

5.4 CAPTAN (007)

TOXICOLOGY

Evaluation for an acute reference dose

Captan, the ISO approved name for *N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide, is a fungicide (CAS No. 133-06-2) registered for the control of fungal diseases in crops. The toxicology of captan was evaluated by the JMPR in 1963, 1965, 1969, 1973, 1978, 1982, 1984, 1990, 1995 and 2004. Toxicological monographs were prepared by the Meeting in 1963, 1965 and 1969, and monograph addenda were prepared in 1973, 1977, 1978, 1982, 1984, 1990, 1995 and 2004. In 1984, an ADI of 0–0.1 mg/kg bw was established based on a NOAEL of 12.5 mg/kg bw per day in studies of reproductive toxicity in rats and monkeys. This ADI was confirmed by JMPR in 1995. In 2004, the Meeting established an ARfD of 0.3 mg/kg bw, for women of childbearing age only, based on a NOAEL of 30 mg/kg bw per day for increased incidences of intrauterine deaths and malformations at 100 mg/kg bw per day in the study in rabbits and a safety factor of 100.

The Meeting concluded that the database was insufficient, particularly with regard to information about the possible developmental effects of the metabolite 1,2,3,6-tetrahydrophthalimide (THPI), to establish the mode of action by which the increased incidences of intrauterine deaths and fetuses with malformations were induced.

The sponsor conducted a study of developmental toxicity with THPI, and studies to evaluate the potential effects of captan and THPI on the intestinal flora of the rabbit. It was known that the rabbit is dependent on the presence of caecotrophs for adequate nutrition. The sponsor suggested that disruption of the intestinal flora might result in maternal malnutrition, with possible consequent adverse effects on foetal development.

At the request of the CCPR at its 39th Session,²² the present Meeting reconsidered the ARfD on the basis of new data.

All pivotal studies with captan and THPI were certified as being compliant with GLP

Toxicological data

Data previously evaluated by the Meeting in 2004

With respect to the developmental toxicity of captan, the following description is quoted from JMPR 2004 (Annex 5, reference 101, 103):

In a study from the published literature, the teratogenic effects of a number of phthalimide derivatives, including captan, were tested in pregnant golden hamsters. The Meeting noted that this study has major limitations (e.g., small number of animals per dose, limited reporting of the data) and is therefore of limited value. It does, however, suggest that developmental effects may occur after a single exposure to captan, albeit at maternally toxic doses.

In a study of developmental toxicity in rats treated by gavage, captan was not teratogenic. The NOAEL for maternal toxicity was 18 mg/kg bw per day on the basis of a reduction in body weight and food consumption. The NOAEL for offspring toxicity was 90 mg/kg bw per day on the basis of the reduction in foetal body weight and an increased incidence of skeletal variations.

In a study in rabbits treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of a markedly reduced body-weight gain and reduced food consumption at 30 mg/kg bw per day. The NOAEL for embryo- and fetotoxicity was 10 mg/kg bw per day on the basis of

²² Codex Alimentarius Commission. *Report of the 39th Session of the Codex Committee on Pesticide Residues, 7–12 May 2007, Beijing, China* (ALINORM07/30/24).

increases in skeletal variations at 30 and 100 mg/kg bw per day. At 100 mg/kg bw per day, increased incidences of early and late intra-uterine deaths were observed, as were increased incidences of several malformations. The NOAEL for these effects was 30 mg/kg bw per day. Multiple malformations observed in two fetuses in the group receiving the intermediate dose were considered to be incidental. In another study in rabbits treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and food consumption at 40 mg/kg bw per day. On the basis of the increase in post implantation losses and the increase in incidence of minor skeletal variations at 160 mg/kg bw per day, the NOAEL for embryo- and fetotoxicity was 40 mg/kg bw per day. In a third study in rabbits treated by gavage, the NOAEL for maternal toxicity was 12 mg/kg bw per day on the basis of reductions in body-weight gain during the initial phase of treatment. The NOAEL for embryo- and fetotoxicity was 25 mg/kg bw per day on the basis of a reduction in foetal body weight at 60 mg/kg bw per day. The Meeting considered that maternal toxicity and the associated increases in skeletal variations and foetal body-weight reductions observed were likely to be caused by high local concentrations of captan produced by administration by gavage, and are not considered to be relevant to dietary exposure.

Toxicokinetics

In a study evaluated by the JMPR in 2004, in mice given captan at a dose of 400 or 3000 ppm (equivalent to 57 and 429 mg/kg bw per day) no captan was found in mouse duodenal tissue or its contents (extracts of 5 cm sections of the duodenum were analysed; limit of quantitation, 0.5–3 nmoles, i.e., 0.150–1 µg), indicating that in mice, captan is largely, if not completely, degraded to THPI in the stomach.

In a toxicokinetic study in rats (previously evaluated by the JMPR in 1995), concentrations of captan and its metabolites were measured in the faeces and urine. In order to establish whether captan reached the distal parts of the gastrointestinal tract in rats, the study was re-evaluated by the present Meeting.

Rats were given [¹⁴C] labelled captan at a dose of 10 or 500 mg/kg bw by gavage. Extracts of urine and faeces were qualitatively analysed by thin-layer chromatography (TLC) through comparison with reference compounds. Quantification of metabolites was performed by linear plate scanner and autoradiography. Subsequently, some metabolites isolated by TLC were further identified by gas chromatography/mass spectrometry (GC/MS). Several compounds were identified in urine and faeces, including THPI, 3- and 5-hydroxylated THPI, THPI-diol, THPI-epoxide, cyclohexene acid amide and hydroxycyclohexene acid amide and parent captan. The presence of captan in extracts of urine and faeces was identified by TLC only (i.e., by comparison with the retention factor, R_f, of captan). No further identification was attempted. Toxicokinetic data are presented in Table 8.

Table 8. Toxicokinetic data for rats given [¹⁴C] labelled captan by gavage.

Dose	Radioactivity excreted (% of administered dose)		Captan in urine (% of radioactivity)	Captan in faeces	
	Urine	Faeces		% of radioactivity (males/females)	% of total administered dose
10 mg/kg bw, single dose	81	8–9	ND	6.5/16.8	0.5–1.5
10 mg/kg bw, repeated dose	88–91	7–9	ND/0.7 (males/females)	ND/4.6	ND/0.4
500 mg/kg bw, single dose	69–73	23–25	1.3–1.6	44.1/40.9	± 12 (males and females)

From Lappin & Havell (1990).

ND, not detected

After a single dose at 10 mg/kg bw, 81% and 8–9% of the administered radioactivity was recovered from urine and faeces, respectively. At this single dose, no captan was detected in the urine. Between 0.5% and 1.5% of the total dose recovered in the faeces was captan.

After 14 consecutive doses at 10 mg/kg bw, 88–91% and 7–9% of the administered radioactivity was recovered from the urine and faeces, respectively. At this repeated low dose, 0.7% of urinary radioactivity was detected as captan in the urine of females (not detected in males). Of the radioactivity recovered from the faeces up to 4.6% was captan.

At 500 mg/kg bw, about 71% and 24% of the administered radioactivity was recovered from the urine and faeces, respectively. At this dose, 1–2% of urinary radioactivity was provisionally identified as intact captan.²³

Developmental toxicity

In a study of developmental toxicity, groups of 25 time-mated female New Zealand White rabbits received THPI (purity, 98.4%) at a dose of 0, 5, 10 or 22.5 mg/kg bw per day by gavage from days 6 to 28 after mating. The vehicle was water containing 0.5% w/v Tween 80 and 0.7% w/v carboxymethylcellulose. In view of the relative molecular masses of captan (300.6) and THPI (151.2), a dose of THPI of 22.5 mg/kg bw per day would be equimolar to a dose of captan of about 45 mg/kg bw per day. All animals were examined twice per day for clinical signs. Body weight was recorded daily from the day of mating until day 29 of gestation, when the animals were killed. Food intake was recorded daily from the first day after mating until day 29 of gestation. On day 29 of gestation, the animals were killed and examined macroscopically. In females of the control group and at the highest dose, the duodenum and sphincter of Oddi (hepatopancreatic sphincter) were examined microscopically. The ovaries and uterus were removed and the foetuses were weighed and examined for visceral and skeletal abnormalities.

One female in the control group died on day 12 of gestation. The cause of death could not be established. One animal at 10 mg/kg bw per day was killed in extremis on day 14 of gestation. At necropsy, in the control group and in the groups at 5, 10 and 22.5 mg/kg bw per day, six, one, three and one female respectively did not appear to be pregnant. In the dams, no treatment-related clinical signs or effects on body weight and food consumption, and no macroscopic or microscopic abnormalities were observed. In the foetuses, no treatment-related embryo/fetotoxicity or effects on visceral and skeletal parameters were observed. The NOAELs for maternal and embryo/fetotoxicity were 22.5 mg/kg bw per day i.e., the highest dose tested.²⁴

²³ Lappin, G.J. & Havell, M.L. (1990) Captan: biotransformation study in the rat. Unpublished report No CTL/P/2951 from ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

²⁴ Blee, M.A.B. (2006) Tetrahydrophthalimide. Prenatal toxicity study in the rabbit by oral gavage administration. Unpublished report No R-18202, MAK 864/053232 from Huntingdon Life Sciences Limited, Woolley Road, Alconbury, Huntingdon, Cambridgeshire. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Inhibition of microbial activity in vitro

A study was performed to determine minimum inhibitory concentrations (MIC) of captan (purity, 95.1%) against two bacterial species (*Bacteroides* sp. and *Enterococcus faecalis*) and one species of yeast (*Candida albicans*). These bacteria were considered to be representative of anaerobic bacteria in the rabbit gut. *Candida albicans* was considered to be representative of yeast that may occur in the rabbit gut. The MIC values for *Bacteroides* sp., *Enterococcus faecalis* and *Candida albicans* were 20–50, 50–500 and 2–5 µg/mL, respectively. The Meeting concluded that captan demonstrates antimicrobial activity against organisms considered representative of rabbit gut flora.²⁵

In a study to determine MIC of THPI (purity, 98.4%), MIC values for *Bacteroides* sp., *Enterococcus faecalis* and *Candida Albicans* were all > 1000 µg/mL. The Meeting concluded that THPI demonstrates no antimicrobial activity against organisms considered representative of rabbit gut flora.²⁶

The Meeting concluded that the existing database (i.e., the new available studies and the previously evaluated studies), was adequate to characterize the potential hazards of captan to fetuses, infants and children.

Toxicological evaluation

In 2004, the Meeting established an ARfD of 0.3 mg/kg bw for captan for women of childbearing age, based on a NOAEL of 30 mg/kg bw per day for increased incidences of intrauterine deaths and malformations at 100 mg/kg bw per day in a study in rabbits and using a safety factor of 100.

The new study of developmental toxicity with THPI did not elucidate the mode of action by which the increased incidences of intrauterine deaths and of fetuses with malformations (observed at 100 mg/kg bw per day expressed as captan) were induced. Because the maximum dose was similar to the NOAEL in the study of developmental toxicity with captan, it was not possible to use this study to determine the contribution of THPI to the developmental toxicity of captan.

Studies in vitro showed that captan, but not its metabolite THPI, has antimicrobial action on gut flora.

A toxicokinetic study in mice indicated that an oral dose of captan is largely, if not completely, metabolized in the stomach in this species. Toxicokinetic studies in rats given captan by gavage indicated that, at a high dose (500 mg/kg bw), a considerable amount of captan reaches the distal part of the gastrointestinal tract. At this dose, intact captan was also present in rat urine. At a single low dose of 10 mg/kg bw given by gavage, captan also reached the distal part of the gastrointestinal tract. It was not detected in urine after a single dose at 10 mg/kg bw, but was detected in the urine after 14 daily doses. No toxicokinetic data in rabbits, the species showing the developmental effect of concern, were provided.

There was some evidence that THPI can reach the caecum and that the developmental effects of captan in rabbits might be secondary to an effect on the gut flora of this species. The doses achieved in the study with THPI did not exclude a systemic role for this compound (and its further metabolites) in the developmental effects seen with captan in rabbits. Furthermore, it could not be excluded that the effects observed in a study of developmental toxicity in rabbits were a result of

²⁵ Akhurst, L.C (2005a) Captan: determination of minimum inhibitory concentration against selected microorganisms representative of rabbit gut microflora. Unpublished report No. R-18666, MAK 0888/052848 from Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

²⁶ Akhurst, L.C (2005b) THPI: determination of minimum inhibitory concentration against selected microorganisms representative of rabbit gut microflora. Unpublished report No. R-18735, MAK 0890/053252 from Huntingdon Life Sciences Limited, Huntingdon, Cambridgeshire, England. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

direct action by captan or the thiocarbonyl chloride moiety released after metabolism to THPI. The presence of captan in the urine indicated that a certain amount of captan is available systemically.

In view of these considerations, the Meeting reconfirmed the ARfD of 0.3 mg/kg bw based on a NOAEL of 30 mg/kg bw per day for increased incidences of intrauterine deaths and malformations at 100 mg/kg bw per day in the study in rabbits and using a safety factor of 100. This ARfD applies to women of childbearing age. The Meeting concluded that it was unnecessary to establish an ARfD for the rest of the general population.

An addendum to the toxicological monograph was not prepared.

Estimate of acute reference dose

0.3 mg/kg bw for women of childbearing age

Unnecessary for the rest of the general population

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

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

5.5 CARBARYL (008)

RESIDUE AND ANALYTICAL ASPECTS

The carbaryl was last evaluated for residues by the 2002 JMPR. Residue data on cranberries and chilli peppers were evaluated by the current Meeting for estimation of maximum residue levels.

Carbaryl is approved for the control of a range of insect pests in cranberries such as Cranberry fireworm, Cranberry fruitworm and Cranberry twig girdler as well various larvae and bugs in chilli peppers.

Results of supervised trials on crops

Supervised trials were carried out following the maximum registered dosage rate in cranberries in the USA and in chilli peppers in Thailand. The residues were determined with HPLC after post-column derivatisation in all trials. The limit of quantification was 0.02 mg/kg. The recoveries ranged between 81% and 110%.

Cranberries

The US GAP permits a maximum of 5 applications at 7 day intervals with a dosage rate of 1.68–2.24 kg ai/ha. Three replicate samples were taken from each plot. The highest residues derived from maximum application rates 7 days (PHI) after the last application was: 0.52, 0.94, 1.85, 2.95 mg/kg.

Taking into account that cranberry is a minor crop, the Meeting considered that four trials performed at maximum GAP were sufficient, and estimated a maximum residue level of 5 mg/kg, an STMR of 1.40 mg/kg and an HR of 2.95 mg/kg.

Chilli peppers

Residues in mature chilli peppers treated according to maximum GAP (0.425–0.6375 kg ai/ha at 7–10 day intervals with a PHI of 14 days) were: 0.05, 0.5, 0.09, 0.09, 0.10, 0.25 mg/kg.