CHLORPROPHAM (addendum)

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Explanation

Chlorpropham is the International Organization for Standardization (ISO) approved name for 1-methylethyl (3-chlorophenyl) carbamate (Figure 1), which is a plant growth regulator used for pre-emergence and early post-emergence control of grass weeds. It is also used to inhibit potato sprouting. The toxicity of chlorpropham was evaluated by the JMPR in 1963, 1965 and 2000. In 2000, the Meeting established an acceptable daily intake (ADI) of 0–0.03 mg/kg bw based on a no-observed-adverse-effect level (NOAEL) of 10 mg/kg bw per day in a 90-day study of toxicity in Wistar rats, this NOAEL being identified on the basis of a significant decrease in erythrocyte counts and an increase in methaemoglobin formation at the next higher dose of 47 mg/kg bw per day. A safety factor of 300 was applied, which included an additional safety factor of 3 to account for inadequacies in the assessment of methaemoglobinaemia (lack of measurements of methaemoglobin formation at early time-points, a concern since adaptation to this effect can occur), the critical toxicological effect. This ADI also provided an adequate margin of safety for the effects on the thyroid observed in dogs (NOAEL, 5 mg/kg bw per day). An acute reference dose (ARfD) equal to the maximum ADI was also established.

The sponsor conducted a study of acute toxicity in female beagle dogs in order to refine the ARfD, to address concerns with respect to the extent of investigation of methaemoglobinaemia. The 2005 JMPR was asked by the Codex Committee for Pesticide Residues to review the ARfD for chlorpropham, and as a consequence of this review, the Meeting also reconsidered the ADI.

The new study of acute toxicity complied with good laboratory practice (GLP).

Evaluation for acute reference dose

Dogs

Groups of four female beagle dogs were given gelatin capsules containing chlorpropham (purity, 99.3%; batch No. 1516-2002) as single oral doses at 0, 50, 125 or 625 mg/kg bw. The dog was chosen since dogs are considered to be more similar to humans than are rodents, particularly with respect to the activity of methaemoglobin reductase, which is higher in rodents (Warren, 2003). Females were selected because no sex-related differences in methaemoglobin formation

were observed at week 4 in a 90-day study of toxicity in dogs given chlorpropham and the use of females rather than males may give marginally greater regulatory confidence if only because it is females that become pregnant; and the fetus, which exists in a state of low oxygen tension, may be considered at particular risk of methaemoglobin formation (Warren, 2003). The test substance was ground to a powder and weighed directly into gelatin capsules. The treated animals were observed for 96 h after dosing. On the day of treatment, animals were examined before dosing, and at 0.5, 1, 2, 4 and 24 h after dosing. On the subsequent days, animals were observed twice daily for mortality and clinical signs of toxicity. Animals were observed daily for evidence of ill health, such as loose faeces. The body weight of each dog was measured at least once per week during the acclimatization period, on the day of treatment (day 1), daily thereafter throughout the observation period, and before necropsy. Food consumption was measured daily for each dog during the acclimatization period and throughout the study. Ophthalmoscopic examinations and urine analyses were not performed. Haematological parameters were measured from blood samples collected from all animals before treatment and at 2, 4, 6, 10, 24, 48, 72, and approximately 78 and 96 h after dosing. Bone marrow samples were obtained from the sternum during necropsy of all animals. Gross necropsies were performed and selected organs were removed, weighed and histopathological examinations were performed at study termination. This study was conducted in accordance with GLP regulations.

No mortality occurred during the study. There were no treatment-related clinical signs of toxicity at the 50 mg/kg bw dose. A total of three out of the four animals in the group receiving a dose of 125 mg/kg bw showed reduced activity between 2 h and 6 h after dosing. At the intermediate dose, vomiting was noted in one animal at about 45 min after dosing and again at about 2 h after dosing and in a further two animals at approximately 2 h after dosing. One animal had a strong pulse between approximately 2 h and 4 h after dosing. A total of two of the four animals in the 625 mg/kg bw dose group showed reduced activity between approximately 2 h and 6 h after dosing. Vomiting was noted in three out of four animals at approximately 30 min after dosing and the last incidence of vomiting was at about 2–2.5 h after dosing. One animal showed unsteady hind gait, head twitching, trembling, rapid pulse, red spots on ears and red spots on abdomen between approximately 2–4 h after dosing. One animal showed vocalizing between 2 h and 4 h after dosing. All clinical signs of toxicity had subsided by 24 h in the treated groups. No treatment-related effects on body weight or body-weight gains were observed (Table 1). One animal in the group receiving the highest dose lost about 0.1 kg of body weight; this was not statistically significant and not considered to be caused by treatment. Food consumption was not affected by treatment.

No treatment-related effects were observed on any of the haematological parameters evaluated except methaemoglobin. Bone-marrow smears were not evaluated because of lack of effects on other haematological parameters. No methaemoglobin formation was observed in any treated group before dosing. One animal in the control group showed methaemoglobin levels of 0.1% and 0.2% at 2 h and 10 h after dosing, respectively.

Figure 1. Chemical structure of chlorpropham

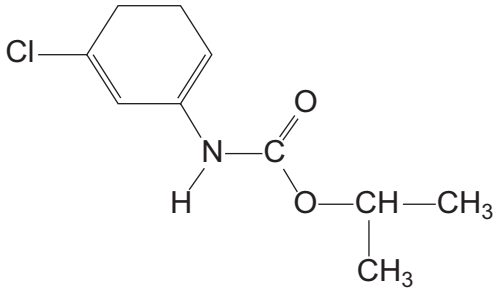

Table 1. Average body weights and body-weight gains of female beagles given single oral doses of chlorpropham

Dose (mg/kg bw)	Body weight (kg)[a]						Total body-weight gain	
	Day –7	Day 1	Day 2	Day 3	Day 4	Day 5	Absolute (kg)	% of control
0	8.4	8.7	8.9	8.9	8.9	9.0	0.2	—
50	9.6	9.8	10.0	10.1	10.1	10.0	0.2	100
125	8.3	8.7	8.7	8.8	8.8	8.8	0.2	100
625	8.7	9.0	9.0	9.1	9.0	9.1	0.1	50

[a] Data obtained from Scott (2003).

No statistical significance ($p > 0.05$)

One animal in the group at 50 mg/kg bw had a value of 0.1% for methaemoglobin formation at 4 h after dosing. This animal and two other animals had values for methaemoglobin formation of 0.2% at 72 h after dosing, while the remaining animal had a value of 0.1%. No methaemoglobin was detected in any animals in the group receiving chlorpropham at a dose 50 mg/kg bw at 78 h and 96 h.

The increase in formation of methaemoglobin seen at 50 mg/kg bw is not considered to be treatment-related because similar levels were also seen in one out of four animals in the control group, and because procedural differences in analysis of the samples in this group at 72 h may have influenced the results.

Three animals in the group receiving chlorpropham at a dose of 125 mg/kg bw had methaemoglobin values of 0.3%, 0.4% and 0.4% at 2 h after dosing; these values returned to zero at 6 h after dosing. One animal at 125 mg/kg bw had a low level of methaemoglobin formation (0.1%) at 10 h and 24 h after dosing. No methaemoglobin was detected in the group receiving chlorpropham at a dose of 125 mg/kg bw at 48, 72, 78 or 96 h after dosing.

All animals at 625 mg/kg bw had detectable levels of methaemoglobin at 2 h after dosing. For two animals, the peak levels of methaemoglobin were observed at 2 h after dosing and returned to zero at 6 h in one animal and at 10 h in another animal. For the remaining two animals, the peak levels were reached at either 4 h or 10 h after dosing and returned to zero by 24 h after dosing, and remaining at zero thereafter. The highest individual value for methaemoglobin formation was 0.8% observed in one animal at the highest dose at 10 h after dosing. Mean values for methaemoglobin formation are summarized in Table 2.

No treatment-related effects on organ weight (either absolute or relative) were observed. Spleen weights were increased in all treated groups compared with those of controls, and thymus weights were decreased in all treated groups compared with those of the controls. However, these changes in organ weights were not considered to be caused by treatment because there was no dose–response relationship, there was overlap in individual values between controls and treated animals, and statistical significance was not achieved. Pale oesophagus was seen in three out of four animals at 50 mg/kg bw and in two out of four animals at 125 mg/kg bw. The significance of this finding is unclear since microscopic examination was not conducted; however, it was not likely to have been caused by treatment since it was not seen in animals at 625 mg/kg bw. No treatment-related effects on thyroid (with parathyroid) were seen in this study. Thyroid toxicity was seen in the previous studies in dogs and thyroid effects were seen at a lower dose than were haematological effects in dogs (Annex 1, reference 91). The NOAEL was 50 mg/kg bw on the basis of clinical signs of toxicity (vomiting, reduced activity). Possible treatment-related increases in the formation of methaemoglobin were seen at the higher doses of 125 and 625 mg/kg bw (Scott, 2003).

Table 2. Mean concentration of methaemoglobin expressed as a percentage of haemoglobin concentration in female beagles given single oral doses of chlorpropham[a,b]

Hours after treatment	Dose (mg/kg bw)			
	0	50	125	625
Pretreatment	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
2	0.03 (0.050)	0.00 (0.000)	0.28 (0.189)	0.30* (0.245)
4	0.00 (0.000)	0.03 (0.050)	0.05 (0.100)	0.28** (0.171)
6	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.35*** (0.289)
10	0.05 (0.100)	0.00 (0.000)	0.03 (0.050)	0.30 (0.383)
24	0.00 (0.000)	0.00 (0.000)	0.03 (0.050)	0.00 (0.000)
48	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
72	0.00 (0.000)	0.18† (0.050)	0.00 (0.000)	0.00 (0.000)
78	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
96	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)

From Scott (2003), Table 3, pp. 27–36.

[a] $n = 4$, except pretreatment values for which $n = 2$

[b] Standard deviation given in parentheses

* $p \leq 0.05$ (Williams' test)

** $p \leq 0.01$ (Williams' test

*** $p \leq 0.05$ (Mantel test for trend in proportions)

† $p \leq 0.05$ (Fisher's exact test)

Comments

In 2000, the JMPR determined that chlorpropham has low acute toxicity: the oral LD_{50} in rats was > 2000–4200 mg/kg bw, and the dermal LD_{50} in both rats and rabbits was > 2000 mg/kg bw. Chlorpropham is also only weakly toxic after inhalation since there were no deaths at 0.47 mg/l, the highest attainable concentration.

Chlorpropham was not irritating to the eyes or skin of rabbits. It did not sensitize the skin of guinea-pigs in a Bühler test, in an open epicutaneous test, or in a Magnusson & Kligman test. Although chlorpropham sensitized the skin of 30% of the guinea-pigs tested in a split adjuvant test, the 2000 JMPR concluded that chlorpropham is unlikely to cause sensitization in humans.

After an evaluation of short- and long-term studies of the effects of chlorpropham in mice, rats, and dogs, the 2000 JMPR determined that the haematopoietic system was the main toxicological target; changes were observed in the morphology and parameters of erythrocytes, including increased formation of methaemoglobin, and changes in the spleen and liver consistent with a haemolytic effect. In a study of dermal toxicity in rabbits, chlorpropham also produced haematopoietic effects. In dogs fed diets containing chlorpropham for 28 days or fed capsules containing chlorpropham for 90 days, effects were also seen on the thyroid gland at doses similar to or lower than those that affected erythrocytes. In dogs given capsules containing chlorpropham for 60 weeks, a NOAEL of 5 mg/kg bw per day was identified on the basis of effects on the thyroid gland, including increased weight, decreased concentrations of thyroxine (in a test for stimulation by thyroid-stimulating hormone), and, occasionally, decreased concentrations of tri-iodothyronine. In 90-day and 2-year dietary studies in rats, reduced thyroid weights were seen at doses higher than those that caused haematotoxic effects.

Chlorpropham was not a reproductive toxicant in rats and was not teratogenic in rats and rabbits. In 2000 the JMPR had concluded that while chlorpropham may be weakly genotoxic in

vitro, it was unlikely to present a risk to humans, although it was noted that this conclusion should be validated in adequate studies of genotoxicity in vivo.

The present Meeting evaluated a study of acute oral toxicity in female dogs given capsules containing chlorpropham as single doses at up to 625 mg/kg bw. Chlorpropham produced clinical signs of toxicity manifested as vomiting and reduced activity at 125 mg/kg bw and above, apparent within 2 h after dosing, but these signs were no longer evident by 4–6 h after dosing. The NOAEL was 50 mg/kg bw. Chlorpropham also produced increased formation of methaemoglobin in all treated groups. However, the increases in methaemoglobin levels were very small, reaching a maximum of 0.8% in one of four animals at the highest dose. The effects were possibly treatment-related at 125 and 625 mg/kg bw, but the small increases at 50 mg/kg bw resulted in levels that were no higher and no more prolonged than those seen in control animals. None of the increases in methaemoglobin levels were toxicologically significant at any dose. With respect to the maximum increase in methaemoglobin seen in this study (0.8%), it should be noted that in 2004 the JMPR recommended that for acute exposure to xenobiotics that induce methaemoglobin formation, only an increase in methaemoglobin formation of 4% (or higher) above background in dogs should be considered to be relevant for setting an ARfD.

The primary effects of repeated doses of chlorpropham appear to be on the haematopoietic system and on the thyroid. In rats, haematological effects appeared at lower doses than thyroid effects, while in dogs thyroid effects appeared at lower doses than did haematological effects. In a 90-day study of toxicity in rats, the NOAEL for increased methaemoglobin formation was 10 mg/kg bw per day, while in a 90-day study of toxicity in dogs, the NOAEL was 25 mg/kg bw per day. These apparent differences in NOAEL are likely to be due to artifacts of dose selection rather than to any increased sensitivity of rats over dogs. Thus the study of acute toxicity in dogs was considered to be adequate to assess the effects of acute dosing with chlorpropham on the formation of methaemoglobin.

Toxicological evaluation

The Meeting reconsidered the previously established ADI on the basis of the new study providing information on methaemoglobin measurements at early time-points. Because the new study in dogs addressed previous concerns about the induction of methaemoglobin at early time-points, the Meeting established an ADI of 0–0.05 mg/kg bw based on the NOAEL of 5 mg/kg bw per day in a 60-week study in dogs fed with chlorpropham, on the basis of changes in the thyroid at 50 mg/kg bw per day, and using a safety factor of 100. This ADI provided an adequate margin of safety for the haematotoxic effects seen in the studies of repeated doses in rats.

The Meeting established an ARfD of 0.5 mg/kg bw, on the basis of a NOAEL of 50 mg/kg bw in the study of acute toxicity in dogs given capsules containing chlorpropham identified on the basis of clinical signs of toxicity at the higher doses of 125 and 625 mg/kg bw, and using a safety factor of 100. Slight increases in methaemoglobin levels in this study were not considered to be toxicologically significant at any dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	90-day study of toxicity[a]	Toxicity	—	190 mg/kg bw per day
	78-week study of toxicity and carcinogenicity[a]	Toxicity	100 mg/kg bw per day	500 mg/kg bw per day
		Carcinogenicity	1000 mg/kg bw per day[b]	—

Rat	90-day study of toxicity[a]	Toxicity	10 mg/kg bw per day	47 mg/kg bw per day
	24-month study of toxicity	Toxicity and carcinogenicity[a]	—	30 mg/kg bw per day
		Carcinogenicity	500 mg/kg bw per day	1000 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Parental and pup toxicity	1000 ppm, equivalent to 50 mg/kg bw per day	3000 ppm, equivalent to 150 mg/kg bw per day
		Reproductive toxicity	10 000 ppm, equivalent to 500 mg/kg bw per dayb	—
	Developmental toxicity[c]	Maternal and fetal toxicity	200 mg/kg bw per day	800 mg/kg bw per day
		Embryotoxicity	200 mg/kg bw per day	800 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	250 mg/kg bw per day	500 mg/kg bw per day
		Embryotoxicity	125 mg/kg bw per day	250 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	125 mg/kg bw per day	250 mg/kg bw per day
		Embryo- and fetotoxicity	250 mg/kg bw per day	500 mg/kg bw per day
Dog	Acute[d]	Toxicity	50 mg/kg bw	125 mg/kg bw
	90-day study of toxicity[d]	Toxicity	25 mg/kg bw per day	125 mg/kg bw per day
	60-week study of toxicity[a,d]	Toxicity	5 mg/kg bw per day	50 mg/kg bw per day

[a] Dietary administration

[b] Highest dose tested

[c] Gavage administration

[d] Capsule

Estimate of acceptable daily intake for humans

0–0.05 mg/kg bw

Estimate of acute reference dose

0.5 mg/kg bw

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

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

Critical end-points for setting guidance values for exposure to chlorpropham

Summary	*Value*	*Study*	*Safety factor*
ADI	0–0.05 mg/kg bw	Dog, 60-week, toxicity	100
ARfD	0.5 mg/kg bw	Dog, acute toxicity	100

References

Scott, A. (2003) Chlorpropham acute reference dose study by oral capsule administration to female beagle dogs. Unpublished report No. MVX 002/033270 from Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, UK. Submitted to WHO by Luxan BV, Netherlands and by Aceto Agricultural Chemicals Corporation, Lake Success, New York, USA.

Warren, S (2003) Chlorpropham rationale for design of study, HLS Study MVX/002. In: Scott, A. (2003) Chlorpropham acute reference dose study by oral capsule administration to female beagle dogs. Unpublished report No. MVX 002/033270 from Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, UK. Submitted to WHO by Luxan BV, Netherlands and by Aceto Agricultural Chemicals Corporation, Lake Success, New York, USA.