Pesticide residues in food - 2002 - Joint FAO/WHO Meeting on Pesticide Residues

CARBOFURAN (addendum)

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Explanation

Carbofuran (2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) was first evaluated by the 1976 JMPR (Annex 1, reference 26). It was last evaluated in 1996 (Annex 1, reference 77), when an ADI of 0–0.002 mg/kg bw was allocated on the basis of the NOAEL in a 4-week study in dogs. Establishment of an acute RfD was requested by the Codex Committee on Pesticide Residues, and that was the basis for the present review.

Carbofuran is a carbamate compound that exerts virtually all its effects by inhibiting cholinesterase activity in nervous tissues. Nevertheless, in one study in dogs, carbofuran also caused testicular degeneration.

Evaluation for acute reference dose

1. Reversibility of inhibition of cholinesterase activity in rats

The reversibility of inhibition of peripheral cholinesterase activity was studied in groups of up to nine Crl:CD(SD)IGS BR rats of each sex with in-dwelling jugular cannulae for blood sampling, although the
groups were usually smaller because the cannula was not patent in some animals. After a preliminary dose-range study, the rats received carbofuran (purity, 98.6%) at a single dose of 0, 0.5 or 1 mg/kg bw by oral gavage in corn oil. Blood samples of 200 µl were taken into containers with EDTA from the catheter 15 min before dosing and 15, 30, 45, 60, 75 and 90 min and 2, 2.5, 3, 4, 6 and 8 h after dosing. Clinical observations were made at these times. After centrifugation of the blood samples, plasma and erythrocytes (the latter diluted 1:1 with Triton X-100 in phosphate buffer) were frozen to –70 °C, and cholinesterase activity was determined by a modified Ellman method (Ellman et al., 1961).

Clinical signs of anticholinesterase poisoning were seen in both treated groups, consisting of tremors and teeth grinding; these signs had nearly completely resolved by 60 min after dosing. The results for cholinesterase inhibition can be analysed by comparing the enzyme activities in the treated groups with those before dosing in that group or with those of concurrent controls. The company preferred a third way of analysing the data. In comparison with the activity before dosing, plasma cholinesterase activity in males was statistically significantly reduced throughout the study at almost all times at both doses. At the lower dose, the activity was > 80% of that before dosing from 90 min onwards, and at the higher dose the activity was < 80% of that before dosing at all times except 6 h. In the females, statistically significant reductions in plasma cholinesterase activity from that before dosing were seen at 45, 60 and 75 min at 0.5 mg/kg bw and throughout the study at 1 mg/kg bw. However, the plasma cholinesterase activity in females was > 80% of that before dosing at all times at the lower dose and > 80% of that before dosing at all times after the higher dose, except 15 min and 8 h after dosing. When the values were compared with those for concurrent controls, because decreases were seen in control activity after dosing (corn oil) and because of biological variability, statistically significant differences were not seen in plasma cholinesterase activity at any time in either sex or at either dose.

Statistically significant reductions were found in erythrocyte cholinesterase activity in males at all times after dosing at both 0.5 and 1 mg/kg bw when compared with pre-dosing activity. In both treated groups, the least activity after dosing was observed at 15 min, representing 58% of the activity before dosing at 0.5 mg/kg bw and 54% at 1 mg/kg bw. At 0.5 mg/kg bw, although the activity was statistically significantly depressed, it was > 80% of that before dosing from 2 h onwards. At the higher dose, the activity was still only 77% of that before dosing at 8 h. In females, reductions in erythrocyte cholinesterase activity in comparison with that before dosing were also found at all times and at both doses up to 8 h, but were no longer statistically significant at 8 h in either group. The greatest reduction in activity in females at 0.5 mg/kg bw was observed at 30 min (when the activity was 69% of that before dosing), and the activity was > 80% of that before dosing at 2.5 h and beyond. At 1 mg/kg bw, the least activity was observed at 15 and 45 min (60% of the activity before dosing). The activity in females at the higher dose was > 80% that before dosing at 6 and 8 h. When erythrocyte cholinesterase activity was compared with that of concurrent controls, significant inhibition of activity was also observed. Thus, in males at the lower dose, erythrocyte cholinesterase activity represented 56% of that in concurrent controls at 15 min and remained at < 80% that of concurrent controls up to and including 2 h. In males at the higher dose, activity was generally more strongly inhibited than at the lower dose and remained at < 80% of that of concurrent controls up to and including 4 h. In females at the lower dose, the greatest depression in activity was also seen at 15 min (69% of that of concurrent controls) and inhibition > 20% was seen at 30, 45 and 60 min and at 6 h. At the higher dose, erythrocyte cholinesterase activity represented 61% that of concurrent controls at 15 min and < 80% up to and including 3 h after dosing. Because erythrocyte cholinesterase activity was inhibited at the lower dose, no NOAEL for this effect could be identified. Furthermore, clinical signs were seen at both doses. The study shows that, at the doses used, the effects were largely reversible in males by 8 h and completely reversible in females by 6 h (Anderson, 2002).
2. Short-term studies of toxicity

Dogs

In a study evaluated by the 1996 JMPR, carbofuran (purity, 99.6%) was administered in the diet to groups of four beagle dogs of each sex at a concentration of 0, 10, 70 or 500 ppm, the highest concentration being reduced to 250 ppm after 6 days on account of toxicity, for 13 weeks. These dietary concentrations were equal to a mean of 0, 0.43, 3.1 and 11 mg/kg bw for males and females combined. Two additional animals of each sex given the control diet and the diet with the highest concentration were kept for a further 4 weeks in order to study the reversibility of the effects. The animals were examined daily for deaths and clinical signs. Food consumption was recorded daily and food consumption weekly. Tests of hearing were carried before the start of the study, at the end of dosing and at the end of the recovery period. Ophthalmic examinations and electrocardiography were similarly carried out, with an additional examination after 6 weeks of dosing. Blood samples (for haematology and clinical chemistry) and urine were taken before the start of the study, at 6 and 13 weeks and after the recovery period. Blood was taken for estimation of plasma and erythrocyte cholinesterase activity before the start of the study, on test days 1 and 3 and at the end of weeks 1, 2, 6 and 13 of the study (also 11 days later for males); furthermore, blood was taken from those animals retained for the study of recovery. The cerebellum was taken at autopsy for assessment of brain cholinesterase activity. Cholinesterase activity was estimated by an Ellman method (Ellman et al., 1961), modified for use on an AutoAnalyser. Selected organs were processed for histopathological examination.

One dog died after 5 days, possibly of intussusception of the jejunum. Animals at all doses showed hyperaemia and salivation, these signs being most severe early in the study. The clinical signs at the highest dietary concentration included ataxia, vomiting and tachypnoea. At this concentration, there was loss of body weight and decreased food consumption, which recovered when the concentration of carbofuran in the diet was reduced. No treatment-related differences were seen in ophthalmic, electrocardiographic, haematological or clinical chemical parameters, other than cholinesterase activity. Inhibition of plasma cholinesterase activity was observed at the two higher dietary concentrations, starting from day 1 in males, but only exiguous, insignificant inhibition was observed at the lowest concentration. In females, biologically significant inhibition of plasma cholinesterase was observed at all dietary concentrations. Inhibition of erythrocyte cholinesterase activity was also seen at all three doses, representing 73%, 35% and 13% of that of concurrent controls at 10, 70 and 500 ppm on day 1 in males, when inhibition was generally maximal. In females at 10, 70 and 500 ppm, the activity on day 1 was 83%, 28% and 14% of that of concurrent controls, respectively. No significant inhibition of brain cholinesterase activity was observed. As biologically significant depression of erythrocyte cholinesterase activity was observed at the lowest dietary concentration, at which clinical signs (salivation) were also observed, no NOAEL could be identified (Bloch et al., 1987).

In a study also evaluated by the 1996 JMPR, groups of six male and six female beagles received diets containing technical-grade carbofuran (purity, 96.1%) at a concentration of 0, 10, 20 or 500 ppm, equivalent to 0, 0.25, 0.5 and 12 mg/kg bw per day, for 1 year. Dogs at 500 ppm showed emesis and weight loss and were given control diet with the test diet from 5 months, in unstated proportions of test and control diet; hence, the equivalence of 12 mg/kg bw per day is likely to be an overestimate of consumption of carbofuran. The animals were observed daily for clinical signs, and deaths were recorded. Ophthalmic examinations were carried out before treatment, at 6 months and just before termination of the study. Blood was taken from all animals for haematology and clinical chemistry before the start of the study and thenceforth monthly. Dogs were weighed before the start of the study and weekly thereafter. Food consumption was measured daily for
the first 5 weeks of the study and weekly thereafter. Blood samples for estimation of cholinesterase activity were taken three times before the start of the study, 3, 7 and 14 days afterwards and then monthly. The samples were taken approximately 1 h after the end of the daily 2-h feeding period and were analysed by an automated method. Urine was analysed before treatment and then at 60-day intervals. At termination, the animals were necropsied. A gross pathological examination was undertaken, and selected organs were weighed and processed for histopathological examination. A sample of brain (cerebellum) was taken for estimation of brain cholinesterase activity.

One death occurred, of a male at the highest dietary concentration. Emesis and loose stools were seen, mostly in animals at the highest concentration. No treatment-related effects were seen on ophthalmic parameters. Decreased erythrocyte volume fraction, haemoglobin concentration and erythrocyte count were seen in males at 500 ppm. Changes in electrolytes were also seen at this concentration. Plasma cholinesterase activity was inhibited in males in all groups, but generally by < 20% in the group at 10 ppm, whereas erythrocyte cholinesterase activity was inhibited only at the highest dietary concentration in males and only sporadically. In females at the lowest concentration, plasma cholinesterase activity was always > 80% of that of concurrent controls. Erythrocyte cholinesterase activity was not significantly inhibited in females. At termination, biologically significant depression of brain cholinesterase activity was seen in males at the highest concentration (76% that of concurrent controls). No depression in brain cholinesterase activity was seen in females. At the highest dietary concentrations, the absolute weights of the brain and heart were decreased in males. On gross pathological examination, decreased body fat and alopecia were found at this dose. Degeneration of the seminiferous tubules and the presence of giant tubules and/or aspermia were seen in four of the five surviving males given the highest dietary concentration and in one of six given 20 ppm. Inflammatory changes in the lungs were considered to be treatment-related. The NOAEL was 10 ppm, equivalent to 0.3 mg/kg bw per day, on the basis of histopathological changes in the testes of one male (Taylor, 1983).

**Comments**

A study of the reversibility of inhibition of plasma and erythrocyte cholinesterase activity, in which groups of up to nine rats of each sex per dose were given single doses of 0, 0.5 or 1 mg/kg bw of carbofuran, was reviewed. Inhibition of erythrocyte cholinesterase activity was maximal within about 15 min and was rapidly reversible within 6 h in females. Although the activity of cholinesterase was still less than 80% of the level before dosing at 8 h in males at the higher dose, the decrease at that time was only marginal. Furthermore, when compared with the activity in concurrent controls, the activity at the higher dose was not biologically significantly depressed in males after 4 h. The LOAEL was 0.5 mg/kg bw.

Three studies carried out in beagle dogs were considered relevant to establishing an acute RfD. In a 13-week study evaluated by the 1996 JMPR, which was re-evaluated at the present Meeting, the LOAEL was 10 ppm in the diet (equal to 0.43 mg/kg bw per day); a NOAEL was not identified. Significant depression of erythrocyte cholinesterase activity and clinical signs were seen on the first day of dosing at the lowest dose. A supplementary study was carried out over 4 weeks in male dogs, which was evaluated by the 1996 JMPR but not by the present Meeting. The NOAEL for cholinesterase inhibition was 5 ppm, equal to 0.22 mg/kg bw per day. An earlier 1-year feeding study in dogs, evaluated by the 1996 Meeting, was reviewed by the present Meeting. The NOAEL was 10 ppm (stated as being equal to 0.3 mg/kg bw per day in the 1996 JMPR monograph), on the basis of concern about the potential for testicular toxicity at 20 ppm.
After considering the data available to the present Meeting as well as the 1996 evaluations, the Meeting established an acute RfD of 0.009 mg/kg bw on the basis of the NOAEL of 0.22 mg/kg bw per day in the 4-week study in dogs and a safety factor of 25, as the relevant toxic effects of carbofuran are dependent on the $C_{\text{max}}$ (see section 2.2 of the report: Annex 1, reference 95).

**References**


See Also:

- [Toxicological Abbreviations](#)
- Carbofuran (ICSC)
- Carbofuran (PDS)
- Carbofuran (Pesticide residues in food: 1979 evaluations)
- Carbofuran (Pesticide residues in food: 1980 evaluations)
- Carbofuran (Pesticide residues in food: 1996 evaluations Part II Toxicological)