SD - Disapproval / Invalidity Reaction of D-15831 (Carbofuran)

18PP

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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MEMORANDUM

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SUBJECT: RfD/Peer Review Report of Carbofuran [2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate].

CASRN: 1463-66-2
EPA Chem. Code: 090601
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Manager, RfD/QA Peer Review Committee
Health Effects Division (7509C)

THRU: William Burnam
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TO: Dennis Edwards, PM 19
Insecticide-Rodenticide Branch
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Chief, Reregistration Branch
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The Health Effects Division-RfD/Peer Review Committee met on February 27, 1997 to discuss and evaluate the existing and or recently submitted toxicology data in support of Carbofuran reregistration and to reassess the Reference Dose (RfD) for this chemical.

Material available for review consisted of data evaluation records (DERs) for a combined chronic toxicity/carcinogenicity study in rats **(83-5 or 83-1a and 83-2a)**, a carcinogenicity study in mice **(83-2b)**, a chronic toxicity study in dogs **(83-1b)**, reproductive toxicity studies in rats **(83-4)**, developmental toxicity studies in rats and rabbits **(83-3a and -3b)**, subchronic toxicity study in rats **(82-1a and -1b)**, acute and subchronic neurotoxicity studies in rats (81- and 82-), non-guideline cholinesterase inhibition studies in human volunteers by oral and dermal routs, and a battery of mutagenicity studies **(84-2)**.

OPTIONAL FORM 99 (7-90)

FAX TRANSMITTAL # of pages ▸ 18

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NSN 7540-01-317-7368 5099-101 GENERAL SERVICES ADMINISTRATION

A. Chronic and Subchronic Toxicity:

The Committee considered the chronic toxicity phase (83-1a) of the combined chronic toxicity/carcinogenicity study in rats (83-5 or 83-1a and -2a, 1979, MRID No. 00030516, 00043745, 00043746, 00058736, 00063629, 00085498) to be acceptable and the data evaluation record (HED Doc. No. 000313) to be adequate.

In males, the NOEL/LOEL for plasma, red blood cell and brain cholinesterase inhibition were 1 and 10 mg/kg/day, respectively. In females, the NOEL/LOEL for plasma and brain cholinesterase inhibition were 1 and 10 mg/kg/day, respectively. The NOEL/LOEL for systemic effects were 1 and 10 mg/kg/day, respectively, based on decreased body weight (>10%) in males, and to a slight but statistically significant extent (>5%), in females. These dose levels were based on a standard conversion food factor. The Committee requested the actual dose levels be expressed based on food intake.

The Committee considered the chronic toxicity study in dogs (83-1b, 1983, MRID No. 003425) to be acceptable and the data evaluation record (HED Doc. No. 003425) to be adequate.

The NOEL/LOEL for plasma cholinesterase inhibition in males and females were 0.25 and 0.5 mg/kg/day, respectively. The NOEL/LOEL for red blood cell and brain cholinesterase inhibition in females were 0.5 and 12.5 mg/kg/day, respectively. The NOEL/LOEL for systemic toxicity were considered to be 0.5 and 12.5 mg/kg/day, respectively, based on toxic signs, decreased body weight and food consumption in both sexes (the high dose group was fed control diet to sustain life), anemia in males (decreased hematocrit, hemoglobin, and red blood cells), decreased total protein, calcium, sodium, and elevated potassium in males, decreased absolute brain and heart weight in males, alopecia in 1/6 of both sexes, decreased body fat in 2/5 males, testicular degeneration with aspermia and giant cell formation in 4/5 males, and lung inflammation in 5/5 males and 2/6 females. The data evaluation record stated that "consultation with the Health Effects Division pathologist regarding the significance of the testicular lesions in the 500 ppm dogs indicates that the testicular degeneration can be attributed to the poor nutritional status of the high-dose dogs rather than to a direct toxic effect of carbofuran in these animals.

The Committee considered the non-guideline cholinesterase inhibition study in human volunteers to be acceptable. The study was performed on a limited number of subjects, i.e. one placebo, two subjects per dose, at 0.05 and 0.1 mg/kg/day and 4 subjects at 0.25 mg/kg/day and only on males. The NOEL/LOEL for plasma and RBC cholinesterase inhibition were established at 0.05 and 0.1 mg/kg/day, respectively. Marginal plasma (32-36%) and red blood cell (11-22%) cholinesterase inhibition was noted at the 0.05 mg/kg/day, but the placebo subject had plasma inhibition also. At

0.1 mg/kg/day, plasma (35-56%) and red blood cell (31-32%) cholinesterase was significantly inhibited. At this level also, some cholinergic symptoms such as headache and lightheadedness were observed. At 0.25 mg/kg/day, severe cholinergic symptoms and signs were associated with plasma and RBC cholinesterase inhibition.

B. Carcinogenicity:

The Committee considered the carcinogenicity phase (83-2a) of the combined chronic toxicity/carcinogenicity study in rats (83-5 or 83-1a and -2a, 1979, MRID No. 00030516, 00043745, 00043746, 00058736, 00063629, 00085498) to be acceptable and the data evaluation record (HED Doc. No. 000313) to be adequate. The Committee considered the high dose level tested in this study (10 mg/kg/day) to be adequate for carcinogenicity testing based on plasma, red blood cell and brain cholinesterase inhibition in males and plasma and brain cholinesterase inhibition in females.

The Committee considered the carcinogenicity study in mice (83-2b, 1980, MRID No. 00030512, 00030513, 00030515) to be acceptable and the data evaluation record (HED Doc. No. 000313) to be adequate. The Committee considered the high dose level tested in this study (12.5 mg/kg/day) to be adequate for carcinogenicity testing based on brain cholinesterase inhibition in males and females.

The treatment did not alter the spontaneous tumor profile in the strains of rat and mouse used in these studies. Since there was no significant increase in any type of tumor in rats or mice under the testing conditions, the chemical was characterized as **"Not likely"** to be a human carcinogen.

C. Developmental and Reproductive Toxicity:

I. Reproductive Toxicity:

The Committee considered the reproductive toxicity study in rats (83-4, 1979, MRID No. MRID 00030514, 00030570, 00079810) to be acceptable and the data evaluation record (HED Doc. No. 000313) to be adequate, provided that the results section be expanded.

In this study, Carbofuran (95.6%) was administered to Sprague-Dawley rats at dietary levels of 20 or 100 ppm (1 or 10 mg/kg/day). The systemic (parental) NOEL/LOEL were established at 20 ppm (1 mg/kg/day) and 100 ppm (10 mg/kg/day), based on decreased pre-mating body weight gain in both sexes and all three generations, decreased P generation food consumption, and decreased gestation body weight gain for F1 and F2 females. The offspring (reproductive/ developmental) NOEL/LOEL were established at 20 ppm (1 mg/kg/day) and 100 ppm (10 mg/kg/day) based on decreased pup survival on postnatal days 0-4 in the first litter of each generation, and decreased day 21 pup weight in both litters of each

generation. The Committee noted that this 3-generation rat reproduction study did not demonstrate reduced fertility, reductions in adult reproductive male organ weights, or histopathological lesions of the testes. A reduction in testes weight was observed in immature F3b weanlings at 100 ppm; this is of unknown toxicological significance. The possibility that it might be related to primary destruction of germinal tissue was not confirmed by mutagenicity data in Drosophila, although a dominant lethal study in rats was not performed.

In a non-guideline study conducted to address the effect of Carbofuran on the male reproductive system in rats (Pant et al., 1995), the test chemical was administered by gavage to male Druckery albino rats at dose levels of 0.1, 0.2, 0.4, or 0.8 mg/kg/day, 5 days/week over 60 days; 10 rats/group were used. The NOEL/LOEL were established at 0.1 and 0.2 mg/kg/day, based on decreased body weight, decreased organ weight (epididymides, seminal vesicles, ventral prostate, coagulating glands), decreased sperm count and motility, increased abnormal sperm morphology (head, neck, and tail), altered activities of marker testicular enzymes [sorbitol dehydrogenase (decr.), glucose-6-P-dehydrogenase (decr.), lactate dehydrogenase (incr.), and gamma-glutamyl transpeptidase (incr.)], and histopathological lesions (edema, congestion, damage to Sertoli cells and germ cells with accumulation of cellular debris, presence of giant cells in the lumen of a few seminiferous tubules). At 0.8 mg/kg/day, the highest dose level tested, mortality was high (approximately 70%).

Although the highest dose tested in the Pant study (0.8 mg/kg/day) was just below the lowest dose tested in the 3-generation reproduction study (1 mg/kg/day), the effects were markedly different and are not corroborated by any other available data. On the other hand, the LD_{50} in rats was reported to be approximately 8 mg/kg, yet the subject publication by Pant et al. demonstrated 70% mortality at doses of 0.8 mg/kg/day. At up to 10 times the dose, the 3-generation reproduction study does not confirm the mortality, adult male reproductive organ weight decreases, or histopathology findings in the testes that were observed in this study. Also, the chronic toxicity study in rats described earlier, does not confirm the organ weight or histopathological findings in testes. In the carcinogenicity study in mice, testes weights were increased by 24% at 18 months in the 500 ppm group (75 mg/kg/day) and there were no histopathological lesions of the testes. In the one-year dog study, a dose-related increase in the testicular degeneration, with aspermia and giant cell formation, occurred in 4/5 males at 500 ppm (12.5 mg/kg/day), however, the HED pathologist stated that the testicular effects could be attributed to the compromised nutritional status of the dogs. It is possible that the difference in effects could be related to the strain of rat used in the subject study by Pant study, although the available data neither prove nor disprove that

hypothesis.

II. Developmental Toxicity:

The Committee considered the developmental toxicity study in rats (83-3a, 1981, MRID No. 00058610, 00058611) to be acceptable and the data evaluation record (HED Doc. No. 000348) to be adequate.

In this prenatal developmental toxicity study, Carbofuran (95.6%) was administered to Sprague-Dawley rats in the diet at dose levels of 20, 60, or 160 ppm (1.0, 3.0, or 8.0 mg/kg/day) on gestation days 6-19. The maternal NOEL/LOEL were established at 20 and 60 ppm (1.0 and 3.0 mg/kg/day), respectively, based on decreased body weight gain during dosing and decreased corrected body weight gain during gestation. The developmental NOEL/LOEL were established at 60 and 160 ppm (3.0 and 8.0 mg/kg/day), respectively, based upon reduced pup body weight at birth and throughout lactation (in a natural delivery subgroup).

The Committee considered the developmental toxicity study in rats (83-3a, 1980, MRID No. 00058609) to be unacceptable due to the lack of maternal and developmental toxicity at doses well below the limit dose. The data evaluation record (HED Doc. No. 000579) was considered to be adequate.

In this prenatal developmental toxicity study, Carbofuran (95.6%) was administered to Sprague-Dawley rats by gavage at dose levels of 0.25, 0.50, or 1.20 mg/kg/day on gestation days 6-15. The NOEL for both maternal and developmental toxicity was established at $\geq$1.20 mg/kg/day, the highest dose level tested. No maternal or developmental toxicity were demonstrated in this study up to the highest dose level tested. These results were consistent with the LOELs established in the 1981 study. Cholinesterase inhibition in fetuses was not measured in this study.

The Committee considered the developmental toxicity study in rabbits (83-3b, 1980, MRID No. 00076762) to be acceptable and the data evaluation record (HED Doc. No. 000000) to be adequate.

In this prenatal developmental toxicity study, Carbofuran (95.6%) was administered to New Zealand White rabbits by gavage at dose levels of 0.12, 0.50, or 2.0 mg/kg/day on gestation days 6-18. The maternal NOEL/LOEL were established at of 0.5 and 2.0 mg/kg/day based on decreased body weight gain during dosing and increased red/brown anogenital staining during and after the treatment period. No developmental toxicity was observed up to the highest dose level tested. In this study, cholinesterase inhibition in fetuses was not measured.

III. Developmental Neurotoxicity:

The Committee considered the developmental neurotoxicity study in rats (83-6, 1984, MRID No. 43378101) to be acceptable and the data evaluation record (HED Doc. No. 011792) to be adequate provided that tables of neurodevelopmental findings in support the LOEL be included.

In this developmental neurotoxicity, Carbofuran (99.1%) was administered to Sprague-Dawley rats at dietary concentrations of 20, 75, or 300 ppm (1.72, 6.91, and 31.0 mg/kg/day) from gestation day 6 through postnatal day 10. The maternal NOEL/LOEL were established at 20 and 75 ppm (1.72 and 6.91 mg/kg/day), respectively, based on decreased body weight gain and food consumption during gestation days 6-10, with an increase in severity at 300 ppm (31.0 mg/kg/day). The developmental neurotoxicity NOEL/LOEL were established at 20 and 75 ppm (1.72 and 6.91 mg/kg/day), respectively, based on increased pup mortality on postnatal days 0-4, decreased pup body weight from days 0-21, delayed maturational and sexual development (pinna unfolding, lower incisor eruption, eye opening, vaginal opening, and preputial separation), delayed swimming angle development, impaired learning and memory in the water maze, and decreased postnatal day 11 absolute brain weight.

D. Neurotoxicity:

The Committee considered the subchronic neurotoxicity study in rats (82-7, MRID No. 43163401) to be acceptable and the data evaluation record (HED Doc. No. 011214) to be adequate, with some revisions.

In this subchronic neurotoxicity study, Carbofuran was administered to Sprague-Dawley rats at dietary levels of 0, 50, 500 and 1000 ppm. Compound intake ranged from 2.4 - 4.7, 27.3 - 46.1, and 55.3 - 92.2 mg/kg/day. The NOEL for neurotoxicity was not established in this study. At 50 ppm (LDT), the effects included splayed hind limbs, and decreased body weight gain by 20% in males and 15% in females.

* The Committee noted that cholinesterase inhibition was not monitored following acute exposures in rats, subchronic feeding exposures in the neurotoxicity study of rats, or the subchronic feeding developmental neurotoxicity study in rats. However, the RfD and other regulatory values for short term exposure limits are currently based on limited human data and additional uncertainty factors have been used to account for the shortcomings of these data. In the event that the human data are no longer seem adequate to support risk assessments, reconsideration or request of these animal data which would require gavage dosing and measurement of cholinesterase data might be necessary.

E. Mutagenicity:

A total of 25 acceptable mutagenicity studies were available for review by the Committee, including: a) 14 studies with carbofuran produced from catechol as the starting material, the currently used manufacturing process, b) 7 studies with carbofuran produced from ortho-nitrophenol as the starting material, and c) 4 studies with carbofuran impurities. The following is a summary of these studies and Committee's conclusions for each study:

I. Catechol-Derived Carbofuran:

a. Gene Mutations:

1) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133510, HED Doc. No. 004333): Weak non-dose related positive response (≈2-fold increases) in S. typhimurium TA 1535 at 5000 and 10,000 µg/plate -S9 (Lot No. RHB-09/10-0112; purity not specified). The test was negative in all other S. typhimurium strains up to the highest dose tested (10,000 µg/plate +/-S9) and in S. typhimurium TA1535 up to 10,000 µg/plate +S9. The test material was insoluble at this level.

2) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133511, HED Doc. No. 004333): The test was considered negative in S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 up to the highest dose tested (10,000 µg/plate +/-S9; Lot No. RHB-11-0202; purity unspecified). The test material was insoluble at this level. However, a 1.9-fold increase in mutant colonies was obtained at 10,000 µg/plate -S9 in strain TA1535.

3) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133512, HED Doc. No. 004333): Weak positive and dose-related response (1.6-2.1 fold increase) in S. typhimurium TA 1535 at 2500 and 5000 µg/plate -S9 (Lot No. E2700-112D; purity not specified). The test was negative in all other S. typhimurium strains up to the highest dose tested (5,000 µg/plate +/-S9) and in S. typhimurium TA1535 up to 5,000 µg/plate +S9.

4) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133513, HED Doc. No. 004333): The test was considered negative in S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 up to the highest dose tested (10,000 µg/plate +/-S9; Lot No. E2700-112E; purity not specified). The test material was insoluble at this level. However, 1.7-fold increases in mutant colonies were obtained at 2500-10,000 µg/plate -S9 in strain TA1535.

5) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133514, HED Doc. No. 004333): Weak positive response (2-fold increase) in S. typhimurium TA 1535 at 5000 and 10,000 µg/mL -S9 (Lot No. E2700-114A; purity not specified). The test was negative in all other S. typhimurium strains up to the highest dose tested

(10,000 µg/plate +/-S9) and in S. typhimurium TA1535 up to 10,000 µg/plate +S9. Test material insolubility was seen at 10,000 µg/plate +/-S9.

6) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133515, HED Doc. No. 004333): The test was considered negative in S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 up to the highest dose tested (10,000 µg/plate +/-S9; Lot No. E2700-116A; purity unspecified). The test material was insoluble at this level. However, 1.9 and 1.7-fold increases in mutant colonies were obtained at 5000 and 10,000 µg/plate -S9, respectively, in strain TA1535.

7) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133516, HED Doc. No. 004333): The test was negative in S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 up to the highest dose tested (10,000 µg/plate +/-S9; Lot No. E2700-154A; 97.5% pure). The test material was insoluble at this level.

It should be noted that several of the studies listed above were considered negative in earlier reviews. It was concluded, however, based on the consistence of the response in S. typhimurium TA1535 at high doses near or at the solubility limits of the test material that all of the studies conducted with lots of carbofuran of unspecified purity yielded positive results. In contrast, the Ames assay conducted with 97.5% pure test substance was clearly negative. Overall, the results suggested that the six uncharacterized lots were probably contaminated with a mutagenic impurity.

8) Mouse lymphoma L5178Y $TK^{+/-}$ forward gene mutation assay (MRID No. 00133518, HED Doc. No. 004333): The test was positive in both the presence and absence of S9 activation with Lot No. E2700-154A; 98% pure. In the absence of S9 activation, dose-related increases in the mutation frequency (MF) were calculated over a dose range (67-211 µg/mL) that caused a 64-95% reduction in total cell growth, respectively. In the presence of S9-activation, increased MFs were observed only at severely cytotoxic doses (1780 and 2373 µg/mL). Lower levels (<67 µg/mL -S9; ≤1335 µg/mL +S9) were negative.

9) Drosophila melanogaster sex-linked recessive lethal mutation assay (MRID No. 000000, HED Doc. No. 004333): The test was negative in the germinal cells of male flies fed 10 ppm (assumed to be equivalent to ≈10 µg/mL) of technical grade (purity unspecified) carbofuran for 24 hours and sequentially mated with untreated females. Overt toxicity (reduced survival) was observed at this level; no effects on fertility were reported.

10) Drosophila melanogaster sex-linked recessive lethal mutation assay (MRID No. 000000, HED Doc. No. 004333): The test

was negative in the germinal cells of male flies fed 7.5 ppm (assumed to be equivalent to ≈7.5 µg/mL; Lot No. E2700-154A; 97.5%) for 22 hours and sequentially mated with untreated females. Overt toxicity (reduced survival) and target cell interaction (reduced fertility) were negligible at this dose. A higher level (10 ppm) was excessively toxic.

b. chromosomal aberrations:

11) In vitro Chinese hamster ovary (CHO) cell chromosome aberration assay (MRID No. 00133006, HED Doc. No. 004333): The test was negative up to the highest doses tested (100 µg/mL -S9; 2500 µg/mL +S9); higher nonactivated doses were cytotoxic. This study is currently listed as Unacceptable because of the low background frequency of aberrations in the negative control groups. However, the Committee recommended that the study be upgraded to Acceptable since background aberration frequencies for this cell line can vary from 0.00-0.06 aberrations/cell and the positive controls induced a powerful clastogenic effect. Additionally, the conclusion that the test material was positive in the presence of S9 activation is not supported. Increased frequencies compared to concurrent control were not dose-related and were well within established background frequencies for CHO cells.

12) In vivo bone marrow cytogenetic assay (MRID No. 00133522, HED Doc. No. 004333): The assay was negative in male Sprague Dawley rats receiving oral gavage administrations of 0.6, 2.0 or 6.0 mg/kg/day Lot No.E2700-154A; 98% for 5 days. Death and other signs of severe toxicity were seen at the high dose; no adverse effects on the target tissue were observed. Only males were evaluated; however, there is no indication in any of the short term studies suggesting that females are more sensitive to the toxicological effects of carbofuran. Therefore, the negative findings from this in vivo cytogenetic assay can be applied to females.

c. Other Mutagenic Mechanisms

13) In vitro sister chromatid exchange in CHO cells assay (MRID No. 00133010, HED Doc. No. 004333): The test was negative with Lot No. E2700-154A; 98% up to the highest doses tested (100 µg/mL -S9; 312.5 µg/mL +S9); higher levels were cytotoxic.

14) Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (MRID No. 00133099, HED Doc. No. 004333): The test was negative with Lot No. E2700-154A; 98% up to the highest dose tested (100 µg/mL); higher concentrations were excessively cytotoxic.

II. Ortho-Nitrophenol - Derived Carbofuran:

a. Gene Mutations:

1) Salmonella typhimurium reverse gene mutation assay (MRID No. 00134506; Doc. No. 004333): The test was considered negative in S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 with Lot No. E2700-154A (purity not specified) up to the highest dose tested (10,000 µg/plate +/-S9). The test material was insoluble at this level. However, ≈1.7- fold increases in mutant colonies were obtained at 2500, 5000 and 10,000 µg/plate -S9 in strain TA1535.

2) Salmonella typhimurium reverse gene mutation assay (MRID No. 00133517; Doc. No. 004333): The test was considered negative in S. typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 with Lot No. E2915-100A (purity not specified) up to the highest dose tested (10,000 µg/plate +/-S9). The test material was insoluble at this level. However, increases (≈1.7-, 2-, and 1.2-fold) in mutant colonies were obtained at 2500, 5000 and 10,000 µg/plate -S9 in strain TA1535 but were not dose-related.

3) Mouse lymphoma L5178Y $TK^{+/-}$ forward gene mutation assay (MRID No. 00133519; Doc. No. 004333): The test was positive in both the presence and absence of S9 activation. In the absence of S9 activation, dose-related increases in the mutation frequency (MF) were calculated for Lot No. E2915-100A (unspecified purity) at moderately cytotoxic (≥34% total survival) doses (75 and 100 µg/mL) and severely cytotoxic (≥9% total survival) concentrations (133-316 µg/mL). Dose-related increased Mfs were also noted in the presence of S9-activation at moderately cytotoxic (1001 and 1335 µg/mL) and severely cytotoxic (1780 µg/mL) doses. Lower levels (≤56 µg/mL -S9; ≤751 µg/mL +S9) were negative.

4) Drosophila melanogaster sex-linked recessive lethal mutation assay (MRID No. 000000, HED Doc. No. 004333): The test was negative in the germinal cells of male flies fed 5 or 10 µg/mL Lot No. RHB-11 (97.6%) for 24 hours and sequentially mated with untreated females. Overt toxicity (reduced survival) and target cell interaction (reduced fertility) were observed at both assayed concentrations.

b. Chromosomal Aberrations:

5) In vitro CHO cell chromosome aberration assay (MRID No. 00133007; Doc. No. 004333): The test was negative with Lot No. E2915-100A (purity unspecified) up to the highest doses tested (1000 µg/mL -S9; 2500 µg/mL +S9); higher nonactivated concentrations were cytotoxic.

c. Other Mutagenic Mechanisms:

6) In vitro sister chromatid exchange in Chinese hamster ovary (CHO) cells assay (MRID No. 00133011; Doc. No. 004333): The test was negative with Lot No. E2915-100A (purity unspecified) up to the

highest concentration tested (100 µg/mL -S9; 2500 µg/mL +S9); higher nonactivated levels were cytotoxic.

7) Unscheduled DNA synthesis (UDS) in WI-38 human fibroblasts (MRID No. 00133008; Doc. No. 004333): The test was negative with Lot No. C-4717-54A technical (purity unspecified) up to a high insoluble concentration (1000 µg/mL +/-S9). The S9-activated portion of the assay was listed as Unacceptable because of reduced sensitivity of the S9-activated positive control. However, information not available at the time the study was initially classified indicates that the response induced by the S9-activated positive control (dimethylnitrosamine) was well within the expected range for this compound. The Committee concluded, therefore, that the S9-activated phase of testing should be upgraded to Acceptable.

d. Other Information

Data from additional studies conducted with impurities found in ortho-nitrophenol-derived carbofuran indicate that two impurities were mutagenic in S. typhimurium TA98 and TA100 in the presence of S9 activation (HED Doc. No. 004534). One of the two impurities was also found to be positive for the induction of SCEs in CHO cells at S9-activated doses of 25, 40 and 50 µg/mL (Doc. No. 004978) but negative for gene mutations in CHO cells up to cytotoxic doses (≥400 µg/mL; ≥75 µg/mL).

III. Conclusions:

Overall, the data from genetic toxicology studies conducted with carbofuran produced by the catechol (CAT) or the ortho-nitrophenol (ONP) process were in good agreement and indicated that the test substance is a mutagen in S. typhimurium TA1535 but only at high concentrations near or at the solubility limit and only in the absence of exogenous metabolic activation. Similarly, there is convincing evidence that carbofuran produced by both methods is mutagenic in mammalian cells in vitro both with and without S9 activation and that the response was lessened when metabolic activation was present. In vivo, carbofuran (CAT and ONP) was negative for the induction of sex-linked recessive lethal mutations in D. melanogaster up to levels that reduced survival and fertility. Confidence in the validity of the in vivo results is high since carbofuran was shown to have reached the germinal cells but failed to induce a genotoxic effect. There was also no evidence of clastogenic activity either in vitro or in vivo with both endproducts. Based on these considerations, the data indicate that carbofuran produced by other method has intrinsic genotoxic potential which is not expressed in whole animals. These conclusions support the lack of an oncogenic effect in the rat and mouse long-term feeding study and also the absence of significant reproductive or developmental toxicity attributable to a mutagenic mode of action (i.e., decreased total implants, increased resorptions).

The Committee further concluded that the submitted test battery satisfies the new and pre-1991 mutagenicity initial testing battery guidelines and no additional testing is warranted at this time.

F. FQPA Considerations:

Under the directive of the Food Quality Protection Act (FQPA) recently enacted as an amendment to the Federal-Fungicide-Insecticide-Rodenticide Act (FIFRA), the Committee examined the data base and concluded that:

1) The data base included an acceptable two-generation reproduction study in rats, acceptable prenatal developmental toxicity studies in rats and rabbits, and an acceptable developmental neurotoxicity study in rats.

2) The data provided no indication of increased sensitivity of rats or rabbits to *in utero* and/or postnatal exposure to carbofuran. When toxicity was observed in the offspring, it invariably occured in the presence of maternal toxicity. In the two-generation reproduction study in rats, the NOEL and LOEL for parental and offspring toxicity were equivalent at 20 and 100 ppm (1 and 10 mg/kg/day, respectively). In the prenatal developmental toxicity study and the developmental neurotoxicity study in rats, the maternal NOEL and functional developmental NOEL were equivalent at 20 ppm (approximately 1 mg/kg/day), while the structural developmental NOEL was identified at 60 ppm (3.0 mg/kg/day). In an oral prenatal developmental toxicity study in rabbits, there was no evidence of developmental toxicity, although maternal toxicity was observed at 2.0 mg/kg/day, with a maternal NOEL of 0.50 mg/kg/day.

G. Reference Dose (RfD):

The Committee recommended that an RfD for this chemical be established based on the cholinesterase study in male human volunteers with a threshold NOEL of 0.05 mg/kg/day.

At 0.05 mg/kg/day, marginal plasma (32-36%) and red blood cell (11-22%) cholinesterase inhibition was observed. However, the Committee discounted the effect on plasma since it was considered within the normal variation of this parameter. The inhibition of the red blood cell cholinesterase at this level was considered to be marginal. The Committee, therefore, considered 0.05 mg/kg/day to be a threshold NOEL.

At the mid-dose level of 0.1 mg/kg/day, which is only two-fold the NOEL, plasma (35-56%) and red blood cell (31-32%) cholinesterase was significantly inhibited. At this level also, cholinergic symptoms such as headache and lightheadedness were observed.

At the high-dose level of 0.25 mg/kg/day, plasma (33-100%) and red blood cell (46-63%) cholinesterase was significantly inhibited. At this level also, severe cholinergic symptoms were observed.

The study was performed on a limited number of subjects, i.e. two subjects per dose for the low- and mid-dose levels and 4 subjects at the high dose level. The study was conducted on males only.

An Uncertainty Factor (UF) of 10 was applied to account for intraspecies variability. An additional UF of 10 was applied to account for the following: 1) study deficiencies; the use of a limited number of subjects (2 subjects/dose) and to less extent, the use of one sex (males only), 2) FQPA considerations; the lack of cholinesterase activity measurementsin the developmental and reproductive toxicity studies which made it impossible to assess any additional sensitivity to infants and children for these parameters, and 3) the steep dose - response of the human study and the presence of clinical symptoms at the LOEL of 0.1 mg/kg/day which is only two-fold the NOEL. On this basis, the RfD was calculated to be 0.0005 mg/kg/day.

It should be noted that this chemical has been reviewed by the WHO/FAO Joint Meeting on Pesticide Residues (JMPR) and an Acceptable Daily Intake (ADI) of 0.01 mg/kg/day has been established in 1982.

H. Individuals in Attendance:

Peer Review Committee members and associates present were George Ghali (Manager, RfD/Peer Review Committee), Karl Baetcke (Chief, TB I), Nancy McCarroll, James Rowe, Susan Makris, Kit Farwell, William Sette, Henry Spencer, and Rick Whiting. In attendance also was Karen Hamernik of HED as an observer.

Scientific reviewers (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report):

William Dykstra William Dykstra

Respective Branch Chief (Committee member; signature indicates concurrence with the peer review unless otherwise stated)

Karl Baetcke

CC: Stephanie Irene
Debra Edwards
Karl Baetcke
William Dykstra
Karen Whitby
Amal Mahfouz (OW)
RfD File
Caswell File

I. Material Reviewed:

1. Goldenthal, E. I. (1979). Two Year Dietary Toxicity and Carcinogenicity Study in Rats. MRID No. 00030516, 00030498, 00043745, 00043746, 00058736, 00063629, 00085498. HED Doc. No. 000313. Classification: Core minimum.

2. Goldenthal, E. et al. (1980). 2-Year Dietary Toxicity and Carcinogenicity Study in Mice. MRID No. 00031512, 00031512, 00030515. HED Doc. No. 000313. Classification: Core guideline.

3. Taylor, G. D. (1983). One-Year Chronic Oral Toxicity Study in Beagle Dogs with Carbofuran. MRID No. 00129507. HED Doc. No. 003425. Classification: Core minimum.

4. Arnold, J. (1977). Evaluation of the safe exposure levels to carbamate, administered orally to healthy adult normal male volunteers. MRID No. 0092826, HED Doc. No. 000000. Classification: Core supplementary.

5. Arnold, J. (1977). Carbamate (carbofuran) human dermal study. MRID No. 00092827, HED Doc. No. 000000. Classification: Core supplementary.

6. Arnold, J. (1978). Comparison of cholinesterase inhibition and effects of Furadan 4F and FMC 35001 4 EC. MRID No. 00092829, HED Doc. No. 000000. Classification: Core supplementary.

5. Goldenthal, E. and Rapp, W. R. (1979). Three Generation Reproduction Study in Rats. MRID No. 00030514, 00030570, 00079810. HED Doc. No. 000313. Classification: Core minimum.

6. Blair, M. (1981). Teratology and Postnatal Study in the Rat with Carbofuran. MRID No. 00058610, 00058611. HED Doc. No. 000348. Classification: Core minimum.

7. Rodwell, D. and Jessup, C. D. (1980). Teratology Study in the Rat with Carbofuran. MRID No. 00058609. HED Doc. No. 000579. Classification: Core minimum.

8. Laveglia, J. and Schardein, J. L. (1980). Teratology Study in the Rabbit with Carbofuran. MRID No. 00076762. HED Doc. No. 000000. Classification: Core minimum.

9. Ponnock, K. S. (1994). A Developmental Neurotoxicity Study of Carbofuran in the Rat Via Dietary Administration. MRID No. 43378101. HED Doc. No. 011792. Classification: Acceptable.

10. Freeman, C. (1994). Carbofuran Technical: Subchronic

Neurotoxicity Screen in Rats. MRID No. 00155429. HED Doc. No. 008419. Classification: Core guideline.

11. Freeman, C. (1995). 21-Day Repeated Dose Dermal Toxicity Study in Rabbits with FMC 10242 Technical (Carbofuran). MRID No. 00155429. HED Doc. No. 008419. Classification: Core guideline.

12. Rand, G. M. and Haworth, S. R. (1983). Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00133511. HED Doc. No. 004333. Classification: Acceptable.

13. Haworth, S. R. and Lawlor, T. E. (1983). Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00133513. HED Doc. No. 004333. Classification: Acceptable.

14. Haworth, S. R. and Lawlor, T. E. (1983). Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00133515. HED Doc. No. 004333. Classification: Acceptable.

15. Haworth, S. R. and Lawlor, T. E. (1983). Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00133517. HED Doc. No. 004333. Classification: Acceptable.

16. Haworth, S. R. and Lawlor, T. E. (1983). Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00134506. HED Doc. No. 004333. Classification: Acceptable.

17. Haworth, S. et al. (1983). Salmonella/Mammalian-microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00133514. HED Doc. No. 004333. Classification: Acceptable.

18. DeGraft, W. G. and Myhr, B. C. 1983). Mutagenicity evaluation of FMC 10242 in the Ames Salmonella/microsome plate test. MRID No. 00133516. Classification: Acceptable.

19. Simon, V. F. (1979). In vitro microbiological mutagenicity and unscheduled DNA synthesis studies of eighteen pesticides. MRID No. 00133008. HED Doc. No. 004333. Classification: Acceptable.

20. Thilager, A. et al. (1983). Chromosome aberrations in Chinese hamster ovary cells with FMC 10242. MRID No. 00133007. HED Doc. No. 004333. Classification: Acceptable.

21. Thilager, A. et al. (1983). Unscheduled DNA synthesis in rat

primary hepatocytes. MRID No. 00133099. HED Doc. No. 004333. Classification: Acceptable.

22. Haworth, S. R. and Lawlor, T. E. (1983). Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00133510. HED Doc. No. 004333. Classification: Acceptable.

23. Haworth, S. R. and Lawlor, T. E. (1983). Salmonella/Mammalian microsome plate incorporation mutagenicity assay (Ames test). MRID No. 00133512. HED Doc. No. 004333. Classification: Acceptable.

24. Thilager, A. et al. (1983). Sister chromatid exchange assay of FMC 10242 in Chinese hamster ovary (CHO) cells. MRID No. 00133011. HED Doc. No. 004333. Classification: Acceptable.

25. Thilager, A. et al. (1983). Chromosome aberrations in Chinese hamster ovary cells with FMC 10242. MRID No. 000000. HED Doc. No. 00133006. Classification: Unacceptable.

26. Thilager, A. et al. (1983). Sister Chromatid Exchange assay of FMC 10242 in Chinese hamster ovary (CHO) cells. MRID No. 00133010. HED Doc. No. 004333. Classification: Acceptable.

27. Kirby, P. E. et al. (1983). L5178y $TK^{+}/-$ Mouse lymphoma mutagenesis assay (FMC 10242). MRID No. 00133518. HED Doc. 004333. Classification: Acceptable.

28. Valencia, R. (1983). Drosophila sex-linked recessive lethal assay of 2,3-dihydro-2,2-dimethyl-7-benzofuranyl-N-methyl carbamate (Carbofuran, T2047). MRID/Accession No. 251983. HED Doc. No. 004333. Classification: Acceptable.

29. Valencia, R. (1981). Mutagenesis screening of pesticides using Drosophila. MRID/Accession No. 251983. HED Doc. No. 004333. Classification: Acceptable.

30. DeGraff, W. G. (1983). Mutagenicity evaluation of FMC 10242 Lot No. RHB-11 for the sex-linked recessive lethal test in Drosophila melangaster. MRID/Accession No. 251983. HED Doc. 004333. Classification: Acceptable.

31. Kirby, P. E. et al. (1983). L5178Y $TK^{+}/-$ Mouse lymphoma mutagenesis assay (FMC 10242). MRID No. 00133519. HED Doc. No. 004333. Classification: Acceptable.

32. Putman, D. L. and Melhorn, J.M. (1983). In vivo cytogenetics assay of FMC 10242. MRID No. 00133523. HED Doc. No. 004333. Classification: Acceptable.

J. References Cited:

1. Pant, N., et al. (1995) Effect of oral administration of carbofuran on male reproductive system of rat. *Human and Experimental Toxicology* 14:889-894.