight were recorded during lactation. Parental rats and offspring were necropsied. Kidney, liver and ovaries or testes were weighed in parental rats and selected F_1 offspring. Achieved doses throughout the study are presented in Table 24.

Table 24. Achieved doses of MCPA in rats

Stage	Achieved dose (mg/kg bw per day)					
	450 ppm		750 ppm		1000 ppm	
	Males	Females	Males	Females	Males	Females
F_0 premating	38.9	41.7	67.1	69.2	88.9	89.0
Gestation	—	35.7	—	57.0	—	75.4
Lactation	—	62.6	—	98.8	—	122.2
Selected F_1 rats	76.1	72.2	114.7	114.9	157.4	156.1

From Milburn (2004a)

There were no treatment-related deaths or clinical signs. During the premating exposure period at 1000 ppm, absolute body weight was significantly lower than the control value during most weeks ($P < 0.01$ or 0.05; up to 9% and 6% lower than the control values in males and females, respectively). At 750 and 450 ppm, the difference in absolute body weight was significant only within the first 3 weeks of treatment ($P < 0.05$; up to 4% lower than the control value). During gestation, the mean body weight of 1000 ppm dams was significantly lower than the control value on day 1 ($P < 0.05$; 5% lower than the control value). There was no treatment-related effect on body weight during lactation. These lower body weights were coincident with significantly lower ($P < 0.01$ or 0.05) feed consumption.

Pup body weights were significantly lower ($P < 0.01$; approximately 8% lower than the control value) only at the highest dietary concentration (667 ppm) on day 29 of lactation.

In selected F_1 rats, mean absolute body weights were significantly lower ($P < 0.01$ or 0.05) than the control value across all doses and in both sexes; the magnitude of the difference relative to the control group was very similar to that occurring during the premating period.

There was no treatment-related effect on reproduction or litter parameters.

There were no treatment-related macroscopic abnormalities.

Absolute liver, ovary and testes weights were significantly lower ($P < 0.01$ or 0.05) than the control values in high-dose F_1 males and females (approximately 10% lower), but when adjusted for body weight, there was no difference relative to the controls. Relative kidney weight was significantly elevated ($P < 0.05$; approximately 10% higher than the control value) in high-dose males.

The NOAEL for reproductive toxicity was 1000 ppm (approximately equal to 160 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was less than 450 ppm (approximately equal to 40 mg/kg bw per day), based on reduced body weight observed at this dose. The NOAEL for offspring toxicity was 750 ppm (approximately equal to 115 mg/kg bw per day), based on reduced body weight at the highest dose of 1000 ppm (approximately equal to 160 mg/kg bw per day) (Milburn, 2004a).

A one-generation reproduction study on MCPA 2-EHE (purity 96.2%) was conducted in parallel with the preceding study by Milburn (2004a) and shared a control group. The experimental conditions were comparable, except that the concentrations of test material in the diet were 0, 700, 1200 and 1600 ppm during the premating period and 2-week exposure period of F_1 rats, decreasing to

0, 467, 800 and 1067 ppm, respectively, during lactation. Achieved doses throughout the study are presented in Table 25.

Table 25. Achieved doses in rats

Stage	Achieved dose (mg/kg bw per day expressed as MCPA acid equivalents)					
	700 ppm		1200 ppm		1600 ppm	
	Male	Female	Male	Female	Male	Female
F_0 premating	64.6	66.3	105.9	112.8	144.9	151.2
Gestation	—	54.0	—	90.3	—	126.4
Lactation	—	91.6	—	162.9	—	217.1
Selected F_1 rats	107.1	106.7	178.4	187.9	232.3	249.1

From Milburn (2004b)

There were no treatment-related deaths or clinical signs. During the premating period (mainly weeks 2–5), the mean body weight of F_0 rats was significantly lower ($P < 0.01$ or 0.05) than the control value at 700 ppm (up to about 3–4% lower), 1200 ppm (up to about 5–6% lower) and 1600 ppm (up to about 5–7% lower). At 1200 and 1600 ppm, the body weight of dams was significantly lower ($P < 0.05$) than the control value on day 15 of gestation (4% and 6% lower, respectively). At 1600 ppm, the mean absolute body weights of F_1 males and females were up to 13% and 11% lower ($P < 0.01$ or 0.05) than the control values, respectively, during the 2-week post-selection exposure period. At 700 and 1200 ppm, body weight was maximally 3–4% and 7–8% lower than the control values, respectively, but was statistically significantly lower ($P < 0.01$) only for low-dose males at week 2. At 1600 ppm, significantly lower ($P < 0.05$) feed consumption was noted in F_0 females during week 1 of the premating period and F_1 males at 1200 ppm (week 2 of the post-selection period) and 1600 ppm (weeks 1 and 2 of the post-selection period).

There was no effect on reproductive performance, pup survival or litter size. At 1600/1067 ppm, male and female pup weights were 5% and 9% lower ($P < 0.05$ in males; $P < 0.01$ in females) than the control values, respectively, on day 29 of lactation. At 1200/800 ppm, female pup weights were 6% lower ($P < 0.05$) than the control weights on day 29 of lactation.

There were no treatment-related macroscopic abnormalities. In F_0 dams, absolute (but not relative) kidney weights were significantly higher ($P < 0.01$ or 0.05) than the control values across all doses, without a clear dose–response relationship (absolute: 2.21, 2.49, 2.46 and 2.48 g at 0, 700, 1200 and 1600 ppm, respectively; relative: 0.69%, 0.77%, 0.75% and 0.78% at 0, 700, 1200 and 1600 ppm, respectively). At 1600 ppm, the absolute weights of the liver and testes of F_1 males were significantly lower ($P < 0.01$ or 0.05) than the control values (liver: 9.8 g versus 11.3 g; testes: 1.80 g versus 1.96 g). Absolute ovary weights were also significantly lower ($P < 0.01$ or 0.05) than the control values across all doses (0.083, 0.069, 0.066 and 0.069 g at 0, 700, 1200 and 1600 ppm, respectively). These apparent reductions in kidney, ovary, liver and testes weights were considered to reflect the reduced body weight of immature rats.

The NOAEL for reproductive toxicity was 1600 ppm (approximately equal to 150 mg/kg bw per day expressed as acid equivalents), the highest dose tested. The NOAEL for parental toxicity was less than 700 ppm (approximately equal to 65 mg/kg bw per day expressed as acid equivalents), based on reduced body weight at this dose. The NOAEL for offspring toxicity was 1200 ppm (equal to a minimum of about 90 mg/kg bw per day expressed as acid equivalents), based on reduced body weight at 1600 ppm (approximately equal to 230 mg/kg bw per day expressed as acid equivalents) (Milburn, 2004b).

(b) Developmental toxicity

Rats

In a pre-GLP/guideline range-finding study, MCPA (unspecified purity) was administered in 1% w/v carboxymethylcellulose by gavage to groups of five mated SD rats (CD strain) at a dose of 25 or 100 mg/kg bw per day from day 0 to day 20 of gestation. Ten control rats received the vehicle only. Observations for clinical signs were made daily throughout the dosing period. Body weight was recorded every 3 days. All dams were sacrificed on day 21 of gestation; fetuses were examined for external, visceral and skeletal abnormalities.

There were no maternal deaths or treatment-related clinical signs. Body weight gain was approximately 22% and 13% lower than the control values at 25 and 100 mg/kg bw per day, respectively. Fetal weight and crown–rump length were significantly lower ($P < 0.05$) than the control values at 100 mg/kg bw per day (fetal weight: 4.9 g versus 5.9 g in the controls; crown–rump length: 40.8 mm versus 44.1 mm in the controls), but all values were stated to be within the normal range for this particular rat strain (Irvine & Tucker, 1978a).

In the follow-up study by Irvine (1980a), MCPA (unspecified purity) was administered by gavage to groups of five mated SD rats (CD strain) in 1% w/v carboxymethylcellulose at a dose of 0, 20, 50 or 125 mg/kg bw per day from day 6 to day 15 of gestation. Observations for clinical signs were made daily, whereas body weight and feed consumption were recorded every 3 days. Surviving dams were sacrificed on day 21 of gestation and fetuses examined for external, visceral and skeletal abnormalities.

There were no treatment-related findings. The small group sizes preclude the establishment of a reliable NOAEL, but when considered with the preceding range-finding study, the results indicate that MCPA was not teratogenic or embryotoxic.

In a range-finding study conducted by Hellwig et al. (1992a), MCPA (purity 94.22%) in 0.5% w/v carboxymethylcellulose was administered by gavage to groups of 10 pregnant Wistar rats at a dose of 0, 80, 120 or 160 mg/kg bw per day from day 6 to day 15 of gestation. Clinical signs, body weight and feed consumption were recorded at various times. Blood was sampled on day 16 of gestation for the analysis of haematology and clinical chemistry parameters. Surviving dams were sacrificed at this same time and examined macroscopically. Liver and kidney weights were recorded. Fetuses (plus the placenta) were weighed and examined for external abnormalities.

There were no deaths. At the highest dose, two of five dams had vaginal haemorrhage on day 15 of gestation. Selected maternal findings are summarized in Table 26. There was a dose-related reduction in body weight gain and feed consumption, which was statistically significant ($P < 0.01$) at 120 and 160 mg/kg bw per day. Perturbations in haematology and clinical chemistry parameters, including increased enzyme activity, occurred at and above 80 mg/kg bw per day and were suggestive of anaemia and impaired liver function. The relative kidney weight of high-dose dams was approximately 9% higher ($P < 0.05$) than the control value, whereas gravid uterus weight was approximately 24% lower ($P < 0.05$). The weight of the placenta was significantly lower than the control value at 120 and 160 mg/kg bw per day. The body weight of viable fetuses was significantly lower ($P < 0.01$ or 0.05) than the control values at every dose (0.53, 0.49, 0.43 and 0.37 g at 0, 80, 120 and 160 mg/kg bw per day, respectively). There were no treatment-related fetal abnormalities.

In the main study, MCPA (purity 94.22%) in 0.5% w/v carboxymethylcellulose was administered by gavage to groups of 22–24 pregnant Wistar rats (Chbb:THOM (SPF) strain) at a dose of 0, 15, 60 or 120 mg/kg bw per day from day 6 to day 15 of gestation. Dams were observed daily throughout gestation for clinical signs of toxicity, with body weight and feed consumption recorded throughout this period. On day 20 of gestation, surviving dams were sacrificed and fetuses examined for external, visceral and skeletal abnormalities.

Table 26. Findings in maternal rats dosed with MCPA from day 6 to day 15 of gestation

Parameter	Mean value			
	0 mg/kg bw per day	80 mg/kg bw per day	120 mg/kg bw per day	160 mg/kg bw per day
Body weight gain, days 6–15 (g)	44.4	38.2	28.2**	26.1**
Feed consumption, days 6–15 (g/rat per day)	25.6	24.2	21.1**	20.1**
Haemoglobin (mmol/l)	7.29	6.87*	6.83*	6.60**
Haematocrit (l/l)	0.39	0.37*	0.036**	0.035**
Mean corpuscular volume (fl)	60.47	58.60	58.51*	58.01**
Platelets ($\times 10^9$/l)	1266	1118	1106*	920**
ALT (µkat/l)	1.24	1.48	1.74**	1.90**
AST (µkat/l)	1.50	1.83	2.29*	2.42**
ALP (µkat/l)	5.35	5.86	6.81	7.21*
Calcium (mmol/l)	3.02	2.94	2.90*	2.88**
Total protein (g/l)	69.59	63.58**	62.59**	61.57**
Globulin (g/l)	30.09	26.40**	24.66**	23.69**
Triglycerides (mmol/l)	4.99	3.92	3.32**	2.60**
Cholesterol (mmol/l)	2.39	1.95**	1.62**	1.58**
Relative kidney weight (%)	0.66	0.66	0.68	0.72*
Gravid uterus weight (g)	21.6	21.6	18.2	16.4*
Placenta weight (from viable fetuses) (g)	0.26	0.25	0.24*	0.20**

From Hellwig et al. (1992a)
* $P < 0.05$; ** $P < 0.01$

There were no deaths and no treatment-related clinical signs. At 120 mg/kg bw per day, body weight gain was approximately 23% lower ($P < 0.01$) than the control value over the dosing period, concomitant with significantly lower ($P < 0.01$ or 0.05) feed consumption (up to 17% lower than the control value). The lower body weight gain and feed consumption were evident within 2 days of the commencement of dosing (days 6–8 body weight gain = 4.1 g versus 7.8 g in the controls, $P < 0.01$; days 6–8 feed consumption = 21.1 g versus 24.8 g in the controls, $P < 0.01$). Body weight gain and feed consumption were unremarkable at 15 and 60 mg/kg bw per day. There were no treatment-related macroscopic findings in dams.

At 120 mg/kg bw per day, mean placenta weight was approximately 10% lower than the control value, but was statistically significant ($P < 0.05$) only for placentas belonging to female fetuses. Also at 120 mg/kg bw per day, mean fetal body weight was approximately 12% lower ($P < 0.01$) than the control value.

External examination revealed severe malformations of the head in two high-dose fetuses (brachygnathia, microglossia, unilateral anophthalmia, proboscis, aglosstomia, caudal displacement of the left ear, bilateral anophthalmia and hydrocephaly). The study author noted that proboscis is a very rare malformation and was likely to have arisen spontaneously. There was no overall treatment-related increase in the incidence of soft tissues or skeletal malformations. Also at the highest dose was a significant ($P < 0.05$) increase in the number of fetuses with an incompletely ossified skull or incompletely or unossified sternebrae (Table 27); this may be attributable to the reduced fetal body weight at this dose.

Table 27. Incidence of skeletal retardations in fetuses

Finding	Incidence (%)			
	0 mg/kg bw per day	15 mg/kg bw per day	60 mg/kg bw per day	120 mg/kg bw per day
Skull incompletely ossified				
Fetal incidence	0.6	0	1.9	7.4**
Litter incidence	4.3	0	9.1	30*
Sternebrae not ossified				
Fetal incidence	6.2	3.4	9.4	45**
Litter incidence	30	21	32	78**
Sternebrae incompletely ossified or reduced in size				
Fetal incidence	22	9.1**	21	39**
Litter incidence	65	50	64	83
Sternebrae: only one ossification centre				
Fetal incidence	5	11	11	16**
Litter incidence	26	50	64*	61*
Total skeletal retardation				
Fetal incidence	63	57*	59	84**
Litter incidence	91	100	95	91

From Hellwig & Hildebrand (1993a)
* $P < 0.05$; ** $P < 0.01$

The NOAEL for maternal toxicity was 60 mg/kg bw per day, based on reduced body weight gain and feed consumption at 120 mg/kg bw per day. The NOAEL for fetal toxicity was also 60 mg/kg bw per day, based on reduced fetal weight and delayed skeletal maturation at 120 mg/kg bw per day (Hellwig & Hildebrand, 1993a).

MCPA 2-EHE (purity 99.9%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 25 pregnant SD rats (Crl:CD®(SD)BR strain) from days 6 to 19 of gestation at a dose of 0, 15, 40 or 120 mg/kg bw per day expressed as acid equivalents. Dams were observed daily throughout gestation for clinical signs. Body weight and feed consumption were recorded during gestation. Surviving dams were sacrificed on day 20 of gestation and fetuses weighed and examined for external malformations. The numbers of corpora lutea and implantation sites were determined. Maternal kidney and brain weights were recorded. Half of the fetuses were prepared and examined for visceral abnormalities, with the remainder examined for skeletal abnormalities.

There were no deaths or treatment-related clinical signs. At the highest dose, maternal body weight gain and feed consumption were significantly lower ($P < 0.01$ or 0.05) than the control values across the entire treatment period (approximately 39% and 13% lower, respectively). The lower body weight gain and feed consumption were evident within 1 day of the commencement of dosing (days 6–7 body weight gain = −3 g versus 2 g in the controls, $P < 0.01$; days 6–7 feed consumption = 19 g versus 22 g in the controls, $P < 0.01$). Also at the highest dose, relative kidney and brain weights were significantly higher ($P < 0.01$) than the control values (kidney: 0.62 g/100 g versus 0.53 g/100 g in the controls; brain: 0.53 g/100 g versus 0.46 g/100 g in the controls), which was attributable to the lower terminal body weight of this group.

Two whole-litter resorptions occurred at 120 mg/kg bw per day. Significantly increased ($P < 0.01$) post-implantation losses (mainly early resorptions) occurred at 120 mg/kg bw per day (15.1% per litter versus 3.5% in the controls; historical control maximum = 13.5%, not statistically

significant), concomitant with a decrease in viable litter size (approximately 85% versus 97% in the controls; not statistically significant). Mean fetal body weight at 120 mg/kg bw per day (2.5 g) was significantly lower ($P < 0.01$) than the control value (3.3 g).

The incidence of malformations (external, visceral and skeletal) was increased at 120 mg/kg bw per day (5.7% versus 0.2% per litter in the control). Treatment-related fetal observations included hydrocephaly (one fetus from each of two litters at 120 mg/kg bw per day) and bent scapula and/or humerus bones (13 fetuses from two litters at 120 mg/kg bw per day). Both of these findings exceeded the performing laboratory's historical control range (0.6% versus 0.3% per litter; 4.9% versus 0.5% per litter, respectively).

The incidence of skeletal variations was significantly increased ($P < 0.01$) at 120 mg/kg bw per day (71.8% versus 35.3% in the control). This was due predominantly to a significant increase ($P < 0.01$) in the incidence of delayed ossification (sternebra 5 or 6 unossified: 61.5% versus 17% per litter in the controls; sternebra 1, 2, 3 or 4 unossified: 9.8% versus 0% per litter in the controls) and bent ribs (6.9% versus 0.3% per litter in the controls; $P < 0.05$).

The NOAEL for maternal toxicity was 40 mg/kg bw per day expressed as acid equivalents, based on reduced body weight gain and feed consumption at 120 mg/kg bw per day expressed as acid equivalents. The NOAEL for fetal toxicity was also 40 mg/kg bw per day expressed as acid equivalents, based on increased post-implantation loss, reduced fetal body weight and fetal anomalies (mainly delayed ossification) at 120 mg/kg bw per day expressed as acid equivalents (Cappon, 1999a).

MCPA DMA (purity 78.2%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 25 pregnant SD rats (Crl:CD®(SD)BR strain) from days 6 to 19 of gestation at a dose of 0, 15, 50 or 150 mg/kg bw per day expressed as acid equivalents. The study design was identical to that described in the preceding study on MCPA 2-EHE.

At 150 mg/kg bw per day, one dam was euthanized in extremis on day 12 of gestation. All remaining dams survived until scheduled termination. Clinical signs observed only at the highest dose during the dosing period included yellow or brown matting or staining of the fur in the urogenital region (up to seven dams), decreased defecation (four dams) and abnormal gait (five dams). Post-dosing clinical signs included wiping the mouth or face on the sides of the cage (all high-dose dams).

At the highest dose, maternal body weight gain and feed consumption were significantly lower ($P < 0.01$ or 0.05) than the control values across the entire treatment period (approximately 57% and 22% lower, respectively). The lower body weight gain and feed consumption were evident within 1 day of the commencement of dosing (days 6–7 body weight gain = −4 g versus 1 g in the controls, $P < 0.01$; days 6–7 feed consumption = 17 g versus 21 g in the controls, $P < 0.01$). Also at the highest dose, mean absolute and relative kidney weights were approximately 10% higher than the control group values; these differences were statistically significant ($P < 0.01$).

Five whole-litter resorptions (mainly early resorptions) occurred at 150 mg/kg bw per day. Significantly increased ($P < 0.01$) post-implantation losses occurred at 150 mg/kg bw per day (41.8% versus 3.8% per litter in the controls; performing laboratory's historical control maximum = 13.5% per litter), concomitant with a significant decrease ($P < 0.01$) in viable litter size (58.3% versus 96.2% in the controls; performing laboratory's historical control minimum = 86.5% per litter). Mean fetal body weight at 150 mg/kg bw per day (2.1 g) was significantly lower ($P < 0.01$) than the control value (3.5 g), which was lower than the performing laboratory's historical control minimum of 3.3 g.

Total malformations were significantly elevated ($P < 0.01$) at the highest dose (0.8%, 0%, 0% and 23.4% per litter at 0, 15, 50 and 150 mg/kg bw per day, respectively). Three fetuses from three litters in the control group and six fetuses from five litters at 150 mg/kg bw per day had external malformations. The authors noted the occurrence of localized oedema of the neck and/or thorax in three high-dose fetuses (4.4%), which was above the historical control maximum.

The incidence of skeletal malformations was increased at 150 mg/kg bw per day (23% of fetuses per litter versus 0% in the controls). Bent limb bones (scapula, humerus and/or clavicle) and rib abnormalities (fused ribs) were significantly increased ($P < 0.01$ or 0.05) at 150 mg/kg bw per day: 22.6% and 3.3% per litter, respectively, versus 0% in the controls, which exceeded the performing laboratory's historical control maxima of 0.5% and 0.6% per litter, respectively.

The incidence of skeletal variations was also significantly increased ($P < 0.01$ or 0.05) at 150 mg/kg bw per day (90.4% versus 41.8% in the controls). These variations included the following: sternebra 5 and/or 6 unossified (79.8% versus 21.4% in the controls, $P < 0.01$); 14th rudimentary rib (14.9% versus 2.5% in the controls, $P < 0.01$); sternebra 1, 2, 3 and/or 4 unossified (40% versus 0.5% in the controls, $P < 0.01$); bent rib (39.3% versus 0% in the controls, $P < 0.01$); and entire sternum unossified (5.9% versus 0.2% in the controls, $P < 0.01$). The majority of these findings were greater than the performing laboratory's historical control range.

The NOAEL for maternal toxicity was 50 mg/kg bw per day expressed as acid equivalents, based on clinical signs, reduced body weight gain and reduced feed consumption at 150 mg/kg bw per day expressed as acid equivalents. The NOAEL for fetal toxicity was also 50 mg/kg bw per day expressed as acid equivalents, based on increased post-implantation losses, reduced fetal body weight and increased fetal anomalies at 150 mg/kg bw per day expressed as acid equivalents (Cappon, 1999b).

Rabbits

In a non-guideline, range-finding study that predated principles of GLP, MCPA (unspecified purity) in 1% w/v carboxymethylcellulose was administered by gavage to groups of five inseminated Dutch Belted rabbits at a dose of 0 ($n = 10$), 25 or 100 mg/kg bw per day from day 1 to day 27 post-insemination. Dams were observed daily throughout gestation for clinical signs of toxicity, and body weights were recorded every 3 days. On day 28, survivors were sacrificed and examined macroscopically. Fetuses were delivered and examined.

At 100 mg/kg bw per day, one rabbit died on day 10, preceded by ataxia and body weight loss (approximately 11%). Ataxia was observed in another high-dose rabbit that survived to scheduled termination. There were no treatment-related findings at 25 mg/kg bw per day. Pregnancy was low across all groups (6/10, 2/5 and 3/5 at 0, 25 and 100 mg/kg bw per day, respectively). At 100 mg/kg bw per day, all fetuses died in utero; however, prenatal mortality was also particularly high at 0 and 25 mg/kg bw per day. There were no treatment-related malformations or variations observed at 25 mg/kg bw per day. Fetal weight (30 g) and length (88 mm) were lower than the control values at 25 mg/kg bw per day (37 g and 94 mm, respectively) (Irvine & Tucker, 1978b).

In the main study by Irvine (1980b), conducted under similar conditions, MCPA (unspecified purity) in 1% w/v carboxymethylcellulose was administered by gavage to groups of 15–18 inseminated Dutch Belted rabbits at a dose of 0 ($n = 30$), 5, 12, 30 or 75 mg/kg bw per day from day 6 to day 18 post-insemination.

Deaths were observed at and above 12 mg/kg bw per day (two, three and five rabbits at 12, 30 and 75 mg/kg bw per day, respectively). The authors noted that these deaths occurred in association with a respiratory infection, which appeared to have been exacerbated by treatment. At 75 mg/kg bw per day, body weight loss occurred during the first half of the dosing period, but was comparable to the control value by the end of pregnancy. Overall (days 0–28) body weight gain was somewhat lower than the control value at the highest dose (10.6%, 10.1%, 4.8%, 9.1% and 8.6% at 0, 5, 12, 30 and 75 mg/kg bw per day, respectively). Feed consumption was unaffected by treatment.

Post-implantation loss was higher than the control value at all doses (1.1%, 2.4%, 8.8%, 3% and 16% at 0, 5, 12, 30 and 75 mg/kg bw per day, respectively). However, only at 75 mg/kg bw per day was post-implantation loss higher than the historical control range (due partly to a dam resorbing a whole litter). The mean number of fetuses per dam was slightly lower than the control value at 75 mg/kg bw per day (7.21, 6.92, 6.89, 6.86 and 6.18 at 0, 5, 12, 30 or 75 mg/kg bw per day,

respectively); however, total litter weight was unremarkable. At 75 mg/kg bw per day, fetal weight and crown–rump length were higher than the control values, but not significantly so (35.9 g versus 34.2 g and 97.5 mm versus 94.6 mm, respectively).

There were no treatment-related external, visceral or skeletal malformations.

The NOAEL for maternal toxicity was 5 mg/kg bw per day, based on mortality at and above 12 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 30 mg/kg bw per day, based on increased post-implantation losses at 75 mg/kg bw per day. MCPA was not teratogenic under the conditions of this study. Because of a number of limitations, including the possible confounding effect of the underlying respiratory illness, these NOAELs are not considered suitable for risk assessment purposes.

In a range-finding study, MCPA (purity 94.22%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of five inseminated Himalayan rabbits (Chbb:HM outbred strain) at a dose of 0, 50, 75 or 100 mg/kg bw per day from day 7 to day 19 post-insemination. Dams were observed daily throughout gestation for clinical signs of toxicity. Body weight and feed consumption were recorded every 2 or 3 days. Blood was sampled on day 20 for the analysis of haematology and clinical chemistry parameters. Surviving dams were sacrificed on day 26 and necropsied, and the kidneys and liver were weighed. Fetuses and the placenta were weighed and examined macroscopically.

Deaths occurred at 75 and 100 mg/kg bw per day (one and two dams, respectively) between days 16 and 21 of gestation, which were preceded by a general decline in health. Feed consumption was significantly lower ($P < 0.01$) than the control value at both 75 and 100 mg/kg bw per day during the dosing and post-dosing periods (approximately 14% and 36% lower, respectively). However, only the body weight gain at 100 mg/kg bw per day was consistently lower than the control value during the treatment period (but not significantly so). There was no treatment-related haematology or clinical chemistry findings. Organ weights and necropsy findings were unremarkable. While post-implantation losses were higher than the control value (5%) at 75 and 100 mg/kg bw per day (17.9% and 17.5%, respectively), they were within the performing laboratory's historical control range of 3–23.1%. There were no treatment-related fetal abnormalities (Hellwig et al., 1992b).

In the main study by Hellwig & Hildebrand (1993b), MCPA (purity 94.22%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 15 pregnant Himalayan rabbits (Chbb:HM outbred strain) at a dose 0, 15, 30 or 60 mg/kg bw per day from day 7 to day 19 post-insemination. Dams were observed daily for clinical signs of toxicity. Body weights were recorded every 2 or 3 days. Survivors were sacrificed on day 29 of gestation and necropsied. Fetuses were delivered and examined for external, visceral and skeletal abnormalities.

At 60 mg/kg bw per day, one dam died on day 20, and another was sacrificed in a moribund condition on day 21. Prior to death/sacrifice, both dams exhibited clinical signs (piloerection, no defecation). At necropsy, ulcerations of the stomach mucosa were detected in both decedents. At 30 mg/kg bw per day, one dam aborted its litter on day 21 and was subsequently sacrificed. At necropsy, focal haemorrhagic oedema of the colon was observed. Blood was detected in the bedding of one dam each at 30 and 60 mg/kg bw per day. At 60 mg/kg bw per day, dams lost a mean of approximately 15 g body weight over the dosing period, while control dams gained approximately 30 g; this difference was not statistically significant. Coincident with this loss of body weight was reduced feed consumption (77.1 g), which was significantly lower ($P < 0.05$) than the control value (85.5 g). There were no other treatment-related effects detected in dams, and no indication of embryo and fetal toxicity or teratogenicity at any dose.

The NOAEL for maternal toxicity was 15 mg/kg bw per day, based on clinical signs and mortality at and above 30 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 60 mg/kg bw per day, the highest dose tested.

In a published study by Ujházy et al. (2006), MCPA (purity unspecified) in 1% w/v aqueous carboxymethylcellulose was administered to groups of 20 New Zealand White rabbits by gavage at a dose of 0, 5, 10 or 25 mg/kg bw per day from days 7 to 27 of gestation. Clinical condition was observed daily, and body weight was measured at appropriate intervals throughout the study. Dams were fed a fixed weight of diet each day (100 g). Rabbits were sacrificed on day 28 of gestation and examined macroscopically. Gravid uterine weight was recorded, and pups were weighed, sexed and examined for external abnormalities. Approximately one third of pups were used for detailed evaluation of viscera, and the remainder were subjected to skeletal examination.

The number of pregnant females at the highest dose (12/20) was lower than the desired minimum (16), which was not attributable to death or visible abortion. There was some evidence of misdosing (lung dosing) across all groups. There were no treatment-related mortalities or clinical signs. When corrected for predosing differences (body weights were unevenly distributed upon allocation and were significantly different prior to the commencement of dosing), there was no treatment-related effect on body weight. There were no treatment-related macroscopic abnormalities in dams.

At 25 mg/kg bw per day, mean fetal weight (31.26 g) was significantly lower ($P < 0.05$) than the control value (32.45 g). Placental weight was significantly lower ($P < 0.05$) than the control value at 5 and 25 mg/kg bw per day (5.38, 4.92, 5.40 and 5.07 g at 0, 5, 10 and 25 mg/kg bw per day, respectively). The authors attributed these differences in body and placental weights to the relatively low body weight of dams allocated to the high-dose group, noting that they were within the range of biological variability. On this basis, these statistically significant differences are not considered to be treatment related.

No skeletal or visceral malformations were detected. In some bones (skull and pelvis), the incidence of delayed ossification was significantly higher ($P < 0.01$) than in the controls, whereas in other bones (sternebrae, forelimbs and hindlimbs), there was no difference relative to the controls, which had a relatively high background incidence of delayed ossification. As the occurrence of delayed ossification across all groups was highly variable and demonstrated no consistent pattern, it was not considered to be treatment related.

The NOAEL for maternal and embryo and fetal toxicity was 25 mg/kg bw per day, the highest dose tested. However, because of a number of limitations to the study, this NOAEL is not considered to be suitable for risk assessment purposes.

(c) In vitro studies

In a published study, inhibition of testicular deoxyribonucleic acid (DNA) synthesis by MCPA (unspecified purity) was investigated following a single gavage dose of 200 mg/kg bw to mice (unspecified strain and group size). One hour before dosing, male mice were injected intraperitoneally with 37 kBq [^{14}C]thymidine and then reinjected 3 hours after dosing with 370 kBq [^{3}H]thymidine. After a further 30 minutes, the animals were sacrificed, the testes were removed and the radioactive uptake was measured. The uptake of thymidine in the testes was inhibited by 54% ($P < 0.01$) (Seiler, 1979).

2.6 Special studies

(a) Neurotoxicity

In a published study, MCPA (purity 99%) in propylene glycol (unspecified concentration) was administered by gavage to groups of four male Sprague-Dawley (SD) rats and NMRI albino mice. Blood–brain barrier damage was examined by measuring the extravasation of albumin using the Evans blue technique. No blood–brain barrier damage was evident in mice following a single dose of 800 mg/kg bw. Following a single dose of 950 mg/kg bw, Evans blue extravasation was observed in the cerebral cortex (2/4 rats) and medulla oblongata (3/4 rats), suggesting that an acute toxic dose of MCPA can damage the blood–brain barrier in rats (Elo et al., 1988).

MCPA (purity 94.2%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage as a single dose to groups of 10 Wistar rats (Chbb:THOM (SPF) strain) of each sex at 0, 200, 400 or 800 mg/kg bw for males and at 0, 150, 300 or 600 mg/kg bw for females and observed for 2 weeks. Rats were observed daily for clinical signs, with a more detailed examination performed weekly. Body weight was recorded weekly. A functional observational battery and motor activity assessment were performed on all rats 7 days prior to dosing and at 24 hours, 2 days and 14 days after dosing. Survivors were sacrificed after 14 days, and tissues from five rats of each sex per group were prepared for neuropathological examinations.

There were no deaths. Mean absolute body weight of high-dose males was approximately 9% and 5% lower than the control values on days 7 ($P < 0.01$) and 14 ($P < 0.05$), respectively. Body weight gain to day 7 and day 14 was approximately 48% and 12% lower ($P < 0.01$) than the control values, respectively, in high-dose males. The mean body weight gain of mid-dose males was approximately 12% lower ($P < 0.05$) than the control value to day 7. Although a similar pattern of lower body weight and body weight gain occurred in high-dose females, albeit of a smaller magnitude (approximately 3% and 20% lower than the control value, respectively), there was no significant difference relative to the control.

Clinical signs were observed only during the functional observational battery performed 24 hours after dosing and are summarized in Table 28. Ataxia and increased abdominal tension were observed at the middle and high doses, whereas impaired activity, reduced rearing and decreased righting response occurred at the highest dose. At the highest dose, motor activity was impaired in both sexes 24 hours after dosing, but not on day 7 or 14. Histopathological examinations detected no treatment-related effect on the nervous system. On this basis, the clinical signs observed during the functional observational battery are attributed to acute systemic toxicity, rather than to a direct neurotoxic effect.

Table 28. Results of functional observational battery and motor activity assessments in rats 24 hours after an acute oral dose of MCPA

Parameter	Absolute number of rats showing the effect ($n = 10$), unless otherwise specified							
	Males				Females			
	0 mg/kg bw	200 mg/kg bw	400 mg/kg bw	800 mg/kg bw	0 mg/kg bw	150 mg/kg bw	300 mg/kg bw	600 mg/kg bw
Open-field observations								
Hypoactivity	0	0	1	4	0	0	0	0
Decreased arousal	0	0	0	1	0	0	0	0
Ataxia								
- slight	0	0	2	3	0	0	0	8
- marked	0	0	1	7	0	0	0	2
Rearing (mean)	3.7	3.6	2.5	0.8**	7.8	6.2	6.3	3.8
Sensorimotor tests/reflexes								
Abdominal tension increased	0	0	2	8	0	0	4	10
Retarded righting response	0	0	0	7	0	0	0	5
Motor activity								
Overall motor activity[a]	173	161	113	65***	220	198	226	121***

From Mellert, Kaufmann & Hildebrand (1994a)
** $P < 0.01$; *** $P < 0.002$
[a] Interruptions per rat per interval.

The NOAEL was 150 mg/kg bw, based on the occurrence of clinical signs observed during the functional observational battery and impaired motor activity at 300 mg/kg bw (Mellert, Kaufmann & Hildebrand, 1994a).

MCPA 2-EHE (purity 93.5%) in 0.5% w/v aqueous carboxymethylcellulose containing Cremophore EL® was administered by gavage to groups of 10 Wistar rats (Chbb:THOM (SPF) strain) of each sex as a single dose of 0, 250, 500 or 1000 mg/kg bw (approximately equal to 0, 160, 320 and 640 mg/kg bw expressed as acid equivalents) and observed for 2 weeks. The study design was consistent with that of the preceding study by Mellert, Kaufmann & Hildebrand (1994a).

There were no deaths and no treatment-related clinical signs observed during general daily observations. In high-dose males, mean absolute body weight was approximately 8% ($P < 0.01$) and 6% ($P < 0.05$) lower than the control values on days 7 and 14, respectively, whereas body weight gain was approximately 41% ($P < 0.01$) and 20% ($P < 0.01$) lower, respectively. In high-dose females, mean absolute body weight and body weight gain were, respectively, approximately 6% ($P < 0.05$) and 43% ($P < 0.01$) lower than the control values at day 7. In mid-dose males, body weight gain was approximately 18% lower ($P < 0.01$) than the control value on day 7. The results of the functional observational battery and motor activity assessment are summarized in Table 29. At every dose, ataxia, increased abdominal tension, impaired motor activity and retarded righting reflex occurred 24 hours after administration. Hypoactivity or ataxia occurred in one of two high-dose males on day 7. There were no treatment-related neuropathological findings.

Table 29. Results of functional observational battery and motor activity assessments in rats 24 hours after an acute oral dose of MCPA 2-EHE

Parameter	Absolute number of rats showing the effect (n = 10), unless otherwise specified							
	Males				Females			
	0 mg/kg bw	250 mg/kg bw	500 mg/kg bw	1000 mg/kg bw	0 mg/kg bw	250 mg/kg bw	500 mg/kg bw	1000 mg/kg bw
Home cage observations								
Impaired activity (apathy)	0	0	0	2	0	0	0	0
Palpebral closure (eyelids permanently closed)	0	0	0	1	0	0	0	0
Open-field observations								
Abnormal posture (abdominal position)	0	0	0	2	0	0	0	0
Impaired activity (hypoactivity or apathy)	0	1	3	4	0	0	0	2
Impaired gait (slight or severe ataxia)	0	5	10	10	0	3	8	10
Urine staining of fur	0	0	0	0	0	0	0	1
Sensorimotor tests/reflexes								
Increased abdominal tension	0	0	3	2	0	3	7	5
Retarded righting response	0	0	4	6	0	1	0	7
Motor activity[a]	57	59	46	36	124	101	87**	67***

From Mellert, Kaufmann & Hildebrand (1994b)
** $P < 0.01$; *** $P < 0.002$
[a] Interruptions per rat per interval.

No NOAEL could be identified. The LOAEL was 250 mg/kg bw (approximately 160 mg/kg bw expressed as acid equivalents), the lowest dose tested, based on clinical signs observed during the functional observational battery and transient impaired motor activity (Mellert, Kaufmann & Hildebrand, 1994b).

In a similar study of acute neurotoxicity, MCPA DMA (purity 91.8%) in 0.5% w/v aqueous carboxymethylcellulose was administered by gavage to groups of 10 Wistar rats (Chbb:THOM (SPF) strain) of each sex as a single dose of 0, 175, 350 or 700 mg/kg bw (approximately equal to 0, 143, 287 and 574 mg/kg bw expressed as acid equivalents) and observed for 2 weeks. The study design was consistent with that of the preceding two studies (Mellert, Kaufmann & Hildebrand, 1994a,b).

There were no mortalities or clinical signs observed during general daily observations. In high-dose males, absolute body weight and body weight gain were significantly lower (approximately 4% and 26% lower, respectively; $P < 0.05$ and 0.01, respectively) than the control values on day 7. In high-dose females, body weight gain was approximately 45% lower ($P < 0.01$) than the control value on day 7. The results of the functional observational battery and motor activity assessment are summarized in Table 30. Ataxia occurred across all treated groups of females and at the middle and high doses in males. Hypoactivity and increased abdominal tension occurred at the middle and/or highest doses. Motor activity was impaired at the highest dose, but only the effect in females was statistically significant ($P < 0.002$). No abnormal functional observational battery findings or effects on motor activity occurred on day 7 or 14. There were no treatment-related neuropathological findings.

Table 30. Results of functional observational battery and motor activity assessments in rats 24 hours after an acute oral dose of MCPA DMA

Parameter	Absolute number of rats showing the effect (n = 10), unless otherwise specified							
	Males				Females			
	0 mg/kg bw	175 mg/kg bw	350 mg/kg bw	700 mg/kg bw	0 mg/kg bw	175 mg/kg bw	350 mg/kg bw	700 mg/kg bw
Open-field observations								
Impaired gait (ataxia)								
- severe	0	0	0	3	0	0	1	5
- slight	0	0	6	7	0	3	7	4
Impaired activity (hypoactivity)	0	0	0	5	0	0	0	1
Sensorimotor tests/reflexes								
Increased abdominal tension	0	0	0	1	0	0	1	2
Motor activity[a]	45	58	40	27	137	126	96	25***

From Mellert, Kaufmann & Hildebrand (1994c)
*** $P < 0.002$
[a] Interruptions per rat per interval.

No NOAEL could be identified. The LOAEL was 175 mg/kg bw (approximately equal to 143 mg/kg bw expressed as acid equivalents), the lowest dose tested, based on the occurrence of ataxia (Mellert, Kaufmann & Hildebrand, 1994c).

MCPA (purity 94.2%) was administered to groups of 15 Wistar rats (Chbb: THOM (SPF) strain) of each sex for 3 months at a dietary concentration of 0, 50, 500 or 2500 ppm (equal to 0, 4, 38

and 183 mg/kg bw per day, respectively). Achieved doses were 0, 3, 34 and 177 mg/kg bw per day for males and 0, 4, 42 and 188 mg/kg bw per day for females at 0, 50, 500 and 2500 ppm, respectively. Although nominally a study of neurotoxicity, the study design incorporated all the elements of a guideline subchronic toxicity study. Rats were observed daily for clinical signs, with body weight and feed consumption recorded weekly. A functional observational battery and motor activity assessment were performed on 10 rats of each sex per dose prior to the commencement of dosing and during weeks 4, 8 and 13. Blood and urine were collected from 10 rats of each sex per group at the end of the study for the analysis of standard haematology, clinical chemistry or urine analysis parameters. Ophthalmoscopy was performed on 10 rats of each sex per group at the end of the study. Following scheduled sacrifice, tissues were prepared from five rats of each sex per group for neuropathological examination. Remaining rats were macroscopically examined; organs were weighed and examined histopathologically.

One high-dose female died on day 57 due to cachexia. The only treatment-related clinical sign observed during general daily observations was paleness in 1 male and 13 females at the highest dose. At the highest dose, absolute body weight was approximately 27% and 21% lower ($P < 0.01$) than the control values in high-dose males and females, respectively, whereas overall body weight gain was approximately 42% and 48% lower ($P < 0.01$) than the control values, respectively. In mid-dose females, body weight gain to day 90 was approximately 12% lower ($P < 0.01$ or 0.05) than the control value. Feed consumption was decreased at the highest dose (approximately 15–33% lower than the control value in males and approximately 18–38% lower in females). Water consumption was increased in mid-dose females (approximately 13–41% higher than the control value) and at the high dose (approximately 22–42% higher than the control value in males and approximately 87–145% higher than the control value in females).

Treatment-related functional observational battery findings were confined to the highest dose and included paleness (two males and five females on day 85), decreased forelimb grip strength in high-dose males on day 50 (4.2 N versus 4.8 N in the control, $P < 0.01$), decreased hindlimb grip strength in females on day 85 (1.6 N versus 2.2 N, not significant) and decreased landing foot spread in males on day 22 (11 cm versus 12.5 cm in the controls, $P < 0.01$). Motor activity was approximately 20–30% lower than the control values in high-dose males and females on days 22, 50 and 85, but not significantly so. Ophthalmoscopy was unremarkable.

Selected haematology, clinical chemistry and urine analysis parameters are summarized in Table 31. Changes in red cell parameters consistent with macrocytic anaemia occurred at the highest dose (reduced erythrocytes, haemoglobin, haematocrit and mean corpuscular haemoglobin concentration). Red blood cell morphology was unremarkable. Significantly reduced ($P < 0.01$) platelet counts also occurred at the highest dose in both sexes. Significantly decreased white blood cell counts occurred in high-dose females; in the males, there was a trend towards reduced leukocyte numbers. The mechanism of the effect on red blood cells and platelets appears to be due to reduced cell production, as indicated by the histological findings in bone marrow. In high-dose females, significantly prolonged ($P < 0.01$) prothrombin times were noted. Effects on clinical chemistry parameters occurred mainly at the highest dose and included significantly reduced ($P < 0.01$ or 0.05) glucose, total proteins and globulins and increased urea, creatinine, ALT, AST and ALP activities. Serum triglyceride concentrations were significantly lower ($P < 0.01$ or 0.05) than the control values at the middle (males) and high doses (both sexes). In high-dose females, urine volume was increased and urine specific gravity significantly decreased ($P < 0.01$).

Absolute organ weights of most organs measured were lower than the control values at the highest dose, most likely due to the reduced body weight at this dose (Table 32). However, the increase in absolute liver weight in high-dose females occurred in conjunction with elevated serum enzymes and histopathology and is therefore considered treatment related. The relative kidney weights of mid- and high-dose males were significantly increased relative to the control values, with the increase in 500 ppm males not attributable to the lower body weight. This may correlate with disturbances of creatinine and urea seen in males, although no histological abnormalities in the kidneys were detected. Lower relative adrenal weights (females) and testes weights (in males) also cannot be attributed to lower body weight.

Table 31. Selected findings in rats following subchronic exposure to MCPA

Parameter	Mean value							
	Males				Females			
	0 ppm	50 ppm	500 ppm	2500 ppm	0 ppm	50 ppm	500 ppm	2500 ppm
WBC (× 10^9/l)	6.56	7.30	7.13	5.44	4.63	4.64	4.66	3.22**
RBC (× 10^{12}/l)	9.13	9.13	8.99	7.75**	7.90	7.84	7.97	6.68**
Hb (mmol/l)	9.8	9.9	9.6	8.8**	9.2	9.0	9.2	8.0**
Hct (l/l)	0.469	0.473	0.466	0.437**	0.423	0.421	0.424	0.386**
MCV (fl)	51.4	51.8	51.8	56.5**	53.6	53.7	53.2	57.9**
MCH (fmol)	1.07	1.08	1.07	1.14**	1.17	1.15	1.16	1.19
MCHC (mmol/l)	20.81	20.91	20.60	20.16*	21.84	21.46	21.74	20.60**
Platelets (× 10^9/l)	781	757	788	591**	736	747	655	601**
Prothrombin time (s)	30.5	31.3	30.0	31.5	27.1	27.7	26.9	29.2**
ALT (µkat/l)	1.11	1.07	2.32	5.33**	0.88	0.81	1.32	2.38**
AST (µkat/l)	1.82	1.81	2.79	5.31**	1.64	1.48	2.06	2.63**
ALP (µkat/l)	4.46	4.73	4.69	9.22**	3.58	3.70	3.89	5.87**
Chloride (mmol/l)	104.8	103.9	105.3	104.1	107.9	106.0	106.6	103.2**
Calcium (mmol/l)	2.89	2.94	2.89	2.75**	2.77	2.77	2.77	2.57**
Urea (mmol/l)	6.55	6.46	6.36	7.22*	7.48	7.52	8.47*	6.91
Creatinine (µmol/l)	54.7	57.9	58.6	66.7**	50.8	52.8	55.6*	54.9
Glucose (mmol/l)	7.98	7.53	7.66	6.36**	7.36	7.18	7.26	6.13*
Total protein (g/l)	66.46	68.34	67.43	61.47*	64.42	63.47	64.55	60.18**
Globulin (g/l)	34.25	35.57	34.81	29.72**	30.20	29.65	30.03	28.12
Triglycerides (mmol/l)	4.15	3.94	2.82*	0.64**	2.50	2.30	2.19	0.65**
Cholesterol (mmol/l)	1.88	1.96	1.92	1.71	2.00	1.95	2.07	2.38*
Urine volume (ml)	5.5	6.4	5.3	4.7	3.1	2.7	3.0	4.5
Urine specific gravity (g/l)								
- < 1040	3	7	4	6	2	0	1	8**
- > 1040	7	3	6	4	8	10	9	1

From Mellert et al. (1994b)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, haemoglobin; Hct, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; WBC, white blood cells; * $P < 0.05$; ** $P < 0.01$

Gross pathological findings occurred mainly at the highest dose and included the following: prominent acinar pattern of the liver (0/10, 2/10, 0/10 and 6/10 males and 0/10, 1/10, 1/10 and 8/10 females at 0, 50, 500 and 2500 ppm, respectively); dilatation of the heart (0/10, 5/10, 3/10 and 10/10 males and 0/10, 1/10, 0/10 and 7/10 females at 0, 50, 500 and 2500 ppm, respectively); reduced kidney (2 high-dose males), testes (all high-dose males), epididymides (5 high-dose males) and seminal vesicle sizes (2 high-dose males); and discoloration of the adrenal cortex (10 high-dose males and 5 high-dose females).

Table 32. Organ weights in rats following subchronic exposure to MCPA

Organ	Mean value							
	Males				Females			
	0 ppm	50 ppm	500 ppm	2500 ppm	0 ppm	50 ppm	500 ppm	2500 ppm
Absolute organ weights (g)								
Liver	16.063	14.848	14.415	11.471**	7.86	7.489	7.393	8.12
Kidneys	3.165	3.207	3.471	2.513**	1.919	1.877	1.883	1.588**
Testes	3.63	3.739	3.582	1.555**	—	—	—	—
Brain	2.04	2.058	2.053	1.963	1.837	1.802	1.809	1.819
Adrenals	0.0813	0.087	0.0854	0.0604**	0.1059	0.1117	0.103	0.063**
Heart	1.444	1.401	1.36	1.045**	0.912	0.937	0.897	0.806**
Relative organ weights (%)								
Liver	3.391	3.198	3.146	3.552	3.193	3.045	3.145	4.233**
Kidneys	0.672	0.696	0.76*	0.773*	0.78	0.765	0.801	0.824
Testes	0.774	0.811	0.787	0.478**	—	—	—	—
Brain	0.434	0.449	0.452	0.609**	0.748	0.734	0.772	0.52**
Adrenals	0.017	0.019	0.019	0.019	0.043	0.046	0.044	0.033**
Heart	0.306	0.305	0.299	0.323	0.371	0.381	0.382	0.42**

From Mellert et al. (1994b)
* $P < 0.05$; ** $P < 0.01$

Histopathology revealed marked hepatocellular cytoplasmic eosinophilia and granular cytoplasm (moderate or severe) in the liver of all high-dose males and nine high-dose females. Single cases of anisokaryosis and increased mitosis (slight) were observed in the liver of high-dose males. Bile duct hyperplasia (minimal) occurred in two high-dose males. Foam cells (minimal to moderate) were detected in the lungs of mid- and high-dose males (one and eight rats, respectively, versus zero in the controls) and high-dose females (nine versus zero in the controls). Hypocellularity of the bone marrow (slight or moderate) (five high-dose females) and hypocellularity of the marrow from the cervical (three high-dose females), thoracic (three high-dose females) and lumbar cord (three high-dose females) and sternum (six high-dose males and six high-dose females, minimal to severe) were consistent with the perturbations in haematological parameters, suggesting impaired red cell production. Increased lipid storage in the adrenal cortex (minimal to severe) was the only histological finding in this tissue, despite markedly lower adrenal weights (1/10, 0/10, 7/10 and 10/10 males and 1/10, 0/10, 6/10 and 9/10 females at 0, 50, 500 and 2500 ppm, respectively). Diffuse atrophy of the testes (graded as severe or extreme) occurred in all high-dose males. Oligozoospermia and aspermia (extreme) were detected in the epididymis at the highest dose (three and seven rats, respectively). Thymic atrophy (moderate or severe) occurred in three high-dose females. There were no treatment-related neuropathological findings.

The NOAEL was 50 ppm (equal to 3 mg/kg bw per day), based on reduced body weight gain in females, increased relative kidney weight in males and increased adrenal lipid in both sexes at 500 ppm (approximately equal to 34 mg/kg bw per day) (Mellert et al., 1994b).

MCPA 2-EHE (purity 93.5%) was admixed in the diet and fed to groups of 15 Wistar rats (Chbb: THOM (SPF) strain) of each sex at a concentration of 0, 75, 750 or 3750 ppm for 13 weeks. The study design and dose regimen (in terms of acid equivalents) were consistent with the previous study by Mellert et al. (1994b). Achieved doses were 0, 5, 54 and 261 mg/kg bw per day for males (approximately equal to 0, 3.2, 34, 56 and 167 mg/kg bw per day expressed as acid equivalents) and 0,

6, 63 and 296 mg/kg bw per day for females (approximately equal to 0, 3.8, 40 and 189 mg/kg bw per day expressed as acid equivalents) at 0, 75, 750 and 3750 ppm, respectively.

There were no mortalities. Clinical signs observed at the highest dose included paleness (one high-dose male and six high-dose females) and cataracts (two high-dose males and one high-dose female). At the highest dose, significantly lower body weight (approximately 34% and 12% lower than the control values in males and females, respectively, on day 91; $P < 0.01$) and overall body weight gain (53% and 28% lower than the control values in males and females, respectively; $P < 0.01$) occurred. At the middle dose, body weight and body weight gain were approximately 8% lower than the control values in males (significant [$P < 0.05$] only in rats used for the functional observational battery on days 53 and 88). Feed consumption was approximately 25–32% and 10–28% lower than the control values in males and females, respectively. Water consumption was significantly increased in mid-dose females (approximately 23–38% higher than the control value) and in both sexes at the highest dose (9–38% higher than the control value in males and 67–112% higher in females).

Home cage, open-field and sensorimotor observations were unremarkable. The forelimb grip strength of high-dose males (4.2 N) was significantly lower ($P < 0.01$) than the control value (5.4 N) on day 88. At the highest dose, landing foot splay (day 25) was significantly lower ($P < 0.01$ or 0.002) than the control value in both sexes (10.8 cm versus 12.6 cm for controls in males; 10.0 cm versus 11.7 cm for controls in females). In males, overall motor activity was significantly lower than the control values at the middle and high doses on day 25 (218, 205, 165** and 144** N at 0, 75, 750 and 3750 ppm, respectively; ** $P < 0.01$) and at every dose on day 88 (182, 138*, 122*** and 107*** N at 0, 75, 750 and 3750 ppm, respectively; * $P < 0.05$ and ** $P < 0.001$). In females, significantly lower ($P < 0.05$, 0.01 or 0.02) motor activity occurred at the highest dose on days 25, 53 and 88 (170 N versus 264 N, 169 N versus 329 N and 153 N versus 257 N in the controls, respectively).

Ophthalmoscopic abnormalities were detected at day 90 or 91 in high-dose rats and included cataracts (2/15 males and 1/15 females), opacity (3/15 males and 3/15 females) and striate thickening of the lens star (11/15 females).

Selected haematology, clinical chemistry and urine analysis parameters are summarized in Table 33. At the end of the study, significantly decreased ($P < 0.01$) red blood cells, mean corpuscular haemoglobin concentration and platelet counts and significantly increased ($P < 0.01$) mean corpuscular volume and mean corpuscular haemoglobin occurred at the highest dose. The anaemia was considered to be a consequence of myeloid atrophy of bone marrow, seen histologically as hypocellularity. Additionally, significantly reduced ($P < 0.01$) haemoglobin and haematocrit occurred in high-dose females. White blood cell count was significantly lower ($P < 0.01$) than the control value in high-dose males. Prothrombin time was significantly increased ($P < 0.01$) in high-dose males. Plasma ALT and ALP were significantly elevated ($P < 0.01$ or 0.05) at the highest dose, with AST also significantly elevated ($P < 0.05$) in high-dose females. Treatment-related effects on clinical chemistry parameters included significantly decreased ($P < 0.01$ or 0.05) calcium, glucose, total bilirubin and triglycerides (both sexes at the highest dose), decreased ($P < 0.01$) inorganic phosphate, total protein and globulin (high-dose males) and increased ($P < 0.01$) serum creatinine (mid- and high-dose females, high-dose males). At the highest dose, there was an increase in urine volume in females (not significant) and an increased number of rats voiding urine with lower specific gravity (not significant).

Treatment-related macroscopic abnormalities included reduced testes and epididymides size (10/10 and 8/10 high-dose males, respectively) and cataracts (2/10 high-dose males). Absolute and relative organ weight findings are presented in Table 34. The absolute organ weights of high-dose males were all significantly lower ($P < 0.01$) than the control values. The magnitude of the difference was approximately 8% for brain, approximately 25% for liver, kidney and heart and approximately 37% for testes and adrenals. With the exception of the adrenals, all corresponding relative organ weights were significantly higher ($P < 0.01$) than the control values (< 20% for liver, kidney and heart, approximately 35% for testes and approximately 45% for brain). The relative kidney weight of mid-dose males was also significantly higher than the control value. Fewer absolute organ weight differences were detected in high-dose females; absolute brain ($P < 0.05$) and adrenal weights ($P <$

0.01) were approximately 5% and 36% lower than the control values, respectively. However, all relative organ weights were significantly lower ($P < 0.01$ or 0.05) than the control values (< 20% lower for kidneys, heart and brain, approximately 36% for liver and 22% for adrenals). Corroborative pathology was detected only in the liver and testes, and therefore all other organ weight differences are attributable to the lower terminal body weight at the highest dose.

Table 33. Selected findings in rats following subchronic exposure to MCPA 2-EHE

Parameter	Mean value							
	Males				Females			
	0 ppm	75 ppm	750 ppm	3750 ppm	0 ppm	75 ppm	750 ppm	3750 ppm
WBC ($\times 10^9$/l)	6.31	5.68	5.31	3.54**	3.65	3.94	3.88	3.54
RBC ($\times 10^{12}$/l)	8.62	8.71	8.99	7.74**	8.24	8.50	8.08	7.09**
Hb (mmol/l)	9.2	9.4	9.6	8.8	9.1	9.3	9.1	8.1**
Hct (l/l)	0.454	0.455	0.464	0.446	0.443	0.455	0.434	0.408**
MCV (fl)	52.7	52.3	51.7	57.7**	53.7	53.5	53.8	57.6**
MCH (fmol)	1.07	1.08	1.06	1.14**	1.11	1.10	1.12	1.15*
MCHC (mmol/l)	20.30	20.62	20.58	19.73*	20.59	20.51	20.83	19.86**
Platelets ($\times 10^9$/l)	745	748	733	473**	756	816	788	577**
Prothrombin time (s)	30.4	30.1	31.1	33.4*	27.5	27.7	27.2	28.6
ALT (µkat/l)	1.36	1.13	1.30	2.59**	0.89	0.89	0.89	1.66*
AST (µkat/l)	2.63	1.84	1.99	3.15	1.76	1.57	1.62	2.26*
ALP (µkat/l)	5.17	4.67	4.82	9.56**	4.18	4.10	4.27	6.29**
Inorganic phosphorus (mmol/l)	2.19	2.04	2.05	1.83**	1.90	1.76	1.86	1.93
Calcium (mmol/l)	2.80	2.80	2.76	2.65**	2.70	2.74	2.74	2.61*
Creatinine (µmol/l)	55.2	51.7	51.6	62.9**	51.1	52.6	58.1**	59.1**
Glucose (mmol/l)	8.10	7.73	7.56	5.92**	7.50	7.80	7.44	5.83**
Total bilirubin (µmol/l)	3.47	2.94	2.96	2.56*	2.72	2.87	2.98	2.07*
Total protein (g/l)	63.94	63.54	62.34	57.63**	62.42	63.79	64.92	60.72
Globulin (g/l)	32.72	32.31	31.31	26.79**	28.20	29.14	29.96	27.82
Triglycerides (mmol/l)	3.14	3.37	3.71	0.57**	2.05	2.27	2.05	0.82**
Urine volume (ml)	4.1	3.9	4.3	3.7	2.0	2.3	2.6	3.6
Urine specific gravity (g/l)								
- < 1040	1	1	3	5	3	1	1	4
- > 1040	9	9	7	5	7	9	9	6

From Mellert et al. (1994c)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, haemoglobin; Hct, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cells; WBC, white blood cells; * $P < 0.05$; ** $P < 0.01$

Histopathology revealed cytoplasmic eosinophilia and granular cytoplasm of hepatocytes (slight to severe) in all high-dose rats (0/10 in the controls). Foam cell accumulations (mainly slight) were observed in the lung of all high-dose rats (minimal observed in one control male). Severe diffuse testicular atrophy (10/10 rats), with associated oligozoospermia (3/10 rats) or aspermia (7/10 rats) in epididymides, was reported in high-dose males (0/10 in the controls). Focal testicular atrophy (minimal to moderate) was observed in four mid-dose males (0/10 in the controls). Hypocellularity of

the bone marrow (grade minimal to moderate) was detected at a number of sites, including the cervical (one high-dose male), thoracic (three high-dose males and females) and lumbar cord (0/10, 0/10, 2/10 and 7/10 males and 1/10, 0/10, 2/10 and 6/10 females at 0, 75, 750 and 3750 ppm, respectively) and sternum (0/10, 0/10, 1/10 and 9/10 males and 2/10, 0/10, 0/10 and 8/10 females at 0, 75, 750 and 3750 ppm, respectively). Hypocellularity of the bone marrow from the femur of high-dose rats was somewhat variable; in males, hypocellularity occurred with similar frequency to the controls, whereas in females, the incidence was increased (3/10, 0/10, 0/10 and 8/10 at 0, 75, 750 and 3750 ppm, respectively). There were no treatment-related microscopic findings in the adrenals, as occurred in the parallel study on MCPA acid. There were no treatment-related neuropathological findings.

Table 34. Organ weights in rats following subchronic exposure to MCPA 2-EHE

Organ	Mean value							
	Males				Females			
	0 ppm	75 ppm	750 ppm	3750 ppm	0 ppm	75 ppm	750 ppm	3750 ppm
Absolute organ weights (g)								
Liver	15.15	15.06	14.4	11.32**	7.86	7.51	7.48	8.71
Kidneys	3.06	3.13	3.25	2.28**	1.98	1.94	1.90	1.85
Testes	3.52	3.79	3.60	1.48**	—	—	—	—
Heart	1.41	1.48	1.39	1.09**	0.95	0.97	0.97	0.92
Brain	2.06	2.11	2.09	1.91**	1.94	1.90	1.91	1.85*
Adrenals	0.086	0.083	0.088	0.055**	0.11	0.10	0.11	0.07**
Relative organ weights (%)								
Liver	3.24	3.28	3.25	3.75**	3.01	2.95	2.94	3.97**
Kidneys	0.65	0.69	0.74**	0.75**	0.76	0.76	0.75	0.84*
Testes	0.76	0.83	0.82	0.49**	—	—	—	—
Heart	0.30	0.32	0.32	0.36**	0.37	0.38	0.38	0.42**
Brain	0.44	0.46	0.47	0.64**	0.75	0.75	0.76	0.85**
Adrenals	0.018	0.18	0.02	0.018	0.041	0.39	0.043	0.032**

From Mellert et al. (1994c)
* $P < 0.05$; ** $P < 0.01$

The NOAEL was 75 ppm (approximately equal to 3 mg/kg bw per day expressed as acid equivalents), based on decreased motor activity, increased serum creatinine and focal testicular atrophy at 750 ppm (approximately equal to 34 mg/kg bw per day expressed as acid equivalents) (Mellert et al., 1994c).

MCPA DMA (purity 91.8%) was admixed in the diet at a concentration of 0, 60, 600 or 3000 ppm and fed to Wistar rats (Chbb:THOM (SPF) strain) under the same experimental conditions (and at similar doses in terms of acid equivalents) described in Mellert et al. (1994b,c). Achieved doses were 0, 4, 42 and 208 mg/kg bw per day for males (approximately equal to 0, 3.3, 34 and 170 mg/kg bw per day expressed as acid equivalents) and 0, 5, 48 and 232 mg/kg bw per day for females (approximately equal to 0, 4, 40 and 190 mg/kg bw per day expressed as acid equivalents) at 0, 60, 600 and 3000 ppm, respectively.

There were no deaths and no treatment-related clinical signs. At the highest dose, reduced feed consumption (10–18% and 6–21% lower than the control values in males and females, respectively), lower absolute body weight (significantly lower [$P < 0.01$] than the control values in both sexes at every week; approximately 15% lower than the control value on day 91) and body

weight gain (significantly lower [$P < 0.01$] than the control values during every week; 26% and 32% lower in males and females, respectively) occurred. Water consumption was not recorded. The functional observational battery was generally unremarkable, with the exception of significantly reduced ($P < 0.01$) forelimb grip strength in high-dose females on day 87 (3.5 N versus 4.5 N in the controls). There was no effect on motor activity. Ophthalmoscopy was unremarkable.

Selected haematology, clinical chemistry and urine analysis parameters are summarized in Table 35. Significantly reduced ($P < 0.01$) white blood cells, red blood cells, haemoglobin and haematocrit occurred in high-dose females, whereas significantly reduced ($P < 0.01$) platelets and mean corpuscular volume occurred in both sexes. These treatment-related effects were attributable to a hypoplastic or hypoproliferative anaemia, confirmed by histopathological findings in the bone marrow. Perturbation in clinical chemistry parameters at the highest dose included the following: significantly decreased ($P < 0.01$) serum calcium, triglycerides (both sexes) and glucose (females); significantly increased ($P < 0.01$) ALT, AST, ALP and creatinine (both sexes); and significantly increased ($P < 0.01$ or 0.05) GGT, serum cholesterol and magnesium (males). Serum creatinine was also elevated ($P < 0.01$) in mid-dose females. Urine volume was increased and urine specific gravity decreased in high-dose females (not statistically significant).

Table 35. Selected findings in rats following subchronic exposure to MCPA DMA

Parameter	Mean value							
	Males				Females			
	0 ppm	60 ppm	600 ppm	3000 ppm	0 ppm	60 ppm	600 ppm	3000 ppm
WBC ($\times 10^9$/l)	6.83	7.92	7.57	6.81	4.14	4.56	4.73	3.29*
RBC ($\times 10^{12}$/l)	8.64	8.78	8.77	8.35	8.20	8.37	8.12	7.18**
Hb (mmol/l)	9.2	9.5	9.5	9.2	9.2	9.4	9.1	8.3*
Hct (l/l)	0.440	0.453	0.454	0.443	0.439	0.448	0.432	0.400**
MCV (fl)	51.0	51.6	51.8	53.2**	53.5	53.6	53.3	55.8**
Platelets ($\times 10^9$/l)	759	729	740	573**	800	735	776	607**
ALT (µkat/l)	1.05	0.87	1.43	10.52**	0.90	0.80	1.01	3.08*
AST (µkat/l)	1.65	1.49	2.01	8.78**	1.53	1.69	2.01	3.52**
ALP (µkat/l)	4.97	5.14	5.17	8.45**	4.03	4.21	4.23	5.55**
GGT (nkat/l)	0	0	2	9**	8	11	2	8
Chloride (mmol/l)	103.2	103.3	102.3	101.0*	105.0	104.6	105.0	103.4
Calcium (mmol/l)	2.70	2.71	2.73	2.60**	2.84	2.90	2.87	2.69**
Creatinine (µmol/l)	58.8	57.9	60.0	68.9**	53.5	55.9	57.4*	58.7**
Glucose (mmol/l)	8.29	7.86	8.26	7.84	7.32	7.04	7.26	5.94**
Total bilirubin (µmol/l)	2.63	2.75	2.78	2.51	2.59	2.95	2.71	1.86*
Triglycerides (mmol/l)	5.57	4.80	4.48	1.54**	3.04	3.02	3.60	0.95**
Cholesterol (mmol/l)	1.73	1.87	1.83	2.52**	1.74	1.70	1.69	1.91
Magnesium (mmol/l)	0.83	0.80	0.84	0.91**	0.91	0.94	0.92	0.89
Urine volume (ml)	5.2	5.1	6.0	4.8	1.6	1.9	2.6	3.1
Urine specific gravity (g/l)								
- < 1040	0	3	5*	2	0	0	1	3
- > 1040	10	7	5	8	10	10	9	7

From Mellert et al. (1994a)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; RBC, red blood cells; WBC, white blood cells; * $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic abnormalities. Absolute and relative organ weights are presented in Table 36. In most instances, significant differences in absolute and/or relative organ weights reflect the body weight differences between treated and control groups. The one exception to this is the significant increase ($P < 0.01$) in relative liver weight in high-dose females compared with the controls (approximately 20% increase in size), concomitant with increased liver enzymes (see Table 35) and histopathology (discussed below).

Table 36. Organ weights in rats following subchronic exposure to MCPA 2-EHE

Organ	Mean value							
	Males				Females			
	0 ppm	60 ppm	600 ppm	3000 ppm	0 ppm	60 ppm	600 ppm	3000 ppm
Absolute organ weights (g)								
Liver	17.00	14.53*	14.18*	11.88**	7.64	7.64	7.08	7.68
Kidneys	3.19	3.21	3.35	3.20	1.97	1.98	1.95	1.75*
Testes	3.78	3.82	3.69	3.35	—	—	—	—
Heart	1.61	1.58	1.60	1.35**	0.96	1.01	0.96	0.85*
Brain	2.15	2.07*	2.11	2.04**	1.87	1.86	1.89	1.86
Adrenals	0.096	0.095	0.092	0.077*	0.12	0.10	0.095	0.068*
Relative organ weights (%)								
Liver	3.44	3.12*	3.04*	2.94**	2.95	3.00	2.89	3.61**
Kidneys	0.65	0.69	0.72*	0.79**	0.76	0.78	0.80	0.82
Testes	0.77	0.83	0.80	0.83	—	—	—	—
Heart	0.33	0.34	0.34	0.33	0.37	0.40	0.39	0.40
Brain	0.44	0.45	0.45	0.51**	0.73	0.73	0.78	0.88**
Adrenals	0.02	0.02	0.02	0.02	0.043	0.041	0.039	0.032**

From Mellert et al. (1994a)
* $P < 0.05$; ** $P < 0.01$

Treatment-related histopathological findings were confined to the highest dose. In the liver, slight eosinophilia occurred in 10 of 10 females, and minimal to slight single-cell necrosis was detected in 10 of 10 males and 9 of 10 females. Minimal bile duct hyperplasia occurred in 5 of 10 females. Foam cell aggregates in the lung (minimal to moderate) were observed in rats of both sexes (10/10 males and 9/10 females). Diffuse testicular atrophy was detected in 2 of 10 males (one of these graded as severe), with oligozoospermia in the epididymides of the rat with severe atrophy. Hypocellularity of the bone marrow occurred variably at a number of sites (bone marrow: 1/10, 0/10, 0/10 and 5/10 males and 2/10, 0/10, 0/10 and 5/10 females at 0, 60, 600 and 3000 ppm, respectively; sternum: 5/10 high-dose males and 5/10 high-dose females versus 0/10 in the controls; femur: 7/10, 0/10, 8/10 and 9/10 males and 0/10, 0/10, 0/10 and 8/10 females at 0, 60, 600 and 6000 ppm, respectively; thoracic cord: 3/10 females versus 0/10 in the controls). There were no treatment-related neuropathological findings.

The NOAEL was 60 ppm (equal to 4 mg/kg bw per day expressed as acid equivalents), based on elevated serum creatinine in females at 600 ppm (equal to 40 mg/kg bw per day expressed as acid equivalents) (Mellert et al., 1994a).

(b) Enzyme induction and peroxisome proliferation

Hietanen et al. (1983) examined the effects of phenoxyacetic acid herbicides on hepatic and intestinal drug metabolizing enzymes in male Wistar rats. MCPA (unspecified purity and vehicle) was administered by gavage to an unspecified number of rats at 100 or 150–200 mg/kg bw on 5 days/week

for 2 weeks. The 200 mg/kg bw dose was reduced to 150 mg/kg bw per day after 4 days due to deaths and clinical signs (drowsiness). Rats were killed 24 hours after the last dose, and the liver and proximal small intestine were prepared for the analysis of microsomal enzymes. Hepatic ethoxycoumarin *O*-deethylase (ECOD) and epoxide hydrolase (EH) activities were significantly increased ($P < 0.01$; approximately 2- and 1.6-fold, respectively) at both 100 and 150–200 mg/kg bw. Intestinal ECOD and EH activities were significantly increased ($P < 0.01$; < 2-fold) at 150–200 mg/kg bw; there was enzyme induction at 100 mg/kg bw. Hepatic glutathione *S*-transferase and intestinal uridine diphosphate–glucuronosyltransferase (UDPGT) activities were significantly lower ($P < 0.05$; < 2-fold) than the control values at 150–200 mg/kg bw. There was no effect on hepatic cytochrome P450, polyphenol oxidase hydroxylase or aryl hydrocarbon hydroxylase (AHH) or intestinal AHH (Hietanen et al., 1983).

In a subsequent study by Hietanen et al. (1985), an unspecified number of male Wistar rats were gavaged with MCPA (isooctyl ester; unspecified purity) in saline at a dose of 1 mmol/kg bw per day for 2 weeks. The dose was reduced to 0.5 mmol/kg bw per day after 4 days due to overt toxicity. Rats were sacrificed after 14 days, and their livers were dissected for the preparation of peroxisomes and microsomes. β-Oxidation in isolated peroxisomes was increased 2-fold compared with the saline control. Microsomal glutathione reductase activity was increased by approximately 40% over the controls, whereas glutathione peroxidase activity was increased by approximately 30%.

MCPA (unspecified purity) in saline was administered by gavage to groups of 4–7 male Wistar rats at 0 or 0.5 mmol/kg bw per day for 14 days. Rats were killed 24 hours after the last dose. and the kidneys and proximal small intestine were prepared for the analysis of microsomal enzymes. Intestinal AHH and kidney ECOD and EH activities were significantly increased ($P < 0.05$) over the control values (1.7-, 2- and 1.6-fold, respectively). There was no induction of intestinal ECOD, EH or UDPGT (Ahotupa et al., 1983).

Male Han Wistar rats ($n = 4$) were gavaged with MCPA (unspecified purity) at 100 mg/kg bw per day, 5 days/week, for 2 weeks. Control rats ($n = 4$) were dosed with the dimethyl sulfoxide (DMSO) vehicle. There was no effect on body weight gain, relative liver weight or the level of non-protein sulfhydryl groups in the liver. Hepatic peroxisomes were significantly increased ($P < 0.01$) compared with the controls (14.2 /100 μm^2 versus 7.7/100 μm^2, respectively), but there was no increase in peroxisome size. In hepatic microsomes, the activities of cytochrome P450, ECOD and propionaldehyde dehydrogenase were significantly higher ($P < 0.01$ or 0.05) than the control values (1.2-, 1.4- and 1.3-fold increases, respectively), whereas the activity of UDPGT was significantly lower ($P < 0.05$) than the control value (approximately 1.3- to 1.4-fold lower). There was no effect on the activities of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase or NADPH diaphorase (Mustonen et al., 1989).

The effects of MCPA on hepatic peroxisomal enzymes were investigated in male Sprague-Dawley rats (five per group) following 2 weeks of daily gavage dosing at 0, 100, 200 or 300 mg/kg bw per day. There was a dose-related reduction in body weight, which was statistically significant ($P < 0.01$ or 0.05) at 200 and 300 mg/kg bw per day (297, 283, 277 and 263 g at 0, 100, 200 and 300 mg/kg bw per day, respectively). Hepatic cholesterol and/or phospholipid levels were significantly lower ($P < 0.01$ or 0.05) than the control values at every dose. Fatty acid β-oxidation was significantly increased at 200 and 300 mg/kg bw per day (2.5- and 3.5-fold higher than the control value, respectively). Significantly increased L-carnitin-dependent acetyltransferase ($P < 0.01$; 2- to 7-fold higher at every dose) and L-carnitin-dependent octanoyl transferase ($P < 0.05$; 3-fold higher than the control value at 300 mg/kg bw per day) also occurred (Inomata et al., 1991a).

Inomata et al. (1991b) further examined the effects of MCPA and MCPA 2-EHE on hepatic peroxisomal enzymes in male Sprague-Dawley rats. MCPA (unspecified purity) or MCPA 2-EHE (purity 96%) in 0.5% w/v carboxymethylcellulose and olive oil, respectively, were administered to groups of five rats by gavage at 0 or 200 mg/kg bw per day for 2 weeks. Body weight and body weight gain (0–2 weeks) were lower than the control values by approximately 12% and 18%, respectively, for MCPA and 36% and 52%, respectively, for MCPA 2-EHE; these differences were statistically significant ($P < 0.01$). Relative liver weight was approximately 10% higher ($P < 0.01$)

than the control value in the MCPA group. The activities of aniline hydroxylase and ECOD in hepatic microsomes were significantly increased ($P < 0.01$ or 0.05) by 15% and 1.5-fold, respectively, over the control with both MCPA and MCPA 2-EHE. MCPA 2-EHE and MCPA significantly increased ($P < 0.01$ or 0.05) microsomal cytochrome P450 activity (approximately 1.5-fold increase over the controls). MCPA also increased cytochrome b_5 activity ($P < 0.05$; 1.2-fold increase) and NADPH cytochrome *c* reductase activity ($P < 0.01$; 1.3-fold increase); NADH-ferricyanide reductase activity was decreased by approximately 12% ($P < 0.01$). There was no effect on aminopyrine *N*-demethylase or UDPGT activities in hepatic microsomes.

The induction of P450-dependent mixed-function oxidase in the livers of male Wistar rats ($n = 6$) was investigated following gavage dosing with MCPA (unspecified purity) in peanut oil at 0 ($n = 15$), 50, 100 or 200 mg/kg bw per day for 3 days. MCPA had no effect on body weight gain or relative liver weight, but increased the microsomal activity of cytochrome P450 (1.6-fold induction over the controls; $P < 0.05$) and specifically the activity of cytochrome P450 4A1 (4.6-fold induction over the controls; $P < 0.001$). Additionally, microsomal ECOD activity was increased by approximately 2-fold ($P < 0.01$) over the controls (Bacher & Gibson, 1988).

To study the effect of MCPA on peroxisome proliferator-activated receptor (PPAR) activation, COS-1 cells transfected with mouse or human PPARα or PPARγ (and the reporter plasmid pHD(x3)-Luc) were incubated with MCPA (unspecified purity) in DMSO at concentrations up to 400 µmol/l. 2,4-D was also tested up to concentrations of 800 µmol/l. At MCPA concentrations of 50 and 400 µmol/l (but not 100 or 200 µmol/l), there was a significant increase ($P < 0.05$) in PPARα activation (2.1- and 1.2-fold higher luciferase activity than the solvent control, respectively). There was no effect on PPARγ activation. No effect was observed for 2,4-D. The authors concluded that MCPA and 2,4-D did not directly transactivate mouse or human PPAR and suggested that metabolism may be required to activate them (Maloney & Waxman, 1999).

(c) Haematology

Pistl et al. (2003) investigated the immunotoxic potential of MCPA (purity 99.1%) (and seven other pesticides) in isolated sheep leukocytes at concentrations of 10^{-1} to 10^{-6} mol/l. Cytotoxicity (measured as a decrease in spontaneous leukocyte migration) occurred at 10^{-1} mol/l (16 cm^2 versus 27.6 cm^2 in the controls; $P < 0.01$). Immunotoxicity (measured as a decrease in lymphocyte activation with phytohaemagglutinin) occurred at concentrations ranging from 10^{-2} to 10^{-6} mol/l ($P < 0.001$). MCPA did not suppress the metabolic activity of sheep phagocytes in the iodonitrotetrazolium reductase test.

MCPA (purity ≥ 99%) (and seven other phenoxyacetic acid herbicides) was tested for its ability to inhibit the aggregation of human platelets in vitro. Platelet aggregation was induced with adenosine diphosphate at 1–32 µmol/l, adrenaline at 0.32–32 µmol/l or collagen at 7.5–30 µg/ml in the presence of MCPA at 0, 0.5, 0.1, 0.5, 1.0 or 2.0 mg/ml. Dose-dependent inhibition of platelet aggregation occurred at MCPA concentrations of 0.1–2.0 mg/ml (Elo et al., 1991).

Bukowska et al. (2000) examined the effect of MCPA sodium salt (purity 98%) (and other phenoxyacetic acid herbicides) on the catalase activity of human erythrocytes in vitro. At concentrations of 100, 500 and 1000 ppm, MCPA had no effect on the activity of standard catalase following incubation for 1 or 3 hours. Following incubation for 24 hours, a slight decrease (approximately 5% compared with the controls; $P < 0.05$) in activity was detected at 1000 ppm only. There was no significant effect of MCPA (100, 500 and 1000 ppm) on the catalase activity of erythrocytes following a 1-hour incubation. Significant ($P < 0.05$) increases (3-hour incubation; < 6% of the control) or decreases (24-hour incubation; < 10% of the control) were considered to be incidental findings due to their inconsistency and small magnitude. In a subsequent study, MCPA (98% purity) at concentrations up to 1000 ppm had no effect on the activity of acetylcholinesterase in human erythrocytes (Bukowska & Hutnik, 2006).

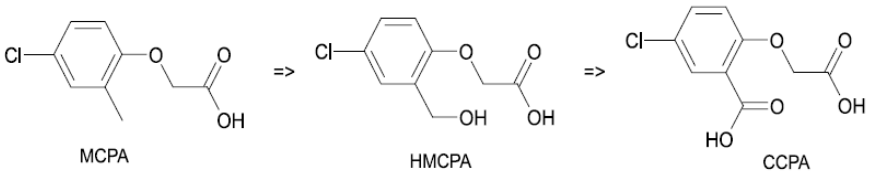
MCPA sodium salt (purity 98%) (and other phenoxyacetic acid herbicides) in phosphate-buffered saline or ethanol, at concentrations up to 4 mmol/l, was tested for its potential to damage human erythrocytes by measuring haemolysis, haemoglobin oxidation and lipid peroxidation. There

was a concentration-related increase in haemolysis that was statistically significant ($P < 0.05$) at 2 and 4 mmol/l (1.1%, 1.2%, 1.8%, 2.6% and 3.5% at 0, 0.5, 1, 2 and 4 mmol/l, respectively). Methaemoglobin formation was also significantly increased ($P < 0.05$) compared with the solvent control (0.8%, 4.1%, 6.1%, 8.7% and 11.7% at 0, 0.5, 1, 2 and 4 mmol/l, respectively). There was also a treatment-related increase in lipid peroxidation that was statistically significant ($P < 0.05$) at and above 1 mmol/l (approximately 0%, 5%, 10%, 40% and 80% at 0, 0.5, 1, 2 and 4 mmol/l, respectively). The manufacturing impurity and bacterial degradant, 4-chloro-2-methylphenol, and the environmental degradant, 2,4-dimethylphenol, were found to cause similar damage to human erythrocytes (albeit a somewhat higher level of haemolysis at 2 and 4 mmol/l) (Duchnowicz, Koter & Duda, 2002).

(d) Novel plant metabolite

4-Chloro-2-carboxyphenoxyacetic acid (CCPA) is a novel plant metabolite of MCPA not identified in animal studies (Figure 3).

Figure 3. Formation of CCPA in plants



From van Ravenzwaay et al. (2005)

CCPA (purity 99.45%) in 0.5% w/v carboxymethylcellulose was administered by gavage as a single dose of 2000 mg/kg bw to five male and five female Crl:CD®(SD)IGS BR rats. There were no deaths, clinical signs or effects on body weight in the 14-day post-dosing observation. The necropsy was unremarkable. The oral LD_{50} was therefore greater than 2000 mg/kg bw (Kern, 1999).

In a bacterial reverse mutation assay using *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2*uvrA*, CCPA (purity 99%) in DMSO was not genotoxic (or cytotoxic) up to concentrations of 5000 µg/plate with and without S9 (Mecchi, 2002).

CCPA (purity 99.7%) was admixed in the diet at a concentration of 0, 2000 or 12 000 ppm and fed to groups of five Wistar rats (CrlGlxBrlHan:WI strain) of each sex for 4 weeks. For comparison, separate groups of rats were concurrently fed a diet containing 2000 ppm MCPA (purity 97%). Mean intakes of CCPA were 0, 176 and 1076 mg/kg bw per day for males and 0, 194 and 1144 mg/kg bw per day for females at 0, 2000 and 12 000 ppm, respectively. The mean intakes of MCPA were 166 mg/kg bw per day for males and 173 mg/kg bw per day for females. Observations for mortality and clinical signs were made daily. Detailed open-field observations were made prior to the commencement of dosing and on a weekly basis thereafter. Body weight and feed and water consumption were recorded weekly. A functional observational battery and motor activity assessment were undertaken after 4 weeks. Blood and urine were collected after 24 or 25 days for the analysis of haematology, clinical chemistry or urine analysis parameters. At the end of the study, all rats were sacrificed and necropsied. Organs were weighed and tissues examined histopathologically. There were no deaths or clinical signs. CCPA had no effect on body weight, body weight gain or feed or water consumption. In contrast, the mean body weight and body weight gain of MCPA-treated rats were significantly lower ($P < 0.01$ or 0.05) than the control values (approximately 17% [day 28] and 37% [days 7–28] lower than the controls, respectively, in males; approximately 10% [day 28] and

31% [days 7–28] lower than the controls, respectively, in females). Feed consumption was up to approximately 23% lower than the control value in MCPA-treated males and up to approximately 16% lower than the control value in females. Feed conversion efficiency was significantly lower than the control value in males on day 7 ($P < 0.01$), day 14 ($P < 0.01$) and day 21 ($P < 0.05$). Water consumption was approximately 40% higher than the control value in MCPA-treated females on day 7 ($P < 0.05$) and day 21 ($P < 0.01$). Functional observational battery and motor activity assessments were unremarkable. There were no treatment-related effects on any haematology parameters.

Selected clinical chemistry and urine analysis parameters are presented in Table 37. At 2000 ppm CCPA, blood glucose was significantly lower ($P < 0.05$) than the control value in males; however, in the absence of a difference at 12 000 ppm, this was not considered treatment related. At 12 000 ppm CCPA, significantly reduced albumin ($P < 0.05$) and increased magnesium levels ($P < 0.05$) occurred in females. In this same group, urine volume was markedly lower than the control value, whereas specific gravity was increased in both sexes, but not significantly. There was a small increase in urobilinogen in the urine of both sexes. The following effects occurred in MCPA-treated rats: significantly elevated ($P < 0.05$) ALT in females (approximately 32% higher than the control value); decreased serum glucose ($P < 0.05$, both sexes), total bilirubin ($P < 0.01$, females) and albumin ($P < 0.05$, females); and increased serum creatinine ($P < 0.05$, males) and magnesium ($P < 0.05$, females). In addition, epithelial casts were detected in the urine of three males.

Table 37. Clinical chemistry and urine analysis findings in rats exposed to CCPA or MCPA for 4 weeks in the diet

Parameter	Mean value							
	Males				Females			
	Control	CCPA 2000 ppm	CCPA 12 000 ppm	MCPA 2000 ppm	Control	CCPA 2000 ppm	CCPA 12 000 ppm	MCPA 2000 ppm
ALT (µkat/l)	0.61	0.54	0.56	0.69	0.50	0.55	0.49	0.66*
Creatinine (µmol/l)	46.2	46.0	47.1	52.5*	47.7	46.3	48.3	47.5
Glucose (mmol/l)	7.92	6.09*	6.68	4.80*	6.34	6.11	5.54	4.81*
Total bilirubin (µmol/l)	2.34	2.16	2.44	1.98	2.40	2.44	2.11	1.34**
Albumin (g/l)	34.81	35.26	35.21	35.08	39.37	38.94	36.95*	36.46*
Magnesium (mmol/l)	0.96	1.00	0.92	0.96	0.93	0.96	1.00*	1.03*
Urine volume (ml)	3.6	3.5	2.4	3.5	2.2	2.0	1.1	1.9
Urine specific gravity (g/l)	1068	1061	1084	1057	1065	1073	1124	1060
Urine urobilinogen								
- normal	3	4	0	0	4	3	1	0*
- > 68 µmol/l	2	1	5	5	1	2	4	5

From Mellert et al. (2004); van Ravenzwaay et al. (2005)
* $P < 0.05$; ** $P < 0.01$

There were no treatment-related macroscopic abnormalities. Because of the lower terminal body weight, the absolute weights of most organs were significantly lower than the control values in males (ranging from approximately 18% lower for the epididymis to approximately 37% for the

thymus), whereas the absolute weights of the thymus, adrenals and ovaries were lower in females (approximately 40%, 29% and 36% lower, respectively). The relative weights of the testes and brain were significantly higher than the control values in males (approximately 14% and 19%, respectively), whereas the relative weights of the ovaries and adrenals of females were significantly lower (approximately 29% and 21% lower, respectively). In CCPA-treated rats, no significant differences in organ weights were determined.

Minimal focal degeneration of seminiferous tubules of the testes was observed in three of five MCPA-treated males, and increased cellularity of bone marrow occurred in two females, consistent with observations from other studies. There was an increase in the severity of renal calcification at the outer/inner medullary transition in high-dose, CCPA-treated females (two females with grade 3 calcification versus zero in the control); however, all females in the control and high-dose CCPA groups had renal calcification.

The NOAEL for CCPA was 2000 ppm (equal to 176 mg/kg bw per day for males and 194 mg/kg bw per for females), based on reduced serum albumin, increased serum magnesium, increased specific gravity and increased renal calcification at 12 000 ppm (equal to 1076 mg/kg bw per day for males and 1144 mg/kg bw per day for females). CCPA was concluded to be less toxic than MCPA (Mellert et al., 2004; van Ravenzwaay et al., 2005).

3. Observations in humans

3.1 Occupational exposure studies

The induction of sister chromatid exchanges (SCEs) was examined in the peripheral lymphocytes of 35 forestry workers exposed to MCPA (500 g/l as an iso-octylester formulation), 2,4-D (550 g/l as an amine salt formulation) or a mixture of both (333 g/l 2,4-D; 167 g/l MCPA). A group of 15 unexposed workers served as the control. Exposure was estimated by analysing the concentration of MCPA and 2,4-D in urine. Three successive blood samples were taken before, during (after 6–26 days of exposure) and after (within 2 days after the cessation of spraying) one 4-month spraying season for the analysis of SCEs. Urinary concentrations of both herbicides were highly variable across the 35 subjects, ranging from 0 to approximately 11 g/l (mean ± 1 standard deviation of 1.80 ± 2.85 g/l). The concentration of MCPA ranged from 0 to 1.14 mg/l. In 7 of 19 subjects, MCPA and 2,4-D were not detectable. There was no significant difference in the frequency of SCEs in the exposed versus the non-exposed subjects (Linnainmaa, 1983).

The effects of short-term exposure (1–3 days) to commercial formulations containing a mixture of MCPA and 2,4-D on peripheral blood lymphocytes were assessed in 10 male farmers. Blood was sampled within 1–7 days before and 1–12 days and 50–70 days after exposure. Lymphocyte subsets were quantified, and mononuclear cells were separated and assayed for natural killer (NK) cell function and mitogen proliferative response. During the first sampling period, the proportions of several T cell subtypes (T helper, suppressor T, cytotoxic T, CD8-DR, NK cells) were significantly lower ($P < 0.01$ or 0.05) than those measured prior to exposure. However, the magnitude of these differences was relatively small (< 10%), and there was no difference in total white blood cell or lymphocyte counts. Given the small sample size, variability in the potential sampling window, the absence of a concurrent unexposed group and the lack of analysis of urine or blood levels of MCPA or 2,4-D (to confirm systemic exposure), the study is considered to be preliminary in nature (Faustini et al., 1996).

Coggon et al. (1986) examined the mortality of 5754 workers involved in manufacturing and spraying of MCPA and other phenoxyacetic acid herbicides from 1947 to 1975 in England. Overall mortality (including that from cancer, heart disease and respiratory disease) was less than that of the national population. One soft tissue sarcoma was detected against the expected 0.6. The authors concluded that any risk of soft tissue sarcoma due to MCPA is less than suggested by earlier studies of 2,4,5-T and 2,4,5-trichlorophenol production and must be small in absolute terms.

A study of cancer incidences among 4459 Danish workers involved in the production of MCPA and other phenoxyacetic acid herbicides was undertaken by Lynge et al. (1985). The overall

cancer incidence among workers was unremarkable, with the exception that 5 soft tissue sarcomas were detected in males versus an expected 1.84 for the Danish population. The 5 soft tissue sarcomas were all different tumour types, and only one case was linked directly to manufacturing. The study did not differentiate between exposure to different pesticides and exposure to precursor chemicals involved in their production.

Wiklund & Holm (1986) investigated the risk of soft tissue sarcoma in 354 620 Swedish male agricultural or forestry workers (identified in the 1960 census) due to exposure to phenoxyacetic acid herbicides. A reference cohort of 1 725 845 Swedish men involved in other industries was used as the comparator. In total, 331 cases of soft tissue sarcoma were detected versus 1508 in the reference cohort (relative risk = 0.9; 95% confidence interval [CI] = 0.8–1.0), which indicated that there was no increase in cancer risk. In a follow-up study by Wiklund et al. (1987), the risk of Hodgkin disease and non-Hodgkin lymphoma in a cohort of 20 245 Swedish pesticide applicators was studied. Seventy-two per cent were estimated to have used phenoxyacetic acid herbicides. Eleven cases of Hodgkin disease (versus an expected 9.1 in the general population; rate ratio = 1.20, 95% CI = 0.6–2.16) and 21 cases of non-Hodgkin lymphoma (versus an expected 20.8 in the general population; rate ratio = 1.01, 95% CI = 0.63–1.54) were detected, with no significant increase in the risk of either malignant lymphoma.

Based on a preliminary finding that the standardized mortality ratio (SMR) for cancers of the biliary tract in Japan was associated with rice production, Yamamoto et al. (1986) examined the association (between 1962 and 1975) with environmental contamination with agricultural chemical products. While the analysis detected statistically significant ecological correlations (predominantly in men) between environmental contamination with MCPA and biliary tract cancer (correlation coefficient of 0.26–0.38), the study is considered to have too many confounding factors to allow a meaningful interpretation.

A case–control study involving 30 males with soft tissue sarcoma and 52 males with malignant lymphoma was undertaken by Smith & Christophers (1992) to test whether there was an association with past exposure to phenoxyacetic acid herbicides or chlorophenols. Exposure was assessed by way of a personal interview; 43 men were definitely or probably exposed and 17 possibly exposed for at least 1 day prior to 5 years previously (exposure ranged from 1 hour to 122 weeks). No significant association was found between exposure to phenoxyacetic acid herbicides or chlorophenols and soft tissue sarcoma or malignant lymphoma.

Using an international register maintained by the International Agency for Research on Cancer of 21 863 workers exposed to phenoxyacetic acid herbicides, Kogevinas et al. (1995) reported an increased risk of soft tissue sarcoma in workers exposed to any phenoxyacetic acid herbicide (odds ratio [OR] = 10.3; 95% CI = 1.2–91) or to MCPA or 4-(4-chloro-*o*-tolyloxy)butyric acid (MCPB) (OR = 11.3; 95% CI = 1.30–97.92). No significant association was determined for non-Hodgkin lymphoma. Exposure to chlorophenols and to other raw materials and process chemicals was not associated with an increased cancer risk. The authors concluded that workers with substantial exposure to phenoxyacetic acid herbicides and their contaminants were at higher risk for soft tissue sarcoma, and the risk was not specifically associated with herbicides contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

In a follow-up analysis, Kogevinas et al. (1997) examined the same register of 21 863 workers using a more refined estimate of exposure to TCDD and higher chlorinated dioxins, verified in some cases by analysis of dioxins in serum and adipose tissue. An SMR of 2.03 (95% CI = 0.75–4.43) was calculated for workers exposed to phenoxyacetic acid herbicides contaminated with TCDD and higher chlorinated dioxins, which was higher than that expected from national mortality rates. Mortality from all malignant neoplasms (SMR = 1.12, 95% CI = 1.04–1.21), non-Hodgkin lymphoma (SMR = 1.39, 95% CI = 0.89–2.06) and lung cancer (SMR = 1.12, 95% CI = 0.98–1.28) was only marginally higher than expected from national mortality rates. No increased risk of mortality from total neoplasms, non-Hodgkin lymphoma or lung cancer was determined in workers exposed to phenoxyacetic acid herbicides with minimal or no contamination by TCDD and higher chlorinated dioxins, whereas mortality from soft tissue sarcoma was marginally elevated (SMR = 1.35). The

authors concluded that "exposure to herbicides containing TCDD and higher chlorinated dioxins may be associated with a small increase in overall cancer risk and in risk for specific cancers".

Vena et al. (1998) analysed non-cancer mortality in the same cohort of pesticide workers as studied by Kogevinas et al. (1995, 1997). SMR analysis did not suggest an increase in non-cancer mortality due to exposure to TCDD and higher chlorinated dioxins. However, Poisson regression analyses suggested an increased risk of ischaemic heart disease (rate ratio = 1.67, 95% CI = 1.23–2.26) and diabetes (rate ratio = 2.25, 95% CI = 0.53–9.50) among TCDD-exposed workers.

In a Swedish population-based, case–control study, the relationship between pesticide exposure and non-Hodgkin lymphoma was examined. Exposure data were obtained from 404 individuals with non-Hodgkin lymphoma (diagnosed from 1987 to 1990) and 741 controls via questionnaires answered by the subjects or next-of-kin (in the case of death). An increased risk for non-Hodgkin lymphoma was calculated for exposure to herbicides (OR = 1.6, 95% CI = 1.0–2.5), phenoxyacetic acid herbicides (OR = 1.5, 95% CI = 0.9–2.4) and MCPA (univariate analysis: OR = 2.7, 95% CI = 1.0–6.9; multivariate analysis: OR = 1.3, 95% CI = 0.4–3.9). For herbicides in total (and for phenoxyacetic acids), the highest risks were calculated when exposure first occurred 10–20 years before diagnosis. In contrast, for MCPA, only exposure during the most recent decade (i.e. 1–10 years) before diagnosis was associated with an increased risk of non-Hodgkin lymphoma (Hardell & Eriksson, 1999).

A case–control study of 910 Swedish patients with non-Hodgkin lymphoma was conducted on cases diagnosed between December 1999 and April 2002. Controls (1016) were chosen randomly from a national population registry. Exposure was evaluated using a questionnaire, with the minimum criterion for inclusion of a single full day. Of the phenoxyacetic acid herbicides, the highest OR (2.81) was calculated for MCPA (95% CI = 1.27–6.22), with all cases having a latency period of greater than 10 years. The OR was 3.76 for less than 32 days of exposure and 1.66 for greater than 32 days of exposure. MCPA appeared particularly associated with histological diffuse large B cell lymphoma (OR = 3.91, 95% CI = 1.48–10.5) or unspecified non-Hodgkin lymphoma (OR = 9.31, 95% CI = 2.11–41.2). The authors concluded that the study confirms an association between exposure to phenoxyacetic acid herbicides and non-Hodgkin lymphoma (Eriksson et al., 2008).

3.2 Poisoning case reports

A 32-year-old Indian male, with a history of tuberculosis and psychosis, was discovered by relatives in a deep coma and unresponsive to painful stimuli. His pupils were small and unreactive and without gross hypertonia; he showed twitching of the face followed by clonic-tonic convulsions. Pulse and respiration were rapid. Liver function test and plasma electrolytes were reportedly within the normal range. Blood pressure declined, and the man died approximately 20 hours after admission to hospital. Based on information provided by relatives, it was estimated that the man ingested 85 ml of a formulation (Verdone) that contained MCPA (equivalent to approximately 22 g MCPA). Autopsy failed to establish the cause of death. Other than evidence of tuberculosis, there were no histopathological abnormalities. MCPA was detected in the gastric washing (0.3 g), liver (0.158 g), urine (0.097 g), stomach (0.041 g), brain (0.038 g), heart (0.037 g) and blood (23 mg/100 ml) (Popham & Davies, 1964).

A 75-year-old retired blacksmith, with a history of congestive cardiac failure and depression, was discovered unconscious near an empty 57 ml bottle of a formulation (Verdone) that contained 25% MCPA. He was admitted to the hospital in a coma and was unresponsive to all stimuli. Blood pressure decreased continuously, and he remained deeply unconscious until death approximately 21 hours after admission (Johnson & Koumides, 1965).

A 61-year-old man, with a recent history of depression, ingested an unknown volume of MCPA. Shortly afterwards, he vomited, his speech became slurred, his face twitched and his limbs jerked. The man was unconscious on arrival at hospital; breathing was laboured, pupils were unresponsive to light and general twitching of the muscles and clonic spasms of the limbs were noted (without major convulsions). Occult blood was observed in the faeces for 21 days. In addition to these central nervous system abnormalities, elevated ALT, ALP and blood urea nitrogen, decreased

haemoglobin and platelets, proteinuria and glycosuria occurred. Serum electrolytes were normal. Hypostatic pneumonia developed. The man recovered completely after prolonged and intensive therapy, including endotracheal intubation, tracheostomy, alkaline diuresis and supportive therapy (Jones et al., 1967).

Roberts et al. (2005) evaluated the toxicity and clinical management of acute MCPA poisoning in a prospective study of 181 self-poisoning cases in Sri Lanka. Patient history, clinical signs and blood (91 patients) were obtained on admission to hospital. Creatine kinase activity was measured in 10 patients who tested positive to MCPA on admission (6 patients had MCPA concentrations in blood > 300 mg/kg, the remainder < 300 mg/kg; limit of detection = 0.05 mg/kg). Minimal toxicity was predominantly observed (85% of patients), manifesting as spontaneous vomiting (44% of patients), with more severe clinical signs (rhabdomyolysis, renal dysfunction and coma) appearing uncommon. Eight patients (4.4%) died, seven within 24–48 hours of poisoning due to cardiorespiratory arrest of an unclear etiology. A delay in the time to hospital admission did not appear to contribute to this outcome. Oliguria or dark-coloured urine was observed in five patients, suggesting renal toxicity. Clinical signs and elevated serum creatine kinase were poorly correlated with plasma concentrations of MCPA. Recommended interventions were limited to correcting acidosis and maintaining adequate urine output.

In a case of fatal poisoning, the highest concentration of MCPA was detected in the stomach contents (10 200 µg/g), the right lung (1362 µg/g), liver (1135 µg/g), heart blood (888 µg/g), brain (771 µg/g), right kidney (756 µg/g) and urine (52 µg/g). 4-Chloro-2-methylphenol was detected as a possible metabolite in body fluids and tissues (Takayasu et al., 2008).

Comments

Biochemical aspects

In studies conducted in rats, dogs and humans using either radiolabelled or unlabelled MCPA, MCPA 2-EHE or MCPA DMA, the time to reach the maximum plasma concentration of radioactivity or of MCPA ion ranged from 1 to 8 hours, depending on the dose. In rats, gastrointestinal absorption was at least 95% of the administered dose, with in vitro data suggesting both a saturable carrier-mediated process and a non-saturable process involving simple diffusion. Following oral dosing of rats, there was no evidence of accumulation of radioactivity in any tissues, with the concentrations in the majority of tissues lower than those in blood. In a study conducted in pregnant rats, the concentration of radioactivity in fetal plasma and amniotic fluid was approximately 3- to 9-fold lower than that in maternal plasma.

MCPA, like other organic acids, is excreted via the kidneys by an active mechanism, with this process saturated at sufficiently high doses in rats, dogs and humans; the threshold of renal saturation is lower in dogs than in rats and humans. In rats, excretion of radioactivity was predominantly via the urine (approximately 90% of the administered dose), with relatively low levels detected in faeces (approximately 5% of the administered dose), although increasing the dose from 5 to 100 mg/kg bw increased the level of radioactivity in the faeces of females (approximately 20% of the administered dose). A different pattern of excretion was evident in dogs, with a greater proportion of radioactivity detected in faeces (17% of the administered dose at 5 mg/kg bw and 49% at 100 mg/kg bw). The plasma elimination half-life was longer in dogs (approximately 45 hours) than in rats and humans (approximately 6–10 hours), with this slower elimination resulting in higher systemic exposure at comparable doses. As a consequence of its longer residence time, MCPA ion undergoes more extensive metabolism in dogs. MCPA ion was the predominant compound detected in rat and dog excreta, followed by HMCPA and glycine-conjugated MCPA. The proportions of MCPA and HMCPA in rat urine ranged from about 51% to 80% and from about 6% to 16% of the administered dose, respectively. In rat faeces, MCPA and HMCPA accounted for approximately 1–2% and 1–7% of the administered dose, respectively. In dogs, MCPA (up to approximately 30% of the administered dose) and HMCPA (up to approximately 15% of the administered dose) were detected in urine, in addition to glycine and taurine conjugates (up to 38% and 10% of the administered dose,

respectively). In dog faeces, MCPA and the glycine and taurine conjugates were identified (up to 28%, 4% and 19% of the administered dose, respectively).

MCPA induced a variety of drug metabolizing enzymes in rats at relatively high oral doses (100–300 mg/kg bw) and increased fatty acid β-oxidation and the number of hepatic peroxisomes. MCPA's peroxisome proliferating potential was not overtly expressed at lower doses in rodents, with a decrease in serum triglycerides the most consistent finding.

Toxicological data

Acute toxicity studies were conducted with MCPA, MCPA 2-EHE and MCPA DMA. The oral LD_{50} in rats was 500–1200 mg/kg bw, depending on the vehicle. Clinical signs generally occurred within hours of dosing and included piloerection, apathy, hunched posture, abnormal gait, decreased respiration, ptosis, pallor and occasionally ataxia, twitching and tonic convulsions; survivors recovered within about 2 days. The dermal LD_{50} in rats and rabbits was greater than 2000 mg/kg bw, whereas the LC_{50} in rats was greater than 4.5 mg/l. Nil to slight skin irritation and slight to severe eye irritation occurred in rabbits, depending on the formulation. No skin sensitization occurred in guinea-pigs (maximization, open epicutaneous and Buehler tests).

The target organs for MCPA ion are the kidney, liver and blood. In laboratory animals, toxicity following repeated dosing typically manifested as perturbations in clinical pathology parameters (increased serum creatinine, urea, liver enzymes and clotting time and reduced red cell parameters and serum protein) and, in some species, increased kidney and liver weights in conjunction with histopathological changes in these organs. Reduced body weight gain and feed consumption, clinical signs and deaths also occurred.

Short-term studies of toxicity of less than 12 months' duration using MCPA, MCPA 2-EHE or MCPA DMA were performed in rats and dogs. In a 3-month study in rats, the NOAEL was 150 ppm (equal to 12 mg/kg bw per day) for increased serum creatinine and kidney weight at 450 ppm (equal to 35 mg/kg bw per day). In dogs, the overall NOAEL from five studies of 13 or 52 weeks' duration was 6 ppm (equal to 0.2 mg/kg bw per day) for increased serum creatinine and urea and increased pigmentation in the proximal tubules at 30 ppm (equal to 1 mg/kg bw per day).

Long-term studies of the toxicity and carcinogenicity of MCPA were conducted in mice and rats, with no indication of any treatment-related neoplastic lesions up to dietary concentrations of 500 ppm in mice and 320 ppm in rats (equal to 83 and 19 mg/kg bw per day, respectively). The chronic NOAEL in mice was 100 ppm (equal to 16 mg/kg bw per day) for increased kidney weight and an increased incidence of intratubular calcification, hyaline casts and tubular epithelial hyperplasia at 500 ppm (equal to 83 mg/kg bw per day). The chronic NOAEL in rats was 80 ppm (equal to 5 mg/kg bw per day), based on increased kidney weight and slight increases in the severity of chronic progressive nephropathy in males and haemosiderin deposition in the spleen of both sexes at 12 but not 24 months at 320 ppm (equal to 19 mg/kg bw per day), findings considered to be of questionable relevance.

The Meeting concluded that MCPA is not carcinogenic in mice or rats.

The genotoxicity of MCPA, MCPA 2-EHE and MCPA DMA has been extensively tested in vitro and in vivo, and all were found to have no genotoxic potential. The Meeting concluded that the MCPA ion is unlikely to be genotoxic to humans.

In the absence of genotoxicity potential and a carcinogenic response in mice and rats, the Meeting concluded that MCPA ion is unlikely to pose a carcinogenic risk to humans.

In multigeneration studies in rats on MCPA or MCPA 2-EHE, there was no evidence of reproductive toxicity up to the highest tested dietary concentrations of 450 and 1600 ppm (equal to 40 and 150 mg/kg bw per day, respectively) over one or two generations. In the two-generation study on MCPA, the NOAEL for parental and offspring toxicity was 150 ppm (equal to 12 mg/kg bw per day) for reduced body weight gain at 450 ppm (equal to 40 mg/kg bw per day). The LOAEL for parental toxicity in this study is consistent with those in four subchronic rat studies. In one-generation studies on MCPA or MCPA 2-EHE, reduced parental body weight gain occurred at the lowest tested dietary

concentrations of 450 and 700 ppm (equal to 40 and 65 mg/kg bw per day, respectively), whereas the NOAEL for offspring toxicity was 750 or 1200 ppm (equal to 115 and 90 mg/kg bw per day, respectively) for reduced body weight gain at 1000 or 1600 ppm (equal to 160 and 230 mg/kg bw per day, respectively).

In rat developmental toxicity studies conducted on MCPA, MCPA 2-EHE and MCPA DMA, the overall NOAEL for maternal toxicity and fetal and embryo toxicity was 60 mg/kg bw per day for reduced maternal body weight gain and feed consumption, reduced fetal body weight and an increase in fetal anomalies (mainly delayed ossification), post-implantation losses and early resorptions at 120 mg/kg bw per day. The reduction in maternal body weight gain and feed consumption was evident 1 or 2 days after the commencement of dosing and persisted throughout gestation. The Meeting noted that the developmental findings occurred only at maternally toxic doses.

In a rabbit developmental toxicity study on MCPA, no developmental effects occurred up to the highest tested dose of 60 mg/kg bw per day. The NOAEL for maternal toxicity was 15 mg/kg bw per day, based on clinical signs (piloerection, no defecation and blood in the bedding) and deaths at 30 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 60 mg/kg bw per day, the highest dose tested.

The Meeting concluded that MCPA ion is not teratogenic in rats or rabbits.

Acute and subchronic neurotoxicity studies were conducted on MCPA, MCPA 2-EHE and MCPA DMA in rats. In the acute studies, clinical signs of toxicity observed 24 hours after dosing and transiently impaired motor activity were attributable to acute systemic toxicity rather than to a direct neurotoxic effect. No pathology of the brain or nervous tissue was observed in these or other toxicity studies. The acute NOAEL for MCPA was 150 mg/kg bw, whereas clinical signs (mainly ataxia) were observed at the lowest tested doses of MCPA 2-EHE and MCPA DMA (160 and 143 mg/kg bw, respectively). The overall NOAEL in the three subchronic studies was 4 mg/kg bw per day, with increased serum creatinine the most consistent effect seen at the next higher dose of 40 mg/kg bw per day.

CCPA is a metabolite of MCPA specific to plants. The oral LD_{50} in rats was greater than 2000 mg/kg bw. CCPA was not mutagenic. In a 4-week dietary study, CCPA was approximately 5-fold less toxic than MCPA. The NOAEL for CCPA was 176 mg/kg bw per day, based on reduced serum albumin, increased serum magnesium, increased urine specific gravity and increased renal calcification at approximately 1100 mg/kg bw per day. The LOAEL for MCPA was approximately 170 mg/kg bw per day. The Meeting concluded that CCPA was less toxic than MCPA ion.

Poisoning case reports described clinical observations in humans following deliberate ingestion of relatively large doses of MCPA formulations. Clinical signs consistent with those observed in laboratory animals and renal toxicity were reported.

The Meeting concluded that the database on MCPA ion was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting concluded that the dog was an unsuitable surrogate for humans because of its relatively low renal capacity to excrete MCPA ion, leading to higher toxicity than in other species. Therefore, the Meeting established an acceptable daily intake (ADI) of 0–0.1 mg/kg bw per day for MCPA ion, based on the overall NOAEL of 12 mg/kg bw per day from four subchronic studies in rats for changes in clinical chemistry parameters indicative of effects on the kidneys at 35 mg/kg bw per day and using a 100-fold safety factor. This overall NOAEL is supported by the NOAEL of 12 mg/kg bw per day for parental and offspring toxicity from the two-generation reproductive toxicity study in rats and the NOAEL of 15 mg/kg bw per day for maternal toxicity in the developmental toxicity study in rabbits. The Meeting considered that this ADI would adequately cover the kidney and spleen effects observed in the 2-year rat study at 19 mg/kg bw per day. The ADI is established for the sum of MCPA and its salts and esters, expressed as MCPA acid equivalents.

The Meeting established an acute reference dose (ARfD) of 0.6 mg/kg bw for MCPA ion, based on the overall NOAEL for maternal and developmental toxicity of 60 mg/kg bw in rats and using a 100-fold safety factor. At 120 mg/kg bw, maternal body weight gain and feed consumption were reduced within 1 or 2 days after commencement of dosing in three rat developmental toxicity studies, in addition to an increase in early resorptions in two of these studies. The Meeting considered that the maternal toxicity observed in the rabbit developmental toxicity study was an unsuitable basis for the ARfD because it was not an acute effect. The ARfD is established for the sum of MCPA and its salts and esters, expressed as MCPA acid equivalents.

Levels relevant to risk assessment based on studies conducted on MCPA, MCPA 2-EHE and MCPA DMA

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 16 mg/kg bw per day	500 ppm, equal to 83 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 83 mg/kg bw per day[b]	—
Rat	Thirteen-week studies of toxicity[a,c]	Toxicity	150 ppm, equal to 12 mg/kg bw per day	450 ppm, equal to 35 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	450 ppm, equal to 40 mg/kg bw per day[b]	—
		Parental toxicity	150 ppm, equal to 12 mg/kg bw per day	450 ppm, equal to 40 mg/kg bw per day
		Offspring toxicity	150 ppm, equal to 12 mg/kg bw per day	450 ppm, equal to 40 mg/kg bw per day
	Developmental toxicity studies[c,d]	Maternal toxicity	60 mg/kg bw per day	120 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day	120 mg/kg bw per day
Rabbit	Developmental toxicity study[d]	Maternal toxicity	15 mg/kg bw per day	30 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day[b]	—

[a] Dietary administration.
[b] Highest dose tested.
[c] Two or more studies combined.
[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.1 mg/kg bw

Estimate of acute reference dose

0.6 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to MCPA ion

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and almost complete
Distribution	Widespread tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid except in dogs
Metabolism in animals	Limited; more extensive in dogs
Toxicologically significant compounds in animals, plants and the environment	MCPA, CCPA (plant metabolite)
Acute toxicity	
Rat, LD_{50}, oral	> 500 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.5 mg/l (4 h, whole body)
Rabbit, dermal irritation	Non-irritating to slightly irritating
Rabbit, ocular irritation	Slightly to severely irritating
Dermal sensitization	Non-sensitizing (guinea-pigs)
Short-term studies of toxicity	
Target/critical effect	Kidney, liver and blood
Lowest relevant oral NOAEL	12 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	160 mg/kg bw per day (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney, liver and blood
Lowest relevant NOAEL	16 mg/kg bw per day (mouse)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Target/critical effect	No evidence of reproductive toxicity (rat)
Lowest relevant parental NOAEL	12 mg/kg bw per day
Lowest relevant offspring NOAEL	12 mg/kg bw per day
Lowest relevant reproductive NOAEL	40 mg/kg bw per day (highest dose tested)
Developmental toxicity	
Target/critical effect	Effects on fetuses at maternally toxic doses (rat)
Lowest relevant maternal NOAEL	15 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	60 mg/kg bw per day (rat and rabbit)
Neurotoxicity	
Acute and subchronic neurotoxicity	Not neurotoxic

Medical data	
	Effects following human poisonings consistent with laboratory animal findings

Summary

	Value	Studies	Safety factor
ADI	0–0.1 mg/kg bw	Short-term repeated-dose studies (rat), two-generation reproductive toxicity study (rat) and developmental toxicity study (rabbit)	100
ARfD	0.6 mg/kg bw	Developmental toxicity studies (rat)	100

References

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