

CHLOROTHALONIL

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EXPLANATION

Chlorothalonil has been evaluated by the Joint Meetings in 1974, 1977, 1979, 1981, 1983, 1985, 1987, and 1990 (Annex I, references 22, 28, 32, 36, 40, 44, 50, 59, and 65). In 1990 the Joint Meeting allocated an ADI of 0.03 mg/kg bw for chlorothalonil, based upon the results of a two-year feeding study in dogs. A WHO Member State has since requested reconsideration of this ADI and a clarification of the basis on which it was established. This request and additional information submitted to the Meeting, including a reproduction study in rats, were considered. The additional data are summarized in this monograph addendum.

EVALUATION FOR ACCEPTABLE DAILY INTAKE

Short-term toxicity studies

Dogs

In a 30-day feeding study, encapsulated chlorothalonil (97.9% purity) was administered orally to groups of 2 male and 2 female beagle dogs at 0, 50, 150, or 500 mg/kg bw/day. The following tissues were examined macroscopically and microscopically at necropsy: brain, liver, kidneys, testes with epididymis, ovaries, adrenals, heart, thyroid and parathyroid. During treatment high-dose dogs exhibited emesis and weight loss and reduced food consumption (males only). Female dogs had slightly reduced bodyweight gains at all doses. At necropsy, liver weights of high-dose females were slightly increased. There were no microscopic changes in the tissues examined. Due to the reduced body-weight gains of treated females, a NOAEL was not established in this study (Fullmore & Laveglia, 1992).

In a two-year study, chlorothalonil (93.6% purity) was fed to groups of four beagle dogs at dietary concentration of 0, 1500, 15 000 or 30 000 ppm equivalent to 0, 37.5, 375 or 750 mg/kg bw/day) for two years. Biochemical and haematological parameters were routinely monitored at days 21 and 45 and months 6, 9, 12, 18, and 24 months. Eight dogs, one of each dose/sex/group, were sacrificed at 12 months and the remainder at 24 months. One dog of each treatment group lost weight during the study. There was a tendency for mild anaemia in four mid-dose dogs at two years and at earlier intervals in two high-dose dogs. Biochemical and urine analyses were unremarkable, apart from slightly

decreased urinary specific gravity at mid- and high-dose. At terminal necropsy, there were compound-related changes in liver, thyroid and kidneys especially at mid- and high-doses. Absolute and relative thyroid and kidney weights were increased and liver/body-weight ratios were increased at mid- and high-dose. Histopathological examination was performed only on liver, thyroid, kidney, stomach, small and large intestine tissues for mid- and low-dose dogs. Treatment-related changes occurred in liver, thyroid, kidney and stomach of mid- and high-dose dogs. Changes in low-dose dogs were equivocal. In the liver, the findings were similar in nature but only slightly more pronounced at low-dose than controls, but increased in severity at mid- and high-dose. They included pericholangitis with associated portal fibrosis, bile duct hyperplasia and pigmentation of hepatic cytoplasm and of macrophages of sinusoids and portal triads. Generalized atrophy of hepatocytes with cytoplasmic vacuolation and nuclei enlargement occurred at mid- and high-doses. Renal glomerulosclerosis and degenerative renal tubular changes (tubular hypertrophy and dilation) were found in the kidneys of mid- and high-dose dogs. In the thyroid, markedly increased pigmentation of follicular epithelia occurred in mid- and high-dose dogs. Moderate to severe gastritis was found irregularly in mid- and high-dose animals.

In summary, administration of chlorothalonil in the diet of dogs at concentrations of 15 000 and 30 000 ppm caused irregular body-weight reduction, borderline anaemia and histopathological changes to liver, kidney, thyroid and stomach. At low-dose, 1500 ppm, the histopathological changes found in the liver were qualitatively similar but minimally to slightly increased in comparison to those found in control animals. Histopathological changes to other tissues were otherwise unremarkable at the low-dose. A NOAEL was not established in this study (Paynter & Busey, 1966).

In a 16-week dietary study, chlorothalonil (purity unspecified) was fed at 0, 250, 500 or 750 ppm to groups of four beagle dogs. There were no compound-related effects on appearance, behaviour, appetite or body-weight. No changes in haematological parameters were found at weeks 0, 4, 13 and 16. At termination, protein-bound iodine was found to be increased in all treated dogs. Urinalysis at weeks 6, 9, 13 and 16 was unremarkable. No compound-related macroscopic or microscopic changes were found at necropsy. In particular, only incidental changes were observed in liver and kidneys. A NOAEL was not established in this study (Paynter & Murphy, 1967).

Groups of beagle dogs (8 males and 8 females per group) were fed chlorothalonil in the diet for 2 years at dosage levels of 0, 60 or 120 ppm. There were no effects noted on behaviour or growth over the course of the study. Clinical chemistry values including haematology, biochemistry and urine analysis, were comparable to the controls at all levels of feeding. Gross and microscopic examination of tissues and organs performed on animals sacrificed at 12 months indicated a compound-related change in the kidney. Further examination of tissues and organs at 24 months did not show chlorothalonil-related abnormalities. A

slight degree of renal tubule vacuolation in two of four animals at 120 ppm after two years in the absence of other changes (urinalyses values) was considered questionable, especially as a slight degree of vacuolation was noted in control as well as other treated animals (Holsing & Voelker, 1970 - cited in Annex 1, reference 23).

Reproduction study

Rats

In a two-generation, two litter per generation, reproduction study in Charles River CD rats, groups of 35 animals of each sex received technical chlorothalonil (98.1% purity) at dietary concentrations of 0, 500, 1500 or 3000 ppm for 10 (F_0) and 14 (F_1) weeks prior to mating and thence continually. At the time of mating, low-dose males consumed approximately 25 mg/kg bw/day and females 32 mg/kg bw/day; mid-dose males consumed approximately 75 mg/kg bw/day and females 100 mg/kg bw/day; for the high-dose groups, males consumed approximately 156 mg/kg bw/day and females 205 mg/kg bw/day. There was no mortality or clinical signs of toxicity in the parental animals. Body-weight depression occurred in the parents of both generations with males being more sensitive than females. The F_0 rats had a dose-related decrease in body-weight of high-dose and mid-dose males and high-dose females, while, in the F_1 parents, the depression of body-weight occurred in the high-dose groups of each sex only. Mating and fertility indices and duration of gestation were unaffected by treatment. Litters were culled at day 4 to 8 pups/litter and litter weights were determined at days 0, 4, 7, 14 and 21. At necropsy the parental animals exhibited similar pathological findings of forestomach and kidneys to those found in previous studies (Annex I: 46); mainly hyperkeratosis and squamous epithelial hyperplasia of the forestomach and epithelia hyperplasia, tubular hypertrophy and clear cell hyperplasia of the kidney. Males were more sensitive to these renal effects than females. There was no treatment-related effect on the incidence of malformations, livebirths or stillbirths, lactation index or sex ratio of the pups. At day 21 only pup weights of all high-dose groups were significantly reduced; the mean body-weights of mid-dose F_{1b} were reduced at days 4, 7, 14 and 21 and there was a slight (ca. 10%) depression of mean pup body-weight of the low-dose F_{2b} at day 21 only. Necropsy findings for all groups were unremarkable. The NOAEL for maternotoxicity in this study was 1500 ppm, equal to 75 mg/kg bw/day (Lucas & Benz, 1990).

Although inadequate by contemporary standards, a previous multigeneration reproduction study with chlorothalonil showed no effect of chlorothalonil on reproduction at high doses that were maternally toxic (suppression of body-weight gain, Annex 1, reference 23). Other reproduction studies with the metabolite of chlorothalonil, 4-hydroxy-2,5,6-trichloroisophthalonitrile, have previously shown diverse effects (reduced fertility index, reduced litter size and weight and increased pup mortality as well as reduced maternal bodyweight gain (Annex 1, references

23 and 33). A NOAEL for reduction of pup bodyweight by the metabolite of the order of 10-30 ppm was indicated (Annex 1, references 37 and 41).

The present 2-generation reproduction study confirmed depression of maternal body-weight, without other adverse effects on reproduction *per se*, as the most sensitive endpoint. The pup body-weight depression seen at 21 days could be attributed to direct consumption of feed containing chlorothalonil. The NOAEL of 1500 ppm for this study does not take into account the toxicity to forestomach and kidneys for which NOAELs have been established in previous studies undertaken at lower doses.

COMMENTS

The new reproduction study in rats showed a NOAEL of 1500 ppm, for maternotoxicity without adverse effects on reproduction, equal to 75 mg/kg bw/day.

The ADI allocated in 1990 was based on the NOAEL of 120 ppm, equivalent to 3.0 mg/kg bw/day, determined by the Joint Meeting in 1974 on review of a two-year feeding study in beagle dogs. This NOAEL was revised by the 1987 Joint Meeting to 60 ppm, equivalent to 1.5 mg/kg bw/day, but it was subsequently restored to its original value, 3.0 mg/kg bw/day, by the 1990 Meeting after consideration of an independent review of the histopathology which indicated that the renal tubular epithelial vacuolation found in the study was in all probability an artifact of fixation.

Concern has been raised recently over the validity of the 1970 study in dogs. The study has again been reviewed by the present Meeting and found to be adequate for evaluation.

Three additional studies with chlorothalonil at higher doses in beagle dogs were considered. None of these showed a no-effect level. In a 30-day study at 0, 50, 150 or 500 mg/kg bw/day, reduced body-weight gain occurred. In a 16-week study at 0, 250, 500 or 750 ppm, protein-bound iodine was increased at all doses. In a two-year study at 0, 1500, 15 000 or 30 000 ppm, weight-loss occurred at all doses. In addition, thyroid and kidney weight and liver/bodyweight ratios were increased at mid- and high-doses. Treatment-related histopathological changes occurred in the liver, kidneys, and stomach of mid- and high-dose dogs.

A range of genotoxicity studies, *in vivo* and *in vitro*, were considered by the Joint Meeting in 1985 and 1987. The present Meeting confirmed that the data previously reviewed did not show a genotoxic hazard of chlorothalonil for humans.

Feeding chlorothalonil to rats for two years produced gastric and renal toxicity, hyperplasia and neoplasia. Renal epithelial hyperplasia and forestomach hyperplasia/hyperkeratosis occurred with a NOAEL of 1.5 mg/kg bw/day. Renal

tumours, adenomas and carcinomas, and non-glandular gastric papillomas and squamous cell carcinomas occurred with a NOAEL for these effects of 3.3 mg/kg bw/day.

Similar findings in a two-year study in mice have increased concern over the carcinogenic potential of chlorothalonil. Mice had demonstrated similar sensitivity to gastric hyperplasia and hyperkeratosis (NOAEL 15 ppm, equal to 1.6 mg/kg bw/day) and papilloma formation (NOAEL 21 mg/kg bw/day) but they are somewhat less susceptible than rats to chlorothalonil renal toxicity (NOAEL for renal epithelial tubular hyperplasia in males, 4.5 mg/kg bw/day) and renal neoplasia (NOAEL for males, 21 mg/kg bw/day).

The 1990 Joint Meeting concluded that the gastric lesions in rats and mice were attributable to the irritancy of chlorothalonil and so had little relevance for humans. The present Meeting confirmed this interpretation and agreed that the gastric lesions occurring in rodents were an inappropriate basis for the estimation of an ADI.

Previous Joint Meetings considered the results of comparative metabolic studies in rats, germ-free rats, monkeys and dogs. Quantitative differences in the absorption, distribution, metabolism, and excretion of chlorothalonil and its metabolites were noted. The urinary metabolites of chlorothalonil differed in each case. Orally-dosed normal rats excreted significantly more urinary thiols than orally-exposed germ-free or dermally-exposed normal rats. Monkeys excreted significantly lower levels of thiols than rats. Thiols were not detected in the urine of treated dogs. This suggested that the intestinal flora of the rat significantly influences the metabolic fate of chlorothalonil in that species and, indirectly, its renal toxicity. Accordingly, the 1990 Joint Meeting considered that these results suggested "that the dog or the monkey may be more suitable models than the rat for predicting the metabolism of chlorothalonil by man." The present Meeting recalled that the rat is well known to have significantly different gastrointestinal flora than humans (WHO, 1987).

Studies reviewed by previous Joint Meetings have shown that chlorothalonil reacts *in vitro* with glutathione (GSH) to produce mono-, di-, tri- and possibly tetra-conjugates with chlorothalonil. Dithiodichloroisophthalonitrile and trithiochloroisophthalonitrile, in both sulphhydryl free and methylated forms, are known to occur as metabolites of chlorothalonil in rat urine. Orally administered monoglutathione conjugates of chlorothalonil are further conjugated with GSH in the gastrointestinal tract of rats prior to absorption. In a 90-day gavage study in rats with the monoglutathione conjugate of chlorothalonil, renal toxicity was induced at 150 mg/kg bw/day. A similar study with equimolar concentrations of chlorothalonil showed renal toxicity at 75 mg/kg bw/day with a similar pattern of urinary metabolites. A mechanism for glutathione conjugation in oncogenesis, and for the causation of nephrotoxicity and renal carcinogenicity by certain chloroalkenes in rats, has been established (Neal *et al.*, 1990; Deleant *et al.*, 1990).

These findings suggest a role for glutathione conjugation in the biotransformation and renal toxicity of chlorothalonil in rats.

Overall, the Meeting considered that there was sufficient concordance between the results of metabolism and toxicity studies to establish that normal rats were sufficiently different from germ-free rats, monkeys and dogs to be discounted as a model for ADI estimation. Accordingly the Meeting used the most sensitive toxicological endpoint that it considered to be appropriate, the NOAEL established in the two-year study in dogs. A safety factor of 100 was applied.

TOXICOLOGICAL EVALUATION

Level causing no toxicological effect

Mouse:	15 ppm in the diet, equal to 1.6 mg/kg bw/day (two-year study reviewed by the 1987 JMPR)
Rat:	1.5 mg/kg bw/day (two-year study reviewed by the 1990 JMPR)
Dog:	120 ppm in the diet, equivalent to 3.0 mg/kg bw/day (two-year study)

Estimate of acceptable daily intake for humans

0-0.03 mg/kg bw

Studies which will provide information valuable in the continued evaluation of the compound

1. Further clarification of the mechanism of nephrotoxicity and renal carcinogenicity in rats and mice
2. Information on the relevance of findings in animal studies to humans, including results of the metabolism study in dogs known to be in progress
3. Observations in humans.

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