5.5 CARBOFURAN (096)

TOXICOLOGY

Carbofuran is the ISO approved common name for 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate, a broad spectrum *N*-methyl carbamate insecticide and nematicide that acts by inhibiting acetylcholinesterase activity in nervous tissues. Carbofuran was previously evaluated by the Joint Meeting in 1976, 1979, 1980, 1982, 1996, and 2002. In 1996, an ADI of 0–0.002 mg/kg bw was established based on the NOAEL for inhibition of erythrocyte acetylcholinesterase at 0.22 mg/kg bw per day in a 4-week dietary study in dogs, and using a safety factor of 100. In 2002, an ARfD of 0.009 mg/kg bw was established based on the NOAEL of 0.22 mg/kg bw per day in a 4-week study in dogs, and using a safety factor of 25, as the relevant toxic effects of carbofuran are dependent on the C_{max}.

The present Meeting evaluated newly submitted studies of acute toxicity in rats (adults and pups) and a newly submitted study in human volunteers (conducted in 1976), and re-examined relevant data from short-term studies of toxicity in dogs, which had been considered by previous Meetings. All pivotal studies were certified as complying with GLP or an approved quality assurance programme.

Toxicological data

Carbofuran is highly toxic after a single oral dose; the LD_{50} values in rats range from 6 to 18 mg/kg bw and in various other species (including mouse, guinea-pig, rabbit, cat and dog) from 3 to 19 mg/kg bw. The clinical signs of toxicity observed were typical of acetylcholinesterase inhibition. In rats, clinical signs were observed starting at about 5 min after administration, and mortality generally occurred within 1 h after dosing.

Two studies of the time course of inhibition of acetylcholinesterase activity were carried out in adult rats and pups aged 11 days (postnatal day 11). In the first study, after a single dose of 0.6 mg/kg bw of carbofuran, the time of maximum incidence and severity of clinical signs and of maximum inhibition of brain acetylcholinesterase was at 15 min after dosing for adults and pups. Recovery of brain acetylcholinesterase activity was achieved in adult males and females within 360 or 240 min after dosing, respectively, while the pups had not fully recovered by 360 min after dosing. In the second study, after a single dose of carbofuran at 0.1 mg/kg bw, no clinical signs were observed, and the time of maximum inhibition of brain acetylcholinesterase activity was at 30 min after dosing for adult rats and at 60 min after dosing for pups aged 11 days. Recovery of brain acetylcholinesterase activity was achieved in adults and in the pups within 240 min after dosing.

Two studies of acute toxicity were conducted to compare acetylcholinesterase inhibition in pups aged 11 days (postnatal day 11) and adult rats, and two range-finding studies of acute toxicity were carried out in rats aged 11 days given carbofuran at doses ranging from 0.03 to 1.0 mg/kg bw. In these studies, a spectrophotometric assay for cholinesterase activity was used. While data on erythrocyte acetylcholinesterase were considered to be unreliable because of unfavourable experimental conditions that led to significant spontaneous enzyme reactivation, the data on brain acetylcholinesterase were considered to be suitable for use in risk assessment because the degree of inhibition of acetylcholinesterase activity agreed with that obtained using the more reliable radiometric assay for cholinesterase activity (see below). Clinical signs (tremors) were observed at doses of 0.3 mg/kg bw and above. On the basis of inhibition of acetylcholinesterase activity in brain (pups, 35–47%; adults, 20–32%) at 0.1 mg/kg bw and above, the overall NOAEL for pups and adults was 0.03 mg/kg bw.

In two studies of acute toxicity designed to compare inhibition of acetylcholinesterase activity in pups (postnatal day 11 or postnatal day 17) and adult rats given carbofuran at doses

ranging from 0.1 to 1.5 mg/kg bw and using a radiometric assay for cholinesterase activity, the overall NOAEL for pups (both postnatal day 11 and postnatal day 17) was < 0.1 mg/kg bw on the basis of inhibition of acetylcholinesterase activity in brain (28–40%) and erythrocytes (50–53%) at 0.1 mg/kg bw and above. The overall NOAEL for adult rats was 0.1 mg/kg bw on the basis of inhibition of acetylcholinesterase activity in brain (28–33%) and erythrocytes (25–49%) at 0.3 mg/kg bw and above.

Using the data from three studies in rat pups aged 11 days, the estimated oral dose resulting in 10% inhibition of brain acetylcholinesterase activity (benchmark dose, BMD_{10}) was 0.04 mg/kg bw, while the lower 95% confidence limit for the BMD_{10} ($BMDL_{10}$) was 0.03 mg/kg bw.

In the latter two studies, inhibition of erythrocyte acetylcholinesterase activity appeared to be a more sensitive end-point than did inhibition of brain acetylcholinesterase activity. In the absence of data on inhibition of acetylcholinesterase activity in peripheral target tissues, the use of data on erythrocyte acetylcholinesterase activity might thus be considered as surrogate for data on the peripheral nervous system. However, given the quantitative dose–response correlation between clinical signs of cholinergic toxicity and inhibition of brain acetylcholinesterase activity by a range of *N*-methyl carbamates including carbofuran, the Meeting concluded that the current data support the use of inhibition of brain acetylcholinesterase activity rather than the surrogate measure of erythrocyte acetylcholinesterase activity as the end-point for the risk assessment of carbofuran.

In a 13-week dietary study in dogs, which was evaluated by the Joint Meeting in 1996 and 2002 and re-evaluated by the present Meeting, the LOAEL was 10 ppm, equal to 0.43 mg/kg bw per day. A NOAEL was not identified since significant inhibition of erythrocyte acetylcholinesterase activity and clinical signs were seen on the first day of dosing at the lowest dose. The data on brain acetylcholinesterase activity in this study were not reliable owing to significant recovery of acetylcholinesterase activity at the time-point of necropsy. In a supplementary 4-week study in male dogs, which was evaluated by the Joint Meeting in 1996 and re-evaluated by the present Meeting, brain acetylcholinesterase activity was not examined. The NOAEL for clinical signs and inhibition of erythrocyte acetylcholinesterase activity was 5 ppm in the diet, equal to 0.22 mg/kg bw per day, the highest dose tested. However, since a spectrophotometric assay for cholinesterase activity was used in both studies in dogs and it was not clear whether the experimental conditions were appropriate to minimize reactivation of the enzyme, the reliability of the data on erythrocyte acetylcholinesterase activity is questionable. The Meeting noted that, on the basis of the data on acute toxicity, dogs are not expected to be more sensitive than other species.

In a study in human volunteers, which met the ethical standards prevalent at the time when the research was conducted (1976), groups of two to four men received carbofuran as a single oral dose at 0.05, 0.1 or 0.25 mg/kg bw, while one man received placebo only. At 0.05 mg/kg bw, erythrocyte acetylcholinesterase activity was inhibited by 22% in one of two subjects; at 0.1 mg/kg bw, erythrocyte acetylcholinesterase activity was inhibited by 31–33% in both subjects; and erythrocyte acetylcholinesterase activity was inhibited by 46–63% and treatment-related clinical signs were seen in all four subjects at 0.25 mg/kg bw. Owing to the small sample size, the study could not be used for identification of a NOAEL or LOAEL, but provided information that was useful for the interspecies comparison of sensitivity for the risk assessment.

Toxicological evaluation

The Meeting established an ARfD of 0.001 mg/kg bw based on the overall NOAEL of 0.03 mg/kg bw per day identified on the basis of inhibition of brain acetylcholinesterase activity in rat pups aged 11 days (postnatal day 11) and a safety factor of 25. This NOAEL was supported by the $BMDL_{10}$ of 0.03 mg/kg bw extrapolated from data on inhibition of brain acetylcholinesterase activity in rat pups aged 11 days (postnatal day 11) in a second study. A safety factor of 25 was considered to be appropriate because the acute toxic effects of carbofuran are dependent on C_{max} rather than area under the curve of concentration–time (AUC) and data indicated that the sensitivity of humans and laboratory animals (rats, dogs) to inhibition of acetylcholinesterase activity by carbofuran was similar

(see general item: *Safety factors for acute C_{max}-dependent effects; specific considerations with respect to carbamates such as carbofuran*). Given the apparent higher sensitivity of younger animals, the ARfD was considered to be adequately protective of infants and children since it was based on the NOAEL from a study in pups aged 11 days.

The Meeting noted that this ARfD was lower than the current ADI of 0–0.002 mg/kg bw. This is plausible in view of the toxicological characteristics of inhibition of acetylcholinesterase activity by carbofuran, which shows very rapid recovery; long-term exposure can thus be likened to a series of acute exposures. The Meeting therefore concluded that the ADI and ARfD for carbofuran should be based on the same NOAEL and revised the ADI to 0–0.001 mg/kg bw based on the overall NOAEL of 0.03 mg/kg bw from the new studies of acute toxicity in rats and using a safety factor of 25.

An addendum to the toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute study of toxicity (pups aged 11 days and adults) [a, b]	Inhibition of pup brain acetylcholinesterase activity	0.03 mg/kg bw [c]	0.1 mg/kg bw
		Clinical signs	0.1 mg/kg bw	0.3 mg/kg bw

[a] Gavage administration.

[b] Results of several studies combined.

[c] Supported by a $BMDL_{10}$ of 0.03 mg/kg bw, based on inhibition of brain acetylcholinesterase activity in pups aged 11 days (postnatal day 11).

Estimate of acceptable daily intake for humans

0–0.001 mg/kg bw

Estimate of acute reference dose

0.001 mg/kg bw

Information that would be useful for continued evaluation of the compound

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

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.001 mg/kg bw	Rat, study of acute toxicity	25
ARfD	0.001 mg/kg bw	Rat, study of acute toxicity	25

DIETARY RISK ASSESSMENT

Long-term intake

The ADI for carbofuran is 0–0.001 mg/kg bw. The International Estimated Daily Intakes (IEDI) for carbofuran was estimated for the 13 GEMS/Food cluster diets using the STMR or STMR-P values estimated by previous Meetings. The results are shown in Annex 3. The IEDI ranged from 20–70% of the maximum ADI. The Meeting concluded that the long-term intake of residues of carbofuran from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The ARfD for carbofuran is 0.001 mg/kg bw. The International Estimated Short-term Intake (IESTI) was calculated for 22 commodities for which STMRs, HRs had been estimated by previous Meetings and for which consumption data was available. The results are shown in Annex 4.

For the general population, the IESTI was higher than the ARfD for banana, cucumber, cantaloupe, milks, orange, potato, summer squash and sweet corn on the cob (from 120 to 510% ARfD). For children, the IESTI was higher than the ARfD also for mandarins (from 280 to 810% ARfD). The information provided to the JMPR precludes an estimate that the short-term intake of residues of carbofuran from the consumption of the above listed commodities will be below the ARfD.

The short-term intake of residues of carbofuran from other uses considered by the JMPR is unlikely to present a public health concern.