

US EPA ARCHIVE DOCUMENT

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✓ Ket: 1-1-80
Pesticide Residues in
Food - FAO - (1980)
CASWELL FILE

CARBOFURAN

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Explanation

Carbofuran was evaluated for an acceptable daily intake in 1978 and 1979 and a temporary ADI for man was estimated to be 0-0.003 mg/kg body weight (FAO/WHO, 1977; FAO, 1980). The available data reflected that carbofuran is a highly toxic carbamate ester whose metabolic profile has been well defined. Carbofuran is a potent, reversible cholinesterase inhibitor. Cholinesterase inhibition and acute toxic signs of poisoning are subject to rapid spontaneous reversal and recovery. The measurement and evaluation of cholinesterase depression induced by carbofuran, because of the rapid reversibility, is difficult and requires substantial care.

In a variety of short-term and long-term studies, in several animal species, cholinesterase depression was the principal effect noted. The 1976 Meeting expressed concern over the lack of appropriate data evaluating the reversible cholinesterase depression in dietary studies and on the apparent sensitivity of brain rather than erythrocyte or plasma cholinesterase. No-effect levels were based on data from a rat reproduction study (short-term) and a short-term dietary study in dogs.

Further studies on cholinesterase depression, induced by dietary administration of carbofuran, were requested. It was suggested that a definition be made of the sensitivity of juveniles, when compared with adults on acute toxicity and cholinesterase depression levels, as observed in preliminary studies and that an additional reproduction study define the highest no-effect level.

Additional data were received with respect to all of the requested information. In addition, further long-term studies in several rodent species were made available. These new data are reviewed in this monograph addendum.

DATA CONSIDERED FOR DERIVATION OF ACCEPTABLE DAILY INTAKE

BIOCHEMICAL ASPECTS

Effects on enzymes and other biochemical parameters

An evaluation was made on the possible differences in brain and erythrocyte cholinesterase activity in adult and juvenile rats of both sexes following acute exposure to carbofuran.

Groups of rats (five rats per sex, using either juvenile or adult animals) were sacrificed and cholinesterase activity measured by a colorimetric procedure suitable for evaluating a rapidly reversible, carbamate-induced inhibition. These animals were untreated and served as baseline values of cholinesterase activity. A further group of eight animals per sex, both juvenile and adult, were treated with carbofuran and sacrificed at various times up to 24 hours after treatment to evaluate the optimal time for depression of both brain and erythrocyte cholinesterase. In all cases, sample preparation was rapid following sacrifice and dilution of tissues was kept to a minimum. Baseline cholinesterase data in brain of juveniles were slightly higher than those noted for adults in both males and females. With erythrocyte cholinesterase, adult values were found to be higher than juvenile values in both males and females. Examination of the optimal sampling time for evaluating maximum erythrocyte cholinesterase depression showed that approximately 30 minutes following acute administration was the optimal in both sexes in adults and juveniles. The optimal sampling time for brain cholinesterase depression was approximately one hour following acute poisoning. The data showed recovery was almost complete four hours following acute carbofuran treatment. Complete recovery was noted at the 24-hour interval. While the cholinesterase depression and recovery studies were difficult to fully evaluate because of large standard deviations of the mean cholinesterase activities, it was quite evident that complete

recovery of all enzyme depression was attained within one day. There were no significant differences in juvenile and adult recovery studies in either brain or erythrocyte cholinesterase. Further, the data did not suggest significant differences in sensitivity to carbofuran-induced inhibition in either adult or juvenile rats (Case, 1980).

Toxicological studies

Special studies on reproduction

Groups of rats (10 male and 20 female rats per group, 12 male and 24 females per group were used in the third generation) were fed carbofuran in the diet at dosage levels of 0, 20, or 100 mg/kg and subjected to a standard 2-litter per generation, 3-generation reproduction study.

Growth, as evidenced by differences in body weight and food consumption, was reduced in parental rats administered 100 mg/kg in the diet. This was consistent throughout three generations at the 100 mg/kg dosage level. No treatment-related growth or food consumption depression was noted with any of the animals administered 20 mg/kg in the diet.

There was no effect associated with carbofuran with respect to the standard reproduction parameters (fertility, gestation, lactation, and viability) with the exception of a slight reduction in viability at day 4 at 100 mg/kg. This was observed in the first litter of all generations. These differences were not noted in the second litters of the 100 mg/kg group and the significance of this reduction is questionable. Mean pup body weights in the 100 mg/kg group were consistently lower than control values. This growth reduction, noted at day 21, was statistically significant in both males and females at the 100 mg/kg dosage level throughout the study.

At the conclusion of the study, parental animals and pups from the second litters of the second and third generations were sacrificed and subjected to gross examination. No pathological lesions or abnormalities, which were considered to be compound-related, were noted at the time of gross examination. Statistically significant mean weight variations occurred in various organs among the F₂ and F_{3b} weanling rats. The significance of these weight variations, which appear to occur frequently in toxicity studies, is questionable in light of the microscopic examination of tissues and organs which did not appear to show substantial effects attributable to the presence in the diet of carbofuran. Microscopic examination of tissues and organs of the parental F_{2b} and F_{3b} showed a variety of morphologic changes, none of which were considered to be related to the presence of carbofuran. All histologic lesions were considered to represent spontaneous occurrences. Based upon the slight, but significant, growth reduction at 100 mg/kg, it may be concluded that 20 mg/kg represents a dietary level that would induce no effect on reproduction (Goldenthal, 1979b; 1980b).

Special studies on teratogenicity

Groups of rats (24 mated females per group) were administered carbofuran (suspended in 0.25% methylcellulose) at dosage levels of 0, 0.1, 0.3, or 1.0 mg/kg body weight/day throughout gestation (days 6-15 of pregnancy). On day 20 of gestation, animals were sacrificed and parents and fetuses were subject to complete internal and external examination to evaluate effects indicative of a teratogenic response of carbofuran.

Toxicological signs of poisoning were evidenced in females at the two higher dose levels. There was one death at the highest dose level. The signs of poisonings were of the typical cholinergic response associated with carbofuran. There were no differences in growth within any treatment group over the 20-day gestation period. At the conclusion of the study, examination of all animals showed a larger number of resorptions in the control group than in any of the treatment groups. There were no differences within the groups administered carbofuran and the high incidence of resorptions in the control group could not be explained. Sonetic and skeletal examinations failed to show teratogenic effects of the administration

of carbofuran. Carbofuran, while inducing toxicological signs of poisoning in maternal rats, induced no teratogenic response in rats (Barron, et al., 1978).

Groups of pregnant rabbits (17 rabbits/group) were administered carbofuran (in 0.25% methylcellulose) at dosage levels of 0, 0.2, 0.6, or 2.0 mg/kg body weight daily from day 6-18 of gestation.

On day 30, all animals were sacrificed and, following laparotomy, an examination of the parents and foetuses was performed to evaluate a potential teratogenic effect. For the parents, this included an examination for early and late resorptions, implantation sites, abnormal placental sites, and for any other abnormalities. Corpora lutea were counted and the placenta was weighed. Gross examinations were made of the parents and somatic and skeletal examinations of foetuses were performed.

Toxic signs of poisoning were observed in rabbits administered the high dose level of carbofuran. Toxic cholinergic signs of poisoning were evident and several animals died during the course of the study. There were no toxicological signs of poisoning evident at the lower dose levels. Growth, over the period of gestation, did not appear to be affected by the administration of carbofuran. There were no significant gross somatic effects observed in either parents or foetuses at any dose level in the study. Pregnancy and viability was unaffected by carbofuran. Foetal body weights, placental weights, and development of foetuses were not affected. Soft tissue and skeletal examinations showed no adverse effect as a result of carbofuran treatment. Under the conditions of this bioassay, carbofuran did not induce a teratogenic effect in rabbits at dose levels up to and including that which induced maternal toxicity (Felton, et al., 1978). i.e. 2.0 mg/kg b.w.

Long-term studies

Mouse

Groups of mice (100 male and 100 female, Charles River CD-1 mice/group) were fed carbofuran in the diet at dosage levels of 0, 20, 125 or 500 mg/kg for two years in an effort to evaluate the carcinogenic potential of carbofuran. Groups of animals were examined at periodic intervals (6, 12, 18, and 24 months) for hematologic parameters, biochemical studies, and urinalyses. At similar intervals over the course of the study, groups of animals were sacrificed and subjected to gross and microscopic examination of tissues and organs for abnormalities, particularly to assess the carcinogenic potential.

There was no mortality in the course of the study that could be attributed to the presence of carbofuran. Additionally, no changes in appearance or behaviour were noted. A localized hair loss and reddening of the ear(s) frequently followed by scabbing or sloughing of portions of the ear, was noted with greater frequency in the treated mice. There was no mortality attributable to carbofuran. A slight decrease in body weight was evidenced through the first year of the study predominantly at the high dose level. During this period where growth reduction was noted, food consumption was also reduced slightly at the high dose level. With the exception of brain cholinesterase depression, there were no specific differences with respect to hematological parameters, clinical chemistry parameters, and urinalysis determinations that could be attributable to carbofuran. Brain cholinesterase was depressed in both males and females at the two highest dose levels and all time intervals recorded.

At the conclusion of the study, gross and microscopic examination of tissues and organs showed several statistically significant weight variations occurring periodically throughout the study. These changes in organ weights were not accompanied by changes observed microscopically which would be indicative of significant morphologic effects. There were no differences with respect to the occurrence of neoplastic or non-neoplastic changes in any of the carbofuran-treated mice. It was concluded that carbofuran was not tumorigenic nor carcinogenic in this strain of mice (Goldenthal, 1980; Brown, 1980). i.e. $> 500 \text{ mg/kg}$

Rat

Groups of rats (90 male and 90 female/group) were fed carbofuran in the diet at dosage levels of 0, 10, 20, or 100 mg/kg for 2 years. Carbofuran was dissolved in acetone and mixed with a small amount of laboratory diet to provide a premix, which was then diluted to provide larger quantities of the appropriate concentration for the test diet. Analytical evaluations of the test diets at periodic intervals verified the presence and concentration of carbofuran.

Rats were observed daily for overt toxicity, mortality, and general behaviour and appearance. Body weights were measured weekly, as was food consumption. At 6, 12, 18 and 24 months, groups of animals were sacrificed for a variety of clinical chemistry studies hematologic examinations, and urinalysis. Ophthalmologic examinations were performed at 0, 12, and 24 months. Groups of 10 animals of each sex were sacrificed at 6, 12, and 18 months and a final sacrifice of all survivors was made at the conclusion of the study. Gross and microscopic examinations of tissues and organs were performed on all animals that died during the study or were sacrificed at periodic intervals.

There was no excessive mortality over the course of the study that could be attributed to the presence of carbofuran. Growth, as evidenced by body weight, was depressed significantly at the high dose level, although food consumption was unaffected.

With the exception of cholinesterase activity depression, there were no significant findings in any of the clinical chemistry, hematology, urinalysis, or ophthalmological examinations. At 100 mg/kg cholinesterase depression was observed in plasma, red blood cell, and brain of both male and female rats. There was no significant cholinesterase depression observed at 20 mg/kg. (Cholinesterase depression was evaluated in the study using specific techniques and procedures that would be applicable for assessing the reversible nature of the inhibition.)

Analysis of the gross and microscopic pathology data did not suggest a compound-related pattern in any of the lesions observed. While there were sporadic lesions observed in a variety of tissues at various sacrifice times with respect to gross changes in weight, these were not accompanied by histological evidence of adverse morphologic effects. It was considered that both neoplastic and non-neoplastic histologic changes represented spontaneous lesions and were unrelated to the presence of carbofuran in the diet. Gross lesions without definitive morphologic change were assumed to have represented physiological variations, artifacts, or other causes that did not induce detectable morphologic alterations on histological examinations.

A no-effect level in this study is 20 mg/kg in the diet equivalent to 1.0 mg/kg body weight. This is based on changes in growth and on depression of cholinesterase activity level observed at the next highest dose level (Goldenthal, 1979a; Rapp, 1980a).

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EVALUATION

COMMENTS

Carbofuran, an anticholinesterase carbamate ester currently in extensive use as an insecticide, has been reviewed at two previous meetings. Additional studies made available to this meeting were reviewed.

There were no differences between juvenile and adult rats with respect to cholinesterase inhibition. Studies on reproduction, including an evaluation of the teratogenic potential of carbofuran, showed that carbofuran was not teratogenic and did not affect reproduction in mammals.

Long-term dietary studies on both rat and mouse failed to demonstrate a carcinogenic potential for carbofuran. In two new long-term studies, a dietary no-effect level was observed. Cholinesterase depression was the most sensitive effect noted in these studies.

The data reviewed met the requirements of previous meetings and allowed the estimation of no-effect levels in two mammalian species (mouse and rat). The rat and the mouse were used in an evaluation of the no-effect levels. Data with the dog (1976 report) showing a no-effect level of 50 mg/kg were based on clinical signs of poisoning where cholinesterase assays were not performed. The available studies with the dog are of limited value in the toxicological evaluation as cholinesterase data were not included.

Level causing no toxicological effect

Rat: 20 mg/kg in the diet equivalent to 1.0 mg/kg bw/day.

Mouse: 20 mg/kg in the diet equivalent to 2.5 mg/kg bw/day.

Estimate of acceptable daily intake for man

0-0.01 mg/kg bw/day. = 0.6 mg/day for 60 kg person

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