

CHLORPYRIFOS-METHYL

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EXPLANATION

Chlorpyrifos-methyl was evaluated by previous Joint Meetings in 1975 and 1991 (Annex 1, references 24 and 62). An ADI of 0.01 mg/kg bw allocated in 1975 was reduced to 0.001 mg/kg bw in 1991. The present Meeting re-evaluated the recently-conducted long-term dietary study in rats on which the ADI of 0.001 mg/kg bw was based in 1991. The major interest was the vacuolation of the *zona fasciculata* of the adrenal gland.

EVALUATION FOR ACCEPTABLE DAILY INTAKE**Long-term toxicity/carcinogenicity studies****Rats**

Five groups of 50 Fischer 344 rats (5-6 weeks old)/sex/dose were fed chlorpyrifos-methyl (average purity $95.4 \pm 0.8\%$) at dietary concentrations of 0, 1, 2, 20 or 1000 ppm for 2 years, equivalent to 0.05, 0.1, 1 or 50 mg/kg bw/day. Ten additional rats/sex/dose were administered the same diets for 53 weeks prior to sacrifice. Analyses of the diet at 9 time intervals during the study showed dietary concentrations to be acceptable. A single set of analyses confirmed adequate homogeneity and stability in the diet for up to 24 days. Rats housed singly with food and water available *ad libitum*, were observed daily, with a detailed examination weekly, throughout the study. Animal weights were recorded weekly for 13 weeks and then once every 4 weeks. Feed intake, using 20 rats/sex/dose followed the same time pattern as body-weight measurement. Blood samples (from fasted animals) for clinical chemistry and haematology and urine for urinalyses were collected at 26, 52-53, 78, and 104 weeks. Brain cholinesterase was measured in half brain samples in all interim sacrifice rates, and in 10 rats/sex/dose at terminal sacrifice. Organ weights (recorded only in scheduled sacrifices) were determined in fasted rats at necropsy. Histopathology was performed on 53 tissues in all rats receiving 0 and 50 mg/kg bw/day for 2 years and on all rats dying or terminated in moribund condition. At 0.05, 0.1 and 1 mg/kg bw/day, histopathological examination was limited to adrenal glands, brain, epididimides, kidneys, liver, lungs, pituitary gland, spinal cord, sciatic nerve, testes, tibial nerve and gross lesions in rats sacrificed at term.

Mortality was not affected. Body-weight reduction (statistically significant but less than 10%) was detected at 50 mg/kg bw/day in both sexes. Food intake was slightly reduced in males at 50 mg/kg bw/day. Clinical signs were comparable in all groups. Haematological parameters (PCV, Hb, RBC counts, WBC total and differential counts and platelet counts) showed no differences attributable to chlorpyrifos-methyl; neither did urinalyses parameters (pH, protein, glucose, ketones, bilirubin, urobilinogen or sediment macroscopic examination). Clinical chemistry values (alanine transaminase, albumin, aspartate transaminase, alkaline phosphatase, glucose, phosphorus, total protein, triglycerides, blood urea nitrogen, chloride, sodium and potassium) were sporadically significantly different, but results were inconsistent, except for depressed levels of ALT and AST in males at 6, 12 and 18 months (but not at 24 months) and in females at 6 and 12 months only. Cholinesterase inhibition was noted in plasma at 1 and 50 mg/kg bw/day in both sexes. Erythrocyte and brain cholinesterase activities were depressed at 50 mg/kg bw/day in both sexes. Incidence of gross lesions were comparable in all groups. Organ absolute and relative weights were comparable in all groups except for the adrenal gland. After deletion of adrenal glands bearing tumours (i.e. where the organ weight was likely to be abnormal) both absolute and relative adrenal weights were increased in both sexes at 50 mg/kg bw/day at interim (1 year) and terminal (2 year) sacrifice. At interim sacrifice, both sexes in the 50 mg/kg bw/day group showed 10/10 rats with moderate vacuolation, consistent with lipid accumulation in the *zona fasciculata* of the adrenal. At terminal sacrifice, in males, the incidence of moderate vacuolation was 49/50, 1/50, 0/50, and 0/50 at 50, 1, 0.1 and 0.05 mg/kg bw/day, respectively. Slight vacuolation was noted in 1/50, 10/50, 9/50, 5/50 and 3/50 at 50, 1, 0.1, 0.05 and 0 mg/kg bw/day. In the females, adrenal *zona fasciculata* vacuolation was noted in 30/50, 0/50, 0/50, 0/50 and 0/50 as being moderate, and in 19/50, 0/50, 1/50, 2/50 and 2/50 as being slight at 50, 1, 0.1, 0.05 and 0 mg/kg bw/day, respectively.

An increased incidence of adrenal vacuolation was observed in both sexes at 50 mg/kg bw/day. This increase was probably compound-related. At lower doses, percentage incidence of adrenal *zona fasciculata* vacuolation, consistent with lipid accumulation were, in males, 22%, 18%, 10% and 6% and in females, 0%, 2%, 4% and 4% at 1, 0.1, 0.05 and 0 mg/kg bw/day. Historical control data from 5 contemporary studies from the same laboratory and in the same strain of rat showed 40%, 10%, 42%, 0% and 20% incidence of vacuolation of the adrenal *zona fasciculata* in males, and 26%, 0%, 10%, 4% and 0% in females. Thus incidences observed in the current study at 1 mg/kg bw/day and below are well within historical control data ranges, and are unlikely to be compound-related. The NOAEL for the study was 1 mg/kg bw/day (Barna Lloyd *et al.*, 1991).

Observations in humans

Fourteen male volunteers were divided into two treatment groups of five men each and a control group of four men. Chlorpyrifos-methyl was administered by gelatin capsule in a single daily dose of 0, 0.03 or 0.1 mg/kg bw/day for four

weeks. Plasma and erythrocyte cholinesterase activities were not depressed at levels tested. Haematology, blood chemistry, urinalysis, blood pressure, pulse rate and ophthalmology were not affected by treatment. The NOAEL was the highest dose tested, 0.1 mg/kg bw/day (Coulston *et al.*, 1975).

COMMENTS

The two-year dietary study in rats, which utilized dietary doses of 0, 0.05, 0.1, 1 or 50 mg chlorpyrifos-methyl/kg bw/day, did not show any carcinogenic potential of the compound.

On histopathological examination, no correlation was found between the incidence of vacuolation of the *zona fasciculata* of the adrenal gland and of other organs. The incidence of vacuolation of the *zona fasciculata* at all dose levels except the high dose was within the range of occurrence noted in contemporary rat studies performed in the same laboratory. The NOAEL for the study was therefore interpreted as being 1 mg/kg bw/day.

The 1975 Joint Meeting reviewed a human study in which five males/test group were given single doses of 0, 0.03, or 0.1 mg chlorpyrifos-methyl/kg bw/day for four weeks. Test groups were comparable to a control group of four males with respect to plasma and erythrocyte cholinesterase activity, haematology, blood chemistry, blood pressure, pulse rate and ophthalmology. The NOAEL was the highest dose tested, 0.1 mg/kg bw/day.

The ADI allocated by the 1991 Joint Meeting was based on the changes in rat adrenal pathology which were interpreted as showing a NOAEL of 0.1 mg/kg bw/day, to which a 100-fold safety factor was applied. With the revision of the NOAEL with respect to the rat adrenal, the present Meeting allocated an ADI based on the human data (NOAEL 0.1 mg/kg bw/day) using a 10-fold safety factor. This ADI is supported by the NOAEL in studies in rats (1 mg/kg bw/day) using a 100-fold safety factor.

TOXICOLOGICAL EVALUATION

Level causing no toxicological effects

Mouse:	50 ppm, equal to 3.9 mg/kg bw/day (78-week study)
Rat:	1 mg/kg bw/day (two-year feeding study)
Human:	0.1 mg/kg bw/day (four-week study).

Estimate of acceptable daily intake for humans

0-0.01 mg/kg bw.

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

Further observations in humans.

REFERENCES

Barna-Lloyd, T., Szabo, J.R. & Davis, N.L. (1991) Chlorpyrifos-methyl (Reldon R) rat chronic dietary toxicity/oncogenicity study. Unpublished report TXT: K-046193-020 from Dow Chemical, Texas, USA. Submitted to WHO by Dow Elanco, Indianapolis, USA.

Coulston, F., Rosenblum, I. & Griffin, T.B. (1975) Study of chlorpyrifos-methyl in human volunteers. Unpublished report from Institute of Comparative and Human Toxicology, Albany Medical College and International Centre of Environmental Safety, Holloman AFB, New Mexico. Submitted to WHO by Dow Chemical Company, Midland, Michigan, USA.