MEMORANDUM

Date: June 29, 2015

SUBJECT: EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals

PC Code: See table, Attachment A
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: See table, Attachment A
MRID No.: NA

FROM: Greg Akerman
Health Effects Division
Office of Pesticide Programs
And
Amy Blankinship
Environmental Fate and Effects Division
Office of Pesticide Programs

THROUGH: Jess Rowland
Deputy Division Director
Health Effects Division
Office of Pesticide Programs

TO: Jolene Trujillo
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Pesticide Re-Evaluation Division
Office of Pesticide Programs

EPA has completed its Weight of Evidence (WoE) assessment evaluating results of the Endocrine Screening Program (EDSP) Tier 1 screening assays for the List 1 chemicals. The WoE documents for the 52 chemicals are listed in Attachment A along with the chemical and report identifiers.
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EDSP: WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL
INTERACTION WITH THE ESTROGEN, ANDROGEN OR
THYROID PATHWAYS

CHEMICAL: CARBOFURAN

OFFICE OF PESTICIDE PROGRAMS
OFFICE OF SCIENCE COORDINATION AND POLICY
U.S. ENVIRONMENTAL PROTECTION AGENCY
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<th>Terminology</th>
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<tbody>
<tr>
<td>A</td>
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<td>ADME</td>
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</table>
Executive Summary

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality were considered in this weight of evidence (WoE) assessment.

In determining whether carbofuran interacts with E, A or T hormone pathways, the number and type of effects induced, the magnitude and pattern of responses observed across studies, taxa, and sexes were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity.

On May 28, 2014, the EDSP Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) conducted a weight-of-evidence (WoE) analysis of the potential interaction of carbofuran with the E, A or T signaling pathways. The T1WoERC conclusions from the WoE evaluation in this report are presented by pathway (E, A and then T) beginning with the results of the Tier 1 in vitro assays followed by in vivo mammalian and wildlife results, then the results of the cited OSRI for mammalian and wildlife studies (40 CFR Part 158 and literature).

For carbofuran, there is no convincing evidence of a potential interaction with the estrogen pathway. The Tier 1 Estrogen Receptor (ER) binding, ER transactivation assay (ERTA) and aromatase assays were negative. The steroidogenesis assays was positive with increased estradiol production observed at the highest test concentration (100 µM); however, no effects on the estrogen pathway were observed in vivo in the uterotrophic or female pubertal assays. In the fish short term reproduction assay (FSTRA), all effects occurred in the presence of overt toxicity, except fertility which was reduced at a concentration without overt toxicity. However, there were no observed effects on vitellogenin (VTG), gonadal somatic index (GSI) or gonadal histopathology. Among the OSRI studies considered, there were no estrogen-related effects observed in the absence of overt toxicity.

For carbofuran, there is no convincing evidence of a potential interaction with the androgen pathway. The Tier 1 in vitro Androgen Receptor (AR) binding and steroidogenesis assays were negative, and no androgen-related effects were observed in the Tier 1 Hershberger or male pubertal assays. Also, there were no androgen-related effects observed in the mammalian Part 158 studies in the absence of overt toxicity. In the FSTRA, male tubercle score and fertility were
decreased at a concentration that was not coincident with overt toxicity, however, there were no treatment-related effects on male VTG, GSI, or gonadal histopathology in this study, and no androgen-related effects observed, in the absence of overt toxicity, for the other Tier 1 battery studies.

There was no convincing evidence of interaction of carbofuran with the thyroid pathway in the mammalian system. No effects on thyroid hormones, weights, or histopathology were seen in rats in the male and female pubertal rat assays. No thyroid-related effects were seen in the mammalian Part 158 studies. In the amphibian metamorphosis assay (AMA), carbofuran significantly increased 7-day normalized hind-limb length (HLL) by 6-7% at the intermediate and high treatment levels and mild to moderate follicular cell hyperplasia and hypertrophy were observed in all treatment groups, with the incidences increasing at the intermediate and high treatment levels as compared to the control. Although there were no thyroid-related effects observed in mammals, based on the effects observed in the AMA (HLL and thyroid histopathological effects) suggest that carbofuran has the potential to interact on the HPT axis in amphibians.

Based on weight of evidence considerations, mammalian EDSP Tier 2 testing is not recommended for carbofuran since there was no evidence of potential interaction with the estrogen, androgen or thyroid pathways. Additionally, no additional testing is recommended for fish or birds based on the lack of evidence for a potential interaction with the estrogen or androgen-pathways.

There is evidence of potential interaction with the thyroid pathway in the AMA. However, at present, all U.S. uses/registrations have been canceled and only import tolerances remain for carbofuran. Consequently, there is no anticipated ecological exposure to carbofuran in the U.S. Therefore, in spite of evidence for potential interaction with the thyroid pathway, EDSP Tier 2 testing is not recommended.
I. Introduction

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality were considered in this weight of evidence (WoE) assessment.

In determining whether carbofuran interacts with E, A or T hormone pathways, the number and type of effects induced, the magnitude and pattern of responses observed across studies, taxa, and sexes were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity.

On May 28, 2014, the EDSP Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) conducted a weight-of-evidence (WoE) analysis of the potential interaction of carbofuran with the E, A or T hormone pathways. The T1WoERC conclusions from the WoE evaluation in this report are presented by pathway (E, A and then T) beginning with the results of the Tier 1 in vitro assays followed by in vivo mammalian and wildlife results, then the results of the cited OSRI for mammalian and wildlife studies (40 CFR Part 158 and literature).

Carbofuran is a contact and systemic insecticide/nematicide belonging to the carbamate class of cholinesterase-inhibiting chemicals. At present, all U.S. uses/registrations have been canceled and only import tolerances remain for carbofuran. Carbofuran has been demonstrated to be highly mobile in many soils and therefore has the potential to leach to ground water in many types of soils or reach surface water via runoff. Carbofuran is very persistent in acidic environments, but dissipation increases as pH increases. Base hydrolysis is the most significant route of degradation in water and soil, with half-lives on the order of a year for pHs less than 7 and on the order of weeks for pHs greater than 7. Carbofuran is moderately resistant to microbial degradation in aerobic soil, with half lives on the order of a year. Near-surface photolysis is significant under laboratory conditions in aqueous solution, with a half-life on the order of days.

The available information considered in determining the potential interaction of carbofuran with the E, A, T pathways include submitted EDSP Tier 1 assays and/or other scientifically relevant information (OSRI) such as general toxicity studies and/or other published articles. These data are summarized in Sections III.A through III.C. An analysis of the data submitted to the agency,
using the WoE approach outlined by the Agency (USEPA, 2011), is presented in Section IV. The EDSP Tier 2 studies recommendations are presented in Section V.

II. Sources of Scientific Data and Technical Information

A. EDSP Tier 1 Screening Assays

The Tier 1 assays and/or other scientifically relevant information (OSRI) submitted to satisfy the agency’s test order are shown below in Table 1. Executive Summaries are presented in Appendix 1.

B. Other Scientifically Relevant Information (OSRI)

In response to the Agency’s Test Orders, data believed to be relevant to one or more of the Tier 1 assays were submitted as OSRI by the Test Order recipients and/or the public. This included studies published in the open literature and/or data submitted to support pesticide registration (e.g., Part 158 guideline studies). The Agency’s review of the initial OSRI is provided in the Report of the Endocrine Disruptor Review Team on Carbofuran (USEPA, 2010). Since then, the Agency has also conducted a more recent search of available scientific literature for any additional relevant information. Summaries of the available OSRI are presented in Appendix 2. Additionally, literature/studies considered but not utilized for the WoE analysis are listed in Appendix 3.

III. Weight of Evidence (WoE) Evaluation

The principles, criteria and approach used in the WoE determination on the potential of a substance to interact with endocrine-related processes (i.e., E, A, or T hormone pathways) were as described in the WoE guidance document (USEPA, 2011) and presented to the 2013 FIFRA Scientific Advisory Panel (SAP) (USEPA, 2013). The weight of evidence process identifies how the individual lines of evidence are assembled and integrated along two concepts (i.e., complementarity and redundancy) within the conceptual framework of an adverse outcome pathway. Broadly, there are four main steps outlined in the guidance which provide the foundation for WoE evaluations. The first step is to evaluate the individual studies for their scientific quality and relevance in evaluating potential endocrine interaction(s). The second step is to integrate the data along different levels of biological organization while examining the extent of complementarity (i.e., the concordance of endpoints within an assay that measures multiple endpoints) and redundancy (i.e., the concordance of endpoints/responses across assays) in the observed responses across these different levels of biological organization. The third step is to characterize the main lines of evidence as well any conclusions. Finally, the last step is to evaluate whether additional testing is needed based on the evidence and conclusions described above.
As mentioned, the first step is to assemble and evaluate the available scientific data. Data for the EDSP Tier 1 WoE evaluation falls into one of two categories: 1) EDSP Tier 1 data, or 2) other scientifically relevant information (OSRI). The EDSP Tier 1 data include a battery of 11 assays consisting of in vitro and mammalian and wildlife in vivo assays. The Tier 1 assays were designed specifically to evaluate a number of key biological events including potential effects on receptor binding (estrogen and androgen agonist and antagonist), steroidogenesis, and other effects on the HPG and HPT axes. OSRI may include published literature studies as well as studies conducted under USEPA (often referred to as Part 158 data) or OECD guidelines submitted in support of pesticide registrations. Each study is evaluated for scientific quality and relevance for informing interactions with the E, A, or T pathway. Additionally, the consistency of the responses in the individual study is evaluated. For the Tier 1 in vivo assays, often multiple endpoints are measured in each assay.

Evaluation of the potential confounding effects of overt toxicity in the study, as well as the relative degree of diagnostic utility of a specific endpoint for discerning whether or not the chemical has interacted with the endocrine system, are considered. The collective response of the individual endpoints, as well as the conditions under which they were expressed, are considered when evaluating an overall indication of potential interaction as measured by the study.

The second step in this WoE process is to formulate hypotheses and integrate the available data along different levels of biological organization. Two key elements in the integration of data, as well as characterizing the extent to which the available data support a hypothesis that a chemical has the potential to interact E, A, or T pathways, are the concepts of complementarity and redundancy. These two concepts provide a basis for considering the plausibility, coherence, strength, and consistency of the body of evidence. The current EDSP Tier 1 screening assays are meant to evaluate whether or not a chemical can interact with E, A and T consisting of different levels of biological organization from a molecular initiating event such as receptor binding through potential adverse effects in apical endpoints such as sexual development and fecundity at the whole organism level. The extent of expression of responses at higher levels of biological organization can indirectly provide information on potential compensatory capabilities of an individual organism.

After the data have been assembled and integrated, the third step is to characterize the main lines of evidence along with the conclusions; this characterization involves three components. The first component is whether the data provide relevant, robust and consistent evidence in terms of complementarity and redundancy as well as biological plausibility. Second, is at what level of biological organization were the responses observed and whether organisms exhibit compensatory responses at higher levels of biological organization? Finally, under what conditions did the responses occur including consideration of whether the responses were observed in the presence of overt or systemic toxicity? The presence of overt and/or systemic toxicity introduces uncertainty in the ability to distinguish effects specifically related to an
endocrine-related effect from a non-endocrine toxic response. This uncertainty in distinguishing whether the responses were endocrine-related was discussed at the FIFRA SAP meeting that evaluated scientific issues associated with the WoE evaluation of the EDSP Tier 1 screening process. In October, 2013, the SAP stated that, “In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis” (USEPA, 2013).

For these WoE analyses, overt toxicity was generally defined in accordance with EPA’s current approach as used by OPP in reviewing 40 CFR Part 158 studies for both human and ecological risk assessments. Specifically, in these analyses, the effects that EPA considered to be potential evidence of overt toxicity included, but were not limited to: mortality; clinical signs such as tremors, ataxia and abnormal swimming (fish and aquatic-phase amphibians); and body weight decreases of ≥10% in mammals. Additionally, other effects including morphological (e.g., organ weights/histopathology), biochemical (e.g., blood chemistry), and other clinical signs (e.g., lethargy) were also considered when evaluating overt toxicity, especially if the effects were extreme. In some instances, one parameter (i.e., death or >10% decrease in mammalian body weight) was sufficient to consider a dose/concentration to be overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. For example, in the FSTRA, generally, body weight decreases were considered along with other responses when assessing potential overt toxicity. Additionally, effects which were considered to be signs of systemic toxicity were also captured and these effects were generally considered as less severe forms of toxicity (e.g., changes in organ weights or blood chemistry). The circumstances for which a dose/concentration was considered overtly toxic for a particular study are described in Section IV.A.

Therefore, EPA considers multiple lines of evidence in including the observed responses in the Tier 1 assays and OSRI in the context of a chemical’s physical/chemical properties and its known modes of action in its overall characterization of a chemical’s potential to interact with the E, A or T pathway. Adequately addressing the three components described above is fundamental to the WoE process and in determining whether additional data are needed. In addition to characterizing the WoE, reviewers also consider: 1) uncertainties and their potential impact to conclusions; 2) discussion of key studies; 3) description of inconsistent or conflicting data; 4) overall strength of evidence supporting a conclusion; and, 5) what, if any, additional data are needed and why. Assessing the need for additional data is based on a case-by-case analysis which took all available toxicity data into account. In summary, the evaluation of the EDSP Tier 1 screening process and ultimate decision for any additional testing is based on a totality of the scientific evidence.
The WoE approach involved consideration of data (i.e., lines of evidence) from the EDSP Tier 1 assays and OSRI which are depicted in Tables 2 - 4. These tables contain data that are considered scientifically and biologically relevant with regard to a treatment-related effect which supports a conclusion of whether a substance has the potential to interact with the E, A, or T pathway. Effects that occurred in the presence of overt toxicity are discussed in the text for each respective pathway (E, A or T) but are not reported in the table for E, A or T.

A. EDSP Tier 1 Screening Assays

The Tier 1 assays submitted in response to the agency’s test order for carbofuran are shown below in Table 1.

Table 1: Tier 1 Screening Assays for Carbofuran.

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<th>Tier 1 Assays</th>
<th>Test Guideline</th>
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<tr>
<td>ER Binding Assay (Rat uterine cytosol)</td>
<td>OCSPP 890.1250</td>
<td>Requirement Satisfied (MRID No. 48615303)</td>
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<td>ERα Transcriptional Activation Assay</td>
<td>OCSPP 890.1300; OECD 455</td>
<td>Requirement Satisfied (MRID No. 48615304)</td>
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<tr>
<td>AR Binding Assay (Rat prostate cytosol)</td>
<td>OCSPP 890.1150</td>
<td>Requirement Satisfied (MRID No. 48615301)</td>
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<tr>
<td>Steroidogenesis Assay (Human cell line H295R)</td>
<td>OCSPP 890.1550; OECD 456</td>
<td>Requirement Satisfied (MRID No. 48615307)</td>
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<tr>
<td>Aromatase Assay (human recombinant microsomes)</td>
<td>OCSPP 890.1200</td>
<td>Requirement Satisfied (MRID No. 48615302)</td>
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<td>Uterotrophic Assay (Rat)</td>
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<td>Pubertal Female Assay (Rat)</td>
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<td>Pubertal Male Assay (Rat)</td>
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B. Effects on Hypothalamic-Pituitary-Gonadal (HPG) Axis

1. Effects on Estrogen Pathway

The potential for carbofuran to interact with the estrogen pathway is summarized in Table 2. The various targets of the estrogen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for estrogenic, anti-estrogenic, or HPG axis effects. This table also includes HPG-relevant findings from data evaluated as OSRI. Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.

Carbofuran was negative in the Tier 1 ER binding, ERTA and aromatase assays. Carbofuran was also negative in an OSRI ERTA in CHO cells (Kojima et al., 2004) and did not induce proliferation in estrogen-responsive MCF-7 cells (Soto et al., 1995). In the steroidogenesis assay, increased estradiol production was observed only at the highest dose tested of 100 µM. Both the in vivo uterotrophic assay and female pubertal assay were negative for estrogen-related effects.

In the fish short-term reproduction assay (FSTRA), fecundity was significantly reduced (↓55%) at the highest treatment concentration (0.435 mg a.i./L). Fertilization success was also significantly reduced by 11 and 21% at the 0.135 and 0.435 mg a.i./L levels. These effects were observed coincident with significant decreases of 12 and 11% in female body weight in the 0.135 and 0.435 mg a.i./L treatment concentrations, respectively. There were no reported treatment-related effects on plasma vitellogenin (VTG), GSI, or gonadal histopathology. In addition to the significant decreases in female body weight, male wet body weights were also reduced by 14 and 9% at the 0.135 and 0.435 mg a.i./L concentrations but were not statistically significant. Furthermore, while no clinical signs of sublethal toxicity were reported in the study at the high test concentration (0.435 mg a.i./L), there were 3 male mortalities (out of 8 exposed) and one female mortality (out of 16 exposed). ChE levels were not determined in the FSTRA. Moreover, previously conducted fish early life stage studies reported significant reductions in growth and survival at levels as low as 0.018 mg a.i./L (one order of magnitude lower than the middle treatment concentration for the FSTRA), thereby, supporting that the high concentration in the FSTRA may have been overtly toxic. Therefore, collectively, the observation of reduced body weight along with the mortality, the high test concentration in the FSTRA is considered to be overtly toxic.

The only potential estrogen-related effect observed in the Part 158 mammalian studies was delayed VO in the developmental neurotoxicity study. A 0.9 and 3.0 day delay was seen at the mid and high doses (6 and 8-31 mg/kg/day), respectively. However, the pups in these dose groups also had substantial reductions in body weight (↓6-38%) during lactation and post-
weaning; therefore, the delay in VO appears to be a secondary effect related to reductions in body weight.

In a Part 158 early life stage (ELS) study with rainbow trout, survival and growth was affected at \( \geq 0.057 \) mg a.i./L. In a Part 158 sheepshead minnow ELS study, hatching was reduced at concentrations \( \geq 0.068 \) mg a.i./L, and survival was affected at \( \geq 0.12 \) mg a.i./L; growth (length and wet weight) was affected at all test concentrations \( \geq 0.018 \) mg a.i./L. In another sheepshead minnow ELS study, hatch was affected at \( \geq 0.006 \) mg a.i./L and survival was reduced at \( \geq 0.016 \) mg a.i./L; there were no effects on growth.

In the Part 158 avian reproduction study with the mallard duck, by the end of the study, 24 of the 43 birds exposed to 2.0 ppm (the lowest treatment concentration) carbofuran had died (56%). A total of 31 of 41 birds exposed to 5.0 ppm had died (75%), and 43 of the 46 birds exposed to 10.0 ppm had died (93%). There were no reproductive endpoints that were significantly reduced from the control however this conclusion is confounded by the high mortality across the treatment groups. In the study with the bobwhite quail, there were no significant effects on any reproductive parameters. Mortality effects were also present in this study with 14% of the middle and high (53 and 180 ppm, respectively) treatment group birds dying during the course of the study. This was also observed with significant reductions in male and female body weight and a high percentage (18%) of cracked eggs in the control group.
Table 2: Estrogenic/Anti-Estrogenic Pathway for Carbofuran

| Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Carbofuran¹ |
|--------------------------------------------------|----------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Study Type/ Literature Citation | ER Binding ER Activation Steroidogenesis Sex Steroid Hormones Uterine Weight Ovarian Weight/GSI Ovarian/Gonad Staging and Histopathology Pituitary Weight Estrous Cyclicity Age & Weight at VO ²° Sex Characteristics Fertility (Frt)/ Fecundity (Fcd) Vitellogenin Systemic Toxicity Observed³ Overt Toxicity Observed² |
| ER Binding (MRID 48615303) | N | | | | | | | | | | | | | |
| ERTA (MRID 48615304) | | N | | | | | | | | | | | | |
| Aromatase (MRID 48615302) | | N | | | | | | | | | | | | |
| Steroidogenesis (MRID 48615307) | | P | | | | | | | | | | | | |
| Uterotrophic (MRID 48615308) | | | N | | | | | | | | | | | |
| Female Pubertal Rat (MRID 48669802) | | | N | N | N | N | N | N | | | | | | |
| FSTRA (MRID 48615305) | | | NE | N | N | | | | N | Frt: ↓11% (M) | | | X⁺ (H) | |
### Table 2: Estrogenic/Anti-Estrogenic Pathway for Carbofuran

<table>
<thead>
<tr>
<th>Study Type/ Literature Citation</th>
<th>ER Binding</th>
<th>ER Activation</th>
<th>Steroidogenesis</th>
<th>Sex Steroid Hormones</th>
<th>Uterine Weight</th>
<th>Ovarian Weight/GSI</th>
<th>Ovarian/Gonad Staging and Histopathology</th>
<th>Pituitary Weight</th>
<th>Estrous Cyclicity</th>
<th>Age &amp; Weight at VO</th>
<th>2° Sex Characteristics</th>
<th>Fertility (Frt)/fecundity (Fcd)</th>
<th>Vitellogenin</th>
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<td>Avian reproduction study (Mallard Duck; MRID 00129500)</td>
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<td>X (M, H)</td>
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1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium high treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that is parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated.
2. The systemic toxicity in the Tier 1 assays are presented in this column (e.g. KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (e.g. ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Decreased female body weight (12 and 11% at 0.135 and 0.435 mg a.i./L). Reduced (NS) male wet body weights (14 and 9% at 0.135 and 0.435 mg a.i./L). At the high test concentration (0.435 mg a.i./L), there were 3 male mortalities (out of 8 exposed) and one female mortality (out of 16 exposed).

P Positive findings
N Negative findings (in vitro)/No effect (in vivo)
NE Not examined
NR Not reported
2. Effects on Androgen Pathway

The potential for carbofuran to interact with the androgen pathway is summarized in Table 3. The various targets of the androgen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for androgenic, anti-androgenic, or HPG axis effects. This table also includes HPG-relevant findings from data evaluated as OSRI. Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.

The Tier 1 in vitro AR binding and steroidogenesis assays were negative. Carbofuran was also negative in an OSRI AR transcriptional activation assay (ARTA) in CHO cells (Kojima et al., 2004). The Tier 1 Hershberger assay was negative and no androgen-related effects were observed in the male pubertal assay.

In the FSTRA study, fertility was significantly reduced by 11 and 21% at the 0.135 and 0.435 mg a.i./L treatment levels. Male median nuptial tubercle scores were significantly lower at the 0.135 and 0.435 mg a.i./L levels (30 in both treatments compared to 39 in control). However, there were no treatment-related effects on male plasma vitellogenin, GSI or gonadal histopathology. Also, as discussed in the estrogen pathway section above, the high concentration was considered to be overtly toxic.

In the Part 158 developmental neurotoxicity study, preputial separation (PPS) was delayed by 3.0 and 3.9 days at the mid and high doses (6 and 8-31 mg/kg/day), respectively. However, the pups in these dose groups also had substantial reductions in body weight (↓6-38%) during lactation and post-weaning; therefore, the delay in PPS appears to be a secondary effect related to reductions in body weight. Additionally, in the chronic study in dogs, treatment related histopathological findings were noted in the testes and consisted of degeneration and giant cell formation in seminiferous tubules and aspermia in 4/5 males at the high dose (vs. 0/6 control). However, overt toxicity noted in the males at the high dose. There were no androgen-related effects observed in the Part 158 avian reproduction studies.
Table 3: Androgenic/Anti-Androgenic Pathway for Carbofuran

| Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for Carbofuran¹ |
|--------------------------------------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Study Type/ Literature Citation                  | AR Binding         | Steroidogenesis & Gene Expression | Sex Steroid Hormones | Testes Weight/GSI | Gonadal Staging and Histopathology | Epididymides Weight | Pituitary Weight | Accessory Sex Organ Weights/2nd Sex Characteristics | Fertility/Fecundity (Fed.) | Age and Weight at PPS | Vitellogenin (VTG) | Systemic Toxicity Observed² | Overt Toxicity Observed³ |
| AR Binding (MRID 48615301)                       | N                   | N                                 | N                   | N                   | N                                 | N                   | N                   | N                   | N                   | N                   | N                   | N                   | N                   |
| Steroidogenesis (MRID 48615307)                  | N                   | N                                 | N                   | N                   | N                                 | N                   | N                   | N                   | N                   | N                   | N                   | N                   | N                   |
| Hershberger (MRID 48615306)                      | NE                  | N                                 | N                   | N                   | N                                 | N                   | N                   | N                   | N                   | N                   | N                   | N                   | N                   |
| Male Pubertal Rat (MRID 48669803)                | N                   | N                                 | N                   | N                   | N                                 | N                   | N                   | N                   | N                   | N                   | N                   | N                   | N                   |
| FSTRA (MRID 48615305)                            | NE                  | N                                 | N                   | N                   | N                                 | N                   | N                   | N                   | N                   | N                   | N                   | N                   | N                   |
| OSRI                                             |                     |                                   |                     |                     |                                   |                     |                     |                     |                     |                     |                     |                     |                     |
| ARTA (Kojima et al., 2004)                       | N                   | N                                 | N                   | N                   | N                                 | N                   | N                   | N                   | N                   | N                   | N                   | X                   | X                   |
| Three-generation reproduction (Rat; MRID 00030514)|                     |                                   |                     |                     |                                   |                     |                     |                     |                     |                     |                     | X                   | X                   |
| Developmental Neurotoxicity (Rat; MRID 43378101) |                     |                                   |                     |                     |                                   |                     |                     |                     |                     |                     |                     | X (M, H)            | X (H)               |
| Chronic toxicity/carcinogenicity (Rat; MRID 00043745)|                     |                                   |                     |                     |                                   |                     |                     |                     |                     |                     |                     | X (H)               | X (H)               |
Table 3: Androgenic/Anti-Androgenic Pathway for Carbofuran

<table>
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<tr>
<th>Study Type/Literature Citation</th>
<th>AR Binding</th>
<th>Steroidogenesis &amp; Gene Expression</th>
<th>Sex Steroid Hormones</th>
<th>Testes Weight/GSI</th>
<th>Gonadal Staging and Histopathology</th>
<th>Epididymides Weight</th>
<th>Epididymides Histopathology</th>
<th>Pituitary Weight</th>
<th>Accessory Sex Organ Weights/2nd Sex Characteristics</th>
<th>Fertility (Frt)/Fecundity (Fcd)</th>
<th>Age and Weight at PPS</th>
<th>Vitellogenin (VTG)</th>
<th>Systemic Toxicity Observed</th>
<th>Overt Toxicity Observed</th>
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<td>Carcinogenicity (Mouse; MRID 00030512)</td>
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1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium high treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that the parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated. Abbreviations for androgen sensitive tissues: Seminal vesicles (SV), Ventral prostate (VP), Dorsal prostate (DP), Prostate (PR), Levator ani-bulbocavernosus (LABC), Epididymides (E), Cowper’s gland (CG), glans penis (GP).

2. The systemic toxicity in the Tier 1 assays are presented in this column (e.g. KW=kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A

3. The overt toxicity in the Tier 1 assays are presented in this column (e.g. ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A

4. Decreased female body weight (12 and 11% at 0.135 and 0.435 mg a.i/L). Reduced (NS) male wet body weights (14 and 9% at 0.135 and 0.435 mg a.i/L). At the high test concentration (0.435 mg a.i./L), there were 3 male mortalities (out of 8 exposed) and one female mortality (out of 16 exposed).

P Positive finding
N Negative findings (in vitro)/No effect (in vivo)
NE Not examined
NR Not reported
C. Effects on Hypothalamic-Pituitary-Thyroidal (HPT) Axis

The current EDSP Tier 1 battery does not have a specific in vitro assay to detect chemicals with the potential to affect hypothalamic or pituitary regulation of thyroid hormone production, but it does include three in vivo assays that provide data to detect changes in the HPT axis, i.e., the female and male rat pubertal assays, and the amphibian metamorphosis assay (frog).

The potential for carbofuran to interact with thyroid regulation is summarized in Table 4. The various targets of the thyroid pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for thyroid or HPT axis effects. This table also includes HPT-relevant findings from data evaluated as OSRI. Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.

There were no thyroid-related effects observed in the Tier 1 male and female pubertal assays or in the mammalian OSRI.

In the amphibian metamorphosis assay (AMA), carbofuran significantly increased 7-day normalized hind-limb length (HLL) by 6-7% at the 0.131 and 0.467 mg a.i./L treatment levels. However, HLL was not significantly different from the control for any treatment group on Day 21. There was no significant acceleration or delay of median developmental stage at Day 7 or 21 in any of the treatment groups, nor was asynchronous development observed. There was no treatment related effect on wet weight or snout-to-vent length (SVL) at Day 7 and Day 21. However, mild to moderate follicular cell hyperplasia and hypertrophy were observed in all treatment groups, with the incidences increasing at the intermediate and high treatment levels as compared to the control.
Table 4. Thyroid Pathway for Carbofuran.

| Lines of Evidence Indicating Potential Interaction with the Thyroid Pathway for Carbofuran¹ |
|---------------------------------|-----------------|----------------|----------------|-----------------|----------------|----------------|-----------------|----------------|
| Study Type/Literature Citation   | Thyroid Weight  | Thyroid: Gross and Histopathology | Serum T₄ Levels | Serum TSH levels | Pituitary Weight | Developmental stage or asynchronous, (HLL) | Growth (BW, SVL) | Systemic Toxicity Observed² | Overt Toxicity Observed³ |
|---------------------------------|-----------------|----------------|----------------|-----------------|----------------|----------------|-----------------|----------------|
| Male Pubertal Rat (MRID 48669803) | N               | N              | N              | N               | N              | N              | ▼ ChE (H)       | N               |
| Female Pubertal Rat (MRID 48669802) | N               | N              | N              | N               | N              | N              | adrenal ▼12% ChE (H) | N               |
| AMA (Frog; MRID 48669801)       | NR              | P(M,H)         |                |                |                | HLL: ▼6-7% (M, H) | N               | N               |
| Developmental toxicity (Rat; MRID 00058611) | NE              | NE             |                |                |                |                | N               | N               |
| Developmental Neurotoxicity (Rat; MRID 43378101) | NE              | NE             |                |                |                |                | N               | X (M, H)        |
| Developmental toxicity (Rabbit; MRID 00076762) | NE              | NE             |                |                |                |                | N               | N               |
| Three-generation reproduction (Rat; MRID 00079810) | N               | NR             |                | NR             |                |                | N               | N               |
| Chronic toxicity/carcinogenicity (Rat; MRID 00043745) | N               | N              |                | N              |                | X (H)          | X (H)           |
| Carcinogenicity (Mouse; MRID 00030512) | N               | N              |                | N              |                | X (M, H)       | N               |
| Chronic toxicity (Dog; MRID 00129507) | NR              | NR             |                | NR             |                | X (M, H)       | X (H)           |

1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium high treatment, H=High treatment. Arrows (▼ or ▼) indicate the direction of the response. A shaded cell indicates that parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated.

2. The systemic toxicity in the Tier 1 assays are presented in this column (e.g. KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A

3. The overt toxicity in the Tier 1 assays are presented in this column (e.g. ▼BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A

P  Positive findings
N  Negative finding (in vitro)/No effect (in vivo)
NE  Not examined
NR  Not reported
IV. Committee’s Assessment of Weight of Evidence

This section of the document describes the weight of evidence (WoE) determination on the potential of carbofuran to interact with endocrine related processes (i.e., E, A or T hormonal pathways) as well as recommendations regarding Tier 2 testing. The results of the Tier 1 assays are considered, along with other scientifically relevant information (e.g., 40 CFR Part 158 test guidelines and published or publicly available peer-reviewed studies). WoE analysis in the context of the EDSP follows the Agency’s guidance (USEPA 2011) and is conducted on a case-by-case basis by first assessing the different lines of evidence (i.e., specific Tier 1 assays and OSRI), then performing an integrated analysis of those lines of evidence.

The WoE evaluation includes considerations of biological plausibility of the findings from the different lines of evidence by examining the consistency, coherence, and interrelationships among the measured endpoints within and across studies. The available findings from standard toxicology studies on the substance may contribute to the WoE evaluation in helping elucidate if effects seen in the Tier 1 assay are related to perturbations of the endocrine system per se or alternatively a sequelae of systemic effects. Endocrine modes of action may elicit a number of phenotypic consequences other than those evaluated in the Tier 1 assays.

Endocrine-related findings in the presence of overt toxicity may result in uncertainty as to whether or not the responses are related through an endocrine pathway, therefore non-endocrine toxic responses (including but not limited to mortality or body weight changes) are also considered in this WoE evaluation. The FIFRA Scientific Advisory Panel (SAP) that evaluated scientific issues associated with weight of evidence evaluation of the results of the Tier 1 assays (July, 2013) stated that “In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis” (USEPA, 2013).

A. Systemic/Overt Toxicity in the in vivo Tier 1 Assays and OSRI

Effects that were considered to be systemic or overt toxicity for the in vivo Tier 1 assays and OSRI studies are described below. In addition to the endocrine-related effects described above for the in vivo Tier 1 assays and OSRI, other effects observed which may be considered overt/systemic toxicity are also describe below. Generally, one parameter (i.e., death or >10% decrease in mammalian body weight) were sufficient for a dose/concentration to be considered overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. Effects which were considered to be signs of systemic toxicity were generally less severe forms of toxicity (e.g., changes in organ weights or blood chemistry).
1. Tier 1 in vivo Assays

No systemic or overt signs of toxicity were noted in the AMA study. ChE levels were not determined in either the FSTRA or AMA. In the FSTRA study, there were significant reductions in wet body weight of females of 4%, 12% and 11% at concentrations of 0.0437, 0.135 and 0.435 mg a.i./L, respectively; male wet body weights were also reduced (NS) by 14 and 9% at the 0.135 and 0.435 mg a.i./L concentrations. While no clinical signs of sublethal toxicity were reported in the study, at the high test concentration (0.435 mg a.i./L), there were 3 male mortalities (out of 8 exposed) and one female mortality (out of 16 exposed).

In the Hershberger assay, no treatment-related effects were observed on mortality, clinical signs of toxicity, body weight, body weight gain or brain weight. However, ChE levels, which were determined only at the high dose (0.3 mg/kg/day) on study days 0 and 9, were substantially decreased in the RBC (87.3% and 45.0%, respectively) and brain (40.4 and 32.1%) compared to the controls.

In the pubertal rat assays, no treatment-related effects were observed on survival, clinical signs, body weight or body weight gain. ChE levels were only determined in the high-dose group (0.3 mg/kg/day) in each pubertal assay. In females, ChE activity was decreased at the high dose on Days 23 and 53 in the RBC (62-70% inhibition) and brain (41-56% inhibition). In males, ChE activity was decreased at the high dose on Days 22 and 42 in the RBC (59-60% inhibition) and brain (29-44% inhibition).

In the uterotrophic assay, all animals survived until scheduled termination and there were no clinical signs of toxicity observed in any carbofuran-treated groups. Body weights and body weight gains in the carbofuran treated groups were comparable to the controls throughout the study. ChE levels, which were only determined at the high dose (0.3 mg/kg/day), were decreased in the RBC (69-73% inhibition) and brain (43-44% inhibition) compared to the controls.

2. OSRI

Overt toxicity was not observed in the rat and rabbit developmental studies, although maternal body weight was reduced at the high dose in both the rat (8 mg/kg/day) and rabbit (2 mg/kg/day) studies, and pup body weights were significantly reduced at the high dose in the rat study. In the developmental neurotoxicity rat study, pup body weights in the mid- and high-dose groups (6 and 8-31 mg/kg/day) were reduced by >10% and pup mortality was increased from PND 0-4 at the mid and high dose, indicating that these doses were overtly toxic. In the rat reproduction study, parental and pup body weights were also significantly reduced at the high dose (5 mg/kg/day), but the reductions were <10%. ChE activity was not determined in any of the developmental studies or the rat reproduction study.
In the chronic rat study, body weight decreases in high dose males were 11% at the 18-month sampling interval, but the decrease was only 5% at the final 24-month interval; female body weight decreases were ≤9%. ChE activity as inhibited in high-dose rats (5 mg/kg/day) at 12, 18 and 24 months by 10-37% in plasma, 11-24% in RBC, and 11-43% in brain. In the mouse carcinogenicity study, brain ChE levels were significantly decreased at the mid- and high-doses (7 and 71 mg/kg/day), but the magnitude of the decrease was not reported. In the chronic dog study, plasma ChE levels were decreased (p<0.01) by 24-31% in mid-dose males (1.0 mg/kg/day) and by 78-87% in high-dose males and 77-84% in high-dose females (12.5 mg/kg/day). The high dose in the dog study was overtly toxic based on body weight decreases >10% at the high-dose; overall body weight gains were also negative (-0.9 kg, males; -0.4 kg, females) at the high dose.

In an early life stage toxicity study conducted with rainbow trout, fish in the top two treatment concentrations (56.7 and 88.7 µg a.i/L) exhibited rapid respiration by Day 20 (larval stage) and scoliosis by Day 90, and both symptoms continued until test termination. Survival was significantly reduced at these top two treatment concentrations at 60, 75 and 90 days post-hatch. Body length at 30 days post-hatch was significantly reduced in highest treatment concentration and was also significantly reduced in the top two treatment concentrations at 60, 75 and 90 days post-hatch. Body weight was also significantly reduced at top two treatment concentrations at 75 days, but only in fish in the highest treatment concentration by 90 days post-hatch.

In another early life stage toxicity study conducted with the sheepshead minnow, there were no embryos that hatched in the highest treatment concentration (51 µg a.i/L) and the percent hatch was reduced to 91, 24 and 14% for the 6.0, 16, and 26 µg/L groups, respectively (3rd, 4th, and 5th treatment concentrations), compared to 100% in the controls. Day 35 survival was reduced to 19 and 9% in the 3rd and 2nd highest treatment concentrations.

In the avian reproduction studies, the critical observed effect was adult survival at all exposure levels tested. For the mallard study, onset of mortality was observed between days 8 and 15 for all exposure levels and continued for the duration of the study. By the end of the period before egg production (12 weeks), 16 of the 43 birds at 2.0 ppm were dead, 18 of the 41 birds at 5.0 ppm were dead, and 26 of the 46 at 10.0 ppm were dead. By the end of the study, 24 of the 43 birds exposed to 2.0 ppm carbofuran had died (56%). A total of 31 of 41 birds exposed to 5.0 ppm had died (75%), and 43 of the 46 birds exposed to 10.0 ppm had died (93%). Similar results were observed in the northern bobwhite study, with 14% of the birds in the middle and high (53 and 180 ppm, respectively) treatment groups suffering mortality. This was observed with significant reductions in male and female body weight. Clinical signs of toxicity included subdued behavior, unsteadiness, and ruffled appearance.
B. Estrogen Pathway

There is no convincing evidence of a potential interaction with the estrogen pathway. The Tier 1 Estrogen Receptor (ER) binding, ER transactivation assay (ERTA) and aromatase assays were negative. The steroidogenesis assays was positive with increased estradiol production observed at the highest test concentration (100 µM); however, no effects on the estrogen pathway were observed in vivo in the uterotrophic or female pubertal assays. In the fish short term reproduction assay (FSTRA), all effects occurred in the presence of overt toxicity, except fertility which was reduced at a concentration without overt toxicity. However, there were no observed effects on vitellogenin (VTG), gonadal somatic index (GSI) or gonadal histopathology. Among the OSRI studies considered, there were no estrogen-related effects observed in the absence of overt toxicity.

C. Androgen Pathway

There is no convincing evidence of a potential interaction with the androgen pathway. The Tier 1 in vitro Androgen Receptor (AR) binding and steroidogenesis assays were negative, and no androgen-related effects were observed in the Tier 1 Hershberger or male pubertal assays. Also, there were no androgen-related effects observed in the mammalian or wildlife Part 158 studies in the absence of overt toxicity. In the FSTRA, male tubercle score and fertility were decreased at a concentration that was not coincident with overt toxicity, however, there were no treatment-related effects on male VTG, GSI, or gonadal histopathology in this study, and no androgen-related effects observed, in the absence of overt toxicity, for the other Tier 1 battery studies.

D. Thyroid Pathway

There was no convincing evidence of interaction of carbofuran with the thyroid pathway in the mammalian system. No effects on thyroid hormones, weights or histopathology were seen in rats in the male and female pubertal rat assays. No thyroid-related effects were seen in the mammalian Part 158 studies. In the AMA, carbofuran significantly increased 7-day normalized hind-limb length (HLL) by 6-7% at the intermediate and high treatment levels and mild to moderate follicular cell hyperplasia and hypertrophy were observed in all treatment groups, with the incidences increasing at the intermediate and high treatment levels as compared to the control. Although there were no thyroid-related effects observed in mammals, based on the effects observed in the AMA (HLL and thyroid histopathological effects), suggest that carbofuran has the potential to interact on the HPT axis in amphibians.

E. Conclusions

Carbofuran demonstrates no convincing evidence of potential interaction with the estrogen, or androgen pathways. While there is also no evidence to suggest potential interaction with the
thyroid pathways in the mammalian system, carbofuran shows the potential to interact with the HPT axis in amphibians.

V.  EDSP Tier 2 Testing Recommendations

Based on weight of evidence considerations, mammalian EDSP Tier 2 testing is not recommended for carbofuran since there was no evidence of potential interaction with the estrogen, androgen or thyroid pathways. Additionally, no additional testing is recommended for fish or birds based on the lack of evidence for a potential interaction with the estrogen or androgen-pathways.

There is evidence of potential interaction with the thyroid pathway in the AMA. However, at present, all U.S. uses/registrations have been canceled and only import tolerances remain for carbofuran. Consequently, there is no anticipated ecological exposure to carbofuran in the U.S. Therefore, in spite of evidence for potential interaction with the thyroid pathway, EDSP Tier 2 testing is not recommended.
VI. References


APPENDIX 1. EDSP Tier 1 Screening Assays

Amphibian Metamorphosis Assay (AMA); (OCSPP 890.1100)

The 21-day assay (MRID 48669801) of carbofuran technical on amphibian metamorphosis of African clawed frogs (*Xenopus laevis*) was conducted under flow-through conditions. Amphibian larvae at Nieuwkoop-Faber (NF) Stage 51 (80 tadpoles per control and treatment group) were exposed to carbofuran (99.4% purity) at nominal concentrations of 0 (negative control), 0.045, 0.15, and 0.50 mg a.i./L; mean-measured concentrations were <0.0142 (<LOQ; control), 0.0366, 0.131, and 0.467 mg a.i./L. The test system was maintained at 21.2 to 22.4°C and a pH of 7.9 to 8.5.

All effects are reported based on comparison to the negative (clean water) control. The survival of tadpoles exposed to carbofuran was not significantly affected (p>0.05) by treatment and was determined to be 100% in the control group and low and high treatment concentrations and 98% in the middle treatment concentration. Tadpoles exhibited spinal curvature ranging from one to five tadpoles in each replicate at test termination. There were no other clinical signs of toxicity noted during the study.

There was no significant acceleration or delay of median NF developmental stage at Day 7 or 21 in any of the treatment groups. There was only one late-stage tadpole (Day 21, NF=61) in replicate D of the 0.467 mg a.i./L treatment group; this individual was excluded from the analysis of Day 21 body weight, snout-vent-length (SVL), hind limb length (HLL), and normalized HLL endpoints.

Carbofuran significantly increased 7-day normalized hind-limb length (HLL) (p<0.05) 6-7% at the mean-measured 0.131 and 0.467 mg a.i./L treatment levels. Day 21 normalized HLL was comparable (p>0.05) between the control and all treatment groups. The study author noted that while the effect on normalized HLL was significant at the middle treatment concentration, this result was not judged to be biologically relevant because the mean value of that endpoint (0.286 mm) was similar to the laboratory’s historical control range (0.299 – 0.496 mm), the low control mean normalized HLL of 0.233 mm (below historical control values) may have contributed to significance, and there were no statistically significant differences in Day 21 normalized HLL between the control and any of the treatment groups.

There was no asynchronous development observed throughout the test. Additionally there were no significant effects (p>0.05) on Day 7 and Day 21 SVL and body weight.

Effects on thyroid gland histopathology included increased incidence and severity of follicular cell hypertrophy and hyperplasia in the middle and high treatment groups. In cases of follicular cell hypertrophy, the incidence was predominately mild at the middle treatment concentration.
and mild to moderate in the high treatment concentration. For follicular cell hyperplasia, the incidence was predominantly mild both for the middle and high treatment concentrations. The severity of these findings appeared to be slightly increased in the middle and high treatment concentrations as compared to the control; the differences were not statistically analyzed.

**Androgen Receptor (AR) Binding Assay; (OCSPP 890.1150)**

In an androgen receptor (AR) binding assay (MRID 48615301), ventral prostate cytosol from Sprague Dawley rats was used as the source of AR. The saturation binding experiment (data submitted separately, MRID 48843501) was conducted to demonstrate that the AR in the rat prostate cytosol was present in reasonable numbers and was functioning with appropriate affinity for the radiolabeled reference androgen (R1881). The competitive binding experiment was conducted to measure the binding of a single concentration (10 nM) of the radio-labeled reference androgen ([³H]-R1881) in the presence of increasing concentrations of carbofuran (logarithmic increase from $10^{-10}$ to $10^{-3}$ M). Dimethyl sulfoxide (DMSO) was used as a vehicle at a final assay concentration of approximately 3.2%. The assay included dexamethasone as a weak positive control, and R1881 as the ligand reference standard. Three independent runs were conducted with 3 replicates per concentration per run.

Saturation binding data were not originally provided in the study report; however, summarized saturation binding data (MRID 48843501) from the performing laboratory were submitted following a request by the Agency. The mean dissociation constant ($K_d$) for [³H]-R1881 was $1.080\pm0.690$ nM, and the estimated B$_{max}$ was $1.071\pm0.167$ fmol/100 µg protein for the single batch of prostate cytosol that was used in the assay. Only one of the three $K_d$ values was within the range reported in the EPA validation program (0.685 to 1.57 nM), and all B$_{max}$ values were below the recommended range (7 to 16 fmol/100 µg protein). Although the goodness of fit for individual runs was acceptable ($R^2 = 0.880-0.986$), confidence in these numbers is low based on the large variation in $K_d$ estimates among runs.

In the competitive binding experiment, the specific [³H]-R1881 binding to the AR was >75% (non-binder) at all test concentrations of carbofuran in all three runs. The estimated log IC$_{50s}$ for R1881 and the weak positive control (dexamethasone) were in the ranges of -9.0 to -9.9 M and -4.4 to -4.6 M, respectively. The mean RBA for the weak positive control was 0.0023%, and the mean RBA for carbofuran could not be determined. The solvent control responses indicated no drift in the study assay. All performance criteria were met, with the exception of the bottom (% binding) of dexamethasone in Run 1; the bottom plateau level could not be accurately calculated because of a deviation where the concentration range for dexamethasone was prepared slightly different than that specified in the guideline. As this was a minor deviation, the reviewers concluded that the performance criteria were met.
Based on the results from the three runs, carbofuran is classified as a Non-Binder in the Androgen Receptor Binding Assay.

**Aromatase Assay; (OCSPP 890.1200)**

In an *in vitro* aromatase (CYP 19) assay (MRID 48615302), carbofuran (99.4% w/w, Batch # PL11-0245) in dimethyl sulfoxide (DMSO) at increasing logarithmic concentrations from $10^{-10}$ to $10^{-3}$ M was incubated with human recombinant aromatase and tritiated androstenedione ([$1\beta\text{-}^3\text{H}(\text{N})$]-androst-4-ene-3,17-dione; $[^3\text{H}]$-ASDN) for 15 minutes at 37°C to assess the potential of carbofuran to inhibit aromatase activity.

Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation period for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting (LSC). Three independent runs were conducted, and each run included a full activity control, a background activity control, a positive control series ($10^{-10}$ to $10^{-5}$ M) using a known inhibitor (4-hydroxyandrostenedione; 4-OH ASDN), and the carbofuran series ($10^{-10}$ to $10^{-3}$ M) with 3 repetitions per concentration.

The response of each full activity control was between 90 to 110% of the average full activity for each run. The response of the full activity controls and background controls was acceptable. The positive control results were within the recommended ranges for the top of the curve, bottom curve, Hill slope, log IC$_{50}$, and coefficient of variation for replicates of each concentration. For 4-OH ASDN, the estimated log IC$_{50}$ averaged $-7.27$ M, and the slope was -1.00. Confidence in these values is very high due to the small variation (<4% CV).

For carbofuran, aromatase activity averaged $0.593\pm0.077$ nmol·mg-protein$^{-1}$·min$^{-1}$ at the lowest tested concentration of $10^{-10}$ M and $0.589\pm0.070$ nmol·mg-protein$^{-1}$·min$^{-1}$ at the highest tested concentration of $10^{-3}$ M. No precipitation was observed at any concentration. The data for carbofuran were not suited for modeling as the mean aromatase activity of $10^{-3}$ M carbofuran was determined to be 99.0% of control. The average lowest portion of the response curve across runs was >75% activity.

Based on the data from the average response curve, carbofuran is classified as a Non-inhibitor of aromatase activity in this assay.

**Estrogen Receptor (ER) Binding Assay; (OCSPP 890.1250)**

In an estrogen receptor (ER) binding assay (MRID 48615303), uterine cytosol from Sprague-Dawley rats was used as the source of ER to conduct binding experiments. A competitive
binding experiment was conducted to measure the binding of a single concentration of \[^{3}H\]-17\(\beta\)-estradiol (1 nM) in the presence of increasing concentrations of carbofuran (logarithmic increase from \(10^{-10}\) to \(10^{-3}\) M). DMSO was used as a vehicle at a final concentration of 2%. The assay included 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control, and 17\(\beta\)-estradiol as the natural ligand reference material.

Saturation binding data were provided via email (to D. McCall 5/18/12). In the competitive binding experiment, the IC\(_{50}\) values were within the expected concentration range for natural ligand (1.3 \(\times\) 10\(^{-9}\) M) and the weak positive control (2.9 \(\times\) 10\(^{-6}\) M). Compared to the natural ligand, the mean relative binding affinity (RBA) was 0.043\% for the weak positive control, with a log RBA of -1.37\%. Although decreases in \[^{3}H\]-ligand binding were observed in the negative control at the highest concentration tested (10\(^{-3}\) M), the decreased binding may have resulted from precipitation of the octyltriethoxysilane.

The curve for the reference material showed that increasing concentrations of unlabeled 17\(\beta\)-estradiol displaced \[^{3}H\]-17\(\beta\)-estradiol in a manner consistent with one-site binding, as indicated by Hill slopes of -1.1 to -0.8. Carbofuran was tested over a concentration range that fully defined the top of the curve, with maximum binding of 97.7-101.8\%.

The minimum percent binding observed for carbofuran at concentrations up to \(10^{-3}\) M was 92.6-97.0\% across all three runs; binding was decreased by <25\% in each run. Based on the results from the three runs, carbofuran is classified as Not Interactive in the Estrogen Receptor Binding Assay.

**ER\(\alpha\) Transcriptional Activation (ERTA) Assay; (OC SSP 890.1300)**

In an estrogen receptor transcriptional activation (ERTA) assay (MRID 48615304), hER\(\alpha\)-HeLa-9903 cells cultured in vitro were exposed to carbofuran (99.4\% a.i., Batch # PL11-0245) at logarithmic concentrations of \(10^{-4}\) to \(10^{-11}\) M in DMSO (0.1\%) for 24 ± 2 hours. The experiments were performed using 96-well plates and each carbofuran concentration was tested with 6 replicates/plate. Cells were exposed to the test agents for 24 ± 2 hours to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor by carbofuran was measured using a proprietary method.

Carbofuran was tested up to the limit of cytotoxicity, \(10^{-5}\) M. The mean RPC\(_{\text{max}}\) for carbofuran was 3.3\% at \(10^{-5}\) M and 3.4\% at \(10^{-7}\) M for the first and second run, respectively. The PC\(_{10}\) could not be calculated as the RPC\(_{\text{max}}\) was less than 10\%.

In the main assays, the responsiveness of the cells to the very weak positive control 17\(\alpha\)-methyltestosterone was lower than the expected values, indicating a decreased sensitivity of the
assay to very weak agonists. The log PC_{10} for 17α-methyltestosterone was not calculable in the first run, and was higher than the acceptable range at −5.9 in the second run. The log PC_{50} for 17α-methyltestosterone was not calculable in either run. Although the conditions of this assay were not optimal to detect very weak activity, carbofuran responses were similar to those of the negative control corticosterone and not comparable to the responses of 17α-methyltestosterone, which was able to reach a maximum of 7.9-35.4% PC. Carbofuran was only able to reach a maximum of 3.3-3.4% PC when tested up to the highest concentration possible based on cytotoxicity. Because the RPC_{Max} < PC_{10} in both assay runs, carbofuran was considered negative for estrogen receptor transcriptional activation in this test system.

**Fish Short Term Reproduction Assay (FSTRA); (OCSPP 890.1350)**

The 21-day short-term reproduction assay (MRID 48615305) of carbofuran technical with fathead minnows (*Pimephales promelas*) was conducted under flow-through conditions. Six-month old fish in 16 spawning groups (2 males and 4 females in each group; four groups per treatment) were exposed to carbofuran (99.4% purity) at nominal concentrations of 0 (control), 0.042, 0.14, and 0.45 mg a.i./L; mean-measured concentrations were <LOQ (<0.0142), 0.0437, 0.135, and 0.435 mg a.i./L. The test system was maintained at 24.4 to 25.0°C and a pH of 7.75 to 8.41.

There were no significant differences in either male or female survival at any treatment level compared to the negative control (p>0.05). However, there were 3 male mortalities in the high treatment concentration (0.435 mg a.i./L) and 1 male mortality each in the control and middle treatment concentration. It was not stated in the study report whether there were notable observations that occurred with abnormal behavior, or other clinical signs of toxicity.

In the control group, spawning frequency was at least every 4 days, fecundity averaged 42 eggs/female/day (range 34-54), and fertilization success averaged 94% (range 92-94%). Fecundity was significantly (p<0.05; Jonckheere-Terpstra) reduced by 55% relative to the control at the 0.435 mg a.i./L level. Fertilization success was significantly lower with 11 and 21% differences from control in the 0.135 and 0.435 mg a.i./L treatments, respectively (p<0.05; Jonckheere-Terpstra).

There were no effects in the gonadal somatic index (GSI) of male or female fish at any treatment concentration. Female body weight was significantly lower in all treatment concentrations, with differences ranging from 4-12% compared to the negative control (p<0.05; Jonckheere-Terpstra). Male body weight was significantly lower 0.135, and 0.435 mg a.i./L, with differences of 14% and 9% versus controls, respectively (p<0.05; Jonckheere-Terpstra). Length was not measured in this study.
Male median nuptial tubercle scores were 34, 30, and 30 in the 0.0437, 0.135, and 0.435 mg a.i./L treatment levels, compared to 39 in controls (Jonckheere-Terpstra; p<0.05). No tubercles were noted for females. Plasma vitellogenin (VTG) was not significantly different (p>0.05) in male or female fish at any treatment level; although high variability was observed in male VTG levels (%CVs 145-200%), with a 478% increase in the low treatment group and a 94 and 99% decrease in the middle and high treatment groups. The high variability was due to plasma VTG concentrations in one fish each of the negative control and 0.042 mg a.i./L treatment group being substantially higher than in the other fish. Plasma sex steroids were not measured.

There were no significant findings on gonadal histopathology observed in male or female fish at any treatment level. There were two findings that were present in the testes of multiple treated male fish but not in those of control fish males, and these were interstitial fibrosis (minimal to mild) and increased testicular degeneration (minimal). However, in both cases, the prevalence and severity was low and there was no apparent concentration-response relationship. Two other findings in male fish were germinal epithelium atrophy (severe) in a one male at the low treatment concentration and the presence of testicular oocytes (minimal) in two control males. Because the atrophy was indistinguishable from a germinal epithelium that never formed at all and because the finding only occurred in one fish in the low concentration group, this finding is not considered to be treatment-related.

There were no findings in carbofuran-treated females that were substantially more prevalent or severe than in control females. Microsporidian infections (minimal) were observed in both control and treated females and were spatially associated with granulomatous inflammation (minimal), although the reverse was not always true. According to the study authors, there was no evidence that these minor infections and associated inflammatory response had any effect on the study outcome. Increased oocyte atresia and ooplasm dysgenesis were present in comparable numbers of the control and treated females. Although secondary sex characteristics were evaluated at study termination (including body color, coloration pattern and body shape), results were not reported.

**Hershberger Assay; (OCSPP 890.1400)**

In a Hershberger assay (MRID 48615306) testing for androgenic activity, carbofuran (99.4% a.i., lot # PL11-0245) was administered daily in corn oil via oral gavage to groups of 55- to 67-day old castrated male Sprague Dawley rats (6/group) at dose levels of 0 (vehicle), 0.03, 0.1, or 0.3 mg/kg/day for 10 consecutive days. The study included an androgenic positive control group of six castrated male rats dosed daily with testosterone propionate (TP) at 0.2 mg/kg/day via subcutaneous (s.c.) injection. The vehicle control and 0.3 mg/kg/day carbofuran dose groups each included 10 extra rats to assess effects of carbofuran on red blood cell (RBC) and whole brain cholinesterase (ChE) activity.
To screen for possible anti-androgenic activity, three additional groups of 55- to 67-day old castrated male Sprague Dawley rats (6/group) were dosed daily via oral gavage with carbofuran (99.4% a.i., lot # PL11-0245) in corn oil at 0.03, 0.1, or 0.3 mg/kg/day for 10 consecutive days in conjunction with a daily s.c. injection of TP at 0.2 mg/kg/day. The control group consisted of six castrated male rats dosed daily with TP (0.2 mg/kg/day) by s.c. injection, and the positive control group consisted of six castrated male rats dosed orally with flutamide (FT) in corn oil at 3 mg/kg/day for 10 days in conjunction with a daily s.c. dose of TP (0.2 mg/kg/day).

Animals in the main groups were euthanized approximately 24 hours after the final dose administration. The five androgen-dependent tissues and other selected organs were weighed and macroscopically examined. Food consumption and serum hormone concentrations were not measured. Five of the extra rats from the vehicle control and 0.3 mg/kg/day carbofuran dose groups were sacrificed approximately 45 minutes post-dose on Study Days 0 or 9, and blood and brain samples were collected for analysis of ChE activity.

No treatment-related effects were observed on mortality, clinical signs of toxicity, body weight, body weight gain, or on weights of adrenals, kidneys or liver in either the agonist or antagonist parts of the assay. There were also no treatment-related effects on the weights of any of the accessory sex organs of animals dosed with carbofuran in the agonist or antagonist assay. The positive controls elicited the expected responses. In the agonist assay, animals in the positive control group (TP) had the following increases (p<0.01) in weights of androgen-dependent tissues compared to the vehicle controls: 381% in seminal vesicles; 638% in ventral prostate; 188% in the levator ani/bulbocavernosus (LABC) muscle complex; 541% in Cowper’s gland; and 52% in glans penis. In the anti-androgen assay, animals in the positive control group (TP + FT) exhibited the following decreases (p<0.01) in weights of androgen-dependent tissues compared to the TP-dosed control: 72% in seminal vesicles; 75% in ventral prostate; 52% in LABC; 67% in Cowper’s gland; and 22% in glans penis.

The %CV values for the androgen-dependent tissues in each dose group fell within the performance criteria provided in the Guidelines, with the exception of the %CV for the glans penis (19%) in the TP + FT group, which slightly exceeded the performance criteria maximum of 17%.

In the animals examined for ChE activity, no treatment-related effects were observed on mortality, clinical signs of toxicity, or brain weight. At 0.3 mg/kg/day on Study Days 0 and 9, ChE activity was significantly (p<0.01) inhibited in RBC by 87.3% and 45.0%, respectively, and in brain by 40.4% and 32.1%, indicating that doses examined in this study were adequate.
No statistically significant changes were seen in the weights of the five androgen sensitive tissues. Carbofuran was negative for androgenicity and anti-androgenicity in the Hershberger assay.

**Female Pubertal Assay; (OCSPP 890.1450)**

In a Female Pubertal Assay (MRID 48669802), 15 Crl:CD(SD) Sprague-Dawley rats/dose group were treated daily via oral gavage with carbofuran technical (99.4% a.i., Lot # PL11-0245) in corn oil at doses of 0, 0.03, 0.1 or 0.3 mg/kg/day from post-natal day (PND) 22 to 42. Animals were examined for vaginal opening (VO) daily beginning on PND 22, and age and weight at day of attainment of VO was recorded. Following sacrifice on PND 42, blood was collected for clinical chemistry analyses, including total thyroxine (T₄) and thyroid stimulating hormone (TSH), which were analyzed using an electrochemiluminescent immunoassays or radioimmunoassays, respectively. Kidney, liver, adrenal gland, thyroid, pituitary, uterus, and ovary weights were recorded and microscopic examination were performed on the thyroid, kidney, ovary, and uterus. An additional 10 female rats/group were used in the control and high-dose groups for analysis of blood and brain cholinesterase (ChE) activity at 45 minutes after dosing on PND 22 and 42 (5 rats/sampling date).

No treatment-related effect was observed on survival, clinical signs, body weight, body weight gain, age at attainment of VO, body weight at VO, or gross pathology. No treatment-related effect was noted on estrous cycle status at necropsy, mean age at first estrus, mean cycle length, or percent cycling. Serum T₄ and TSH levels were not affected by treatment. No treatment-related microscopic lesions were observed on the thyroid, ovary, uterus, or kidney.

Unadjusted adrenal gland weight at 0.3 mg/kg/day was decreased (p<0.05) by 11.7% compared to the controls. Unadjusted wet and blotted uterus weights at 0.3 mg/kg/day were decreased by 11.1 and 13.5%, respectively (not significant, NS). Organ weights adjusted for body weight in the treated groups were similar to controls. Additionally, the apparent effect on the uterus may have been due to fewer females in the 0.3 mg/kg/day group being in the estrus phase compared to the control group at necropsy. It was stated in that these changes were considered to be within the range of normal individual biological variation rather than due to test substance administration because only a few individual animal values were outside the range of the control group. No correlating histologic lesions were noted in the uterus. No other effects on organ weights were reported.

The mean bile acid level in the 0.3 mg/kg/day group was increased (p<0.01) by 70.2% compared to the controls. This difference was primarily due to 2 individual outliers that were markedly high (3- to 5-fold higher than the mean control group value). At 0.3 mg/kg/day, ChE levels were
decreased (p<0.01) on Days 22 and 42 in the red blood cells (59-60% inhibition) and brain tissue samples (29-44% inhibition) compared to the controls. The cholinesterase data indicate that 0.3 mg/kg/day was an adequate high dose.

**Male Pubertal Assay; (OCSPP 890.1500)**

In a Male Pubertal Assay (MRID 48669803), 15 Crl:CD(SD) Sprague-Dawley rats/dose group were treated daily via oral gavage (2.5 mL/kg) with carbofuran technical (99.4% a.i., Lot # PL11-0245) in corn oil at doses of 0, 0.03, 0.1 or 0.3 mg/kg/day from post-natal day (PND) 23 to 53. Animals were examined for preputial separation (PPS) daily beginning on PND 30, and age and weight at day of attainment was recorded. Following sacrifice on PND 53, blood was collected for clinical chemistry analyses, including total thyroxine (T4), testosterone, thyroid stimulating hormone (TSH). Testosterone and T4 were analyzed using an electrochemiluminescent assay and TSH levels were analyzed using radioimmunoassay. Liver, adrenals, pituitary, and urogenital organ weights were recorded, and microscopic examinations were performed on the thyroid, kidney, testis and epididymis. An additional 10 male rats/group were used in the control and high-dose groups for analysis of blood and brain cholinesterase (ChE) activity at 45 minutes after dosing on PND 23 and 53 (5 rats/sampling date).

No treatment-related effects were observed on mortality, body weights, body weight gain, clinical chemistry, organ weights, or gross pathology. Age and body weight at PPS in the treated groups were similar to controls. Thyroid hormone levels, colloid area, and follicular cell height scores were similar in the treated groups to the control group. Serum testosterone levels were similar in the treated groups to the control group.

At 0.3 mg/kg/day, one animal was observed with tremors on PND 34 and clear material around the mouth. These signs are characteristic of cholinesterase inhibitors, such as carbofuran, and are considered treatment-related. As there was only a single incidence of tremors in 454 observations, this effect was not considered adverse. The clinical signs of wet yellow material in the ventral abdominal area, clear/red material around the mouth, and scabbing around the mouth were observed during the study; however, the occurrence was infrequent, involved 3 or less animals (usually only 1), and/or a dose-response relationship was not apparent.

At 0.3 mg/kg/day, one male was observed with renal carcinoma; however, there were no preneoplastic or benign neoplastic changes in the kidneys of other animals in the study. One 0.1 mg/kg/day male was observed with moderate sperm granuloma and moderate hypospermatia in the right epididymis, mild degeneration of seminiferous tubules, and hypospermatogenesis in the right testis. The report stated that sperm granulomas are encountered with some frequency in the epididymides of laboratory rats; therefore, the epididymal lesion was considered to be an
incidental finding. There were no treatment-related findings in the testes, epididymides, or kidneys.

At 0.3 mg/kg/day, the ChE levels were decreased (p<0.01) on Days 23 and 53 in the red blood cells (62-70% inhibition) and brain tissue samples (41-56% inhibition) compared to the controls. The cholinesterase data indicated that the high dose was adequate for testing.

**Steroidogenesis Assay; (OCSPP 890.1550)**

In a steroidogenesis assay (MRID 48615307), H295R cells cultured in vitro in 24-well plates were incubated with carbofuran (99.4% purity, Batch # PL11-0245) at concentrations of 100, 10, 1, 0.1, 0.01, 0.001, and 0.0001 µM for 48 hours in triplicate in four independent experiments. Run 1 was not analyzed because one of the forskolin concentrations on the quality control plate was incorrect (3.3 µM was used instead of 1 µM). Dimethyl sulfoxide (DMSO) was used as the vehicle, at a final concentration of 0.05%.

Testosterone and estradiol levels were measured using HPLC-MS/MS. A Quality Control (QC) plate was run concurrently with each independent run of a test chemical plate to demonstrate that the assay responded properly to positive control agents at two concentration levels. Positive controls included a known inhibitor (prochloraz) and inducer (forskolin) of estradiol and testosterone production.

All guideline acceptability requirements were met, including lack of cytotoxicity, adequate production of testosterone and estradiol, acceptable reproducibility (low %CV), and appropriate induction and inhibition with positive controls.

Testosterone concentration was not affected by carbofuran. A decrease (p≤0.05) of 8% in testosterone concentration was observed in a single run at 100 µM of carbofuran, but a statistically significant decrease did not occur in the other two runs in which minor decreases of 6 and 11% were observed. Conversely, estradiol concentration was increased (p≤0.05) in all three runs by 45–55% at 100 µM. Therefore, a 1.45 to 1.55-fold increase of estradiol was observed at the highest concentration tested in all three assays. No effect was observed on testosterone or estradiol at lower concentrations.

Based on the hormone responses in each of the three independent runs, carbofuran treatment resulted in statistically significant and reproducible increases in estradiol production, but carbofuran treatment did not result in statistically significant and reproducible alterations in testosterone production.
In a uterotrophic assay (MRID 48615308) conducted to screen for potential estrogentic activity, carbofuran (99.4% a.i., lot # PL11-0245) in corn oil was administered daily via oral gavage to groups of 6 ovariectomized female Sprague-Dawley rats at dose levels of 0 (vehicle), 0.03, 0.1, and 0.3 mg/kg/day on post-natal days (PND) 73-75. A positive control group was treated with 17α-ethylnyl estradiol (EE) by daily subcutaneous injection at a dose level of 0.003 mg/kg/day. To also assess effects on cholinesterase (ChE), an additional 10 ovariectomized female Sprague-Dawley rats were dosed at 0 (vehicle) and 0.3 mg/kg/day on PND 70-72. All main study animals were terminated and necropsied approximately 24 hours after the final dose on PND 76-78 to determine wet and blotted uterine weights. The ChE activity in red blood cells (RBC) and brain of control and 0.3 mg/kg/day carbofuran dosed rats was determined from samples collected from 5 rats/group on Days 1 or 3, approximately 45 minutes after dosing.

All animals survived until scheduled termination. No clinical signs of toxicity were observed in animals from any carbofuran treated groups. Body weights and body weight gains in the carbofuran treated groups were comparable to the controls throughout the study, and uterine weights in the carbofuran treated groups were comparable to the vehicle control. Absolute wet and blotted uterus weights for the EE group were increased (p<0.05) by 834% and 253%, respectively, as expected. At 0.3 mg/kg/day, the ChE levels were decreased (p<0.01) in the RBC (69-73% inhibition) and brain (43-44% inhibition) compared to the controls, indicating that the dose levels tested were adequate.

No statistically significant changes were seen in uterine weights in this assay. Carbofuran was negative in the uterotrophic assay.
APPENDIX 2: Other Scientifically Relevant Information (OSRI)

Published literature and Part 158 studies considered relevant for determining the potential interaction of carbofuran with the endocrine system are summarized below. The only Part 158 studies and/or published literature articles discussed in this appendix are those that fulfill current guideline requirements or are directly relevant to assessing potential endocrine effects (i.e. two generation rodent reproductive studies, development studies, chronic toxicity studies, etc.).

ER /AR Activation

In a published, non-Guideline study (Kojima et al., 2004), the potential of 200 chemicals (including carbofuran, purity 95-100%) to interact with two human ER subtypes (hERα and hERβ) and one human androgen receptor (hAR) in transactivation assays with Chinese hamster ovary cells (CHO-K1 cells) was investigated. For detection of hERα or hERβ activity, cells were transfected with pcDNAERα or pcDNAERβ, pGL3-tkERE, and pRL-SV40. For detection of hAR activity, cells were transfected with pZeoSV2AR, pIND-ARE, and pRL-SV40. Flutolanil was tested over a concentration range from 10⁻⁸ to 10⁻⁵ M, and the responses were compared to the activity of 17β-estradiol (E₂; purity >97%) or 5α-dihydrotestosterone (DHT; purity 95%) for the ER or AR assays, respectively. For measurement of antagonistic activity to hERα, hERβ and hAR, either 10⁻¹¹ or 10⁻¹⁰ M E₂ or 10⁻¹⁰ M DHT was added to the cell cultures along with the test compound, respectively. Agonistic activities were evaluated by the relative activity expressed as 20% relative effective concentration (REC₂₀), the concentration of the compound demonstrating 20% of the activity of 10⁻¹⁰ M E₂, 10⁻⁹ M E₂, or 10⁻⁹ M DHT for ERα, ERβ, and AR, respectively. Antagonistic activities were evaluated similarly by the relative activity expressed as 20% relative inhibitory concentration (RIC₂₀), the concentration of the compound showing 20% inhibition of the activity of E₂ or DHT against ERα, ERβ, and AR, respectively. Carbofuran did not show agonist or antagonist activity for ERα, ERβ, or AR in this study.

Estrogen-mediated Proliferation

In a published non-guideline study, Soto et al, 1995, using the E-SCREEN assay assessed the potential estrogenicity of environmental chemicals using the proliferative effect of estrogen and estrogenic chemical on and estrogen-sensitive target cell line. This quantitative in vitro assay compares the cell number achieved by similar inocula of human breast cancer estrogen-sensitive (MCF-7) cells in the absence of estrogens (negative control), and in the presence of 17β-estradiol (E₂, positive control) and a range of concentrations of chemicals suspected to be estrogenic. The assay was developed based on the premise that a human serum-borne molecule specifically inhibits the proliferation of human estrogen sensitive cells and estrogens or estrogen-like chemicals induce cell proliferation by blocking this inhibitory effect. The aim of the work was to: 1) validate the E-SCREEN Assay, 2) screen a variety of chemicals, 3) examine whether the effects of different chemicals act cumulatively and 4) assess the reliability of the assay for use as a screening tool.
Proliferation rates are measured by comparing the cell yield achieved by similar inocula harvested simultaneously during the late exponential phase of proliferation. The proliferative effect is measured as the ratio between the highest cell yield obtained with the test chemical and the hormone free control. In this experimental design, MCF-7 cell yields were measured after 6 days of exposure and differences between treatment and positive (E2) control were apparent after 4 days. Estrogenic activity was assessed by 1) relative proliferative potency (RPP) which is the ratio between the minimal concentration of estradiol needed for maximum cell yield and the minimum dose of the test compound needed to achieve a similar effect, and 2) measuring the relative proliferative effect (RPE) which is 100 times the ratio between the highest cell yield obtained with the chemical and with E2l. The RPE allows one to quantitatively compare the agonist effect relative to E2. Results were expressed as mean ± SE. Proliferation yield experiments were conducted in duplicate wells with a minimum of 5 replicated. Differences between groups were assessed by analysis of variance and the a posteriori Shaffe’s test. A p value ≤ 0.05 was regarded as significant. Carbofuran was tested over a concentration range of 10^-9 to 10^-3 M and found to have no activity in the E-SCREEN Assay.

**Developmental Toxicity (Rat, OCSPP 870.3700)**

In a prenatal developmental toxicity study (MRID 00058609) groups of 25 pregnant Crl:COBS CD rats were administered carbofuran (purity not reported) in corn oil by gavage at dose levels of 0, 0.25, 0.50 or 1.20 mg/kg/day during gestation days (GD) 6 through 15. The pregnant females were euthanized on GD 20, and the pups were removed by cesarean section and weighed, sexed and examination for malformations.

All females survived until study termination. No treatment-related changes were seen in the mean number of implantations/litter, pre- or post-implantation loss, early or late resorptions, number of live fetuses/litter, fetal sex ratio, or soft tissue abnormalities. Mean fetal body length and the number of litters, and fetuses with malformations and variations were also comparable to the control group. There was a slight increase in developmental variations occurring at the mid-dose group, consisting of unossified sternabrae or ribs, but the effect on not dose-dependent. The maternal and developmental NOAELs are 1.2 mg/kg/day (highest dose tested).

In another prenatal developmental toxicity study (MRID 00058611), groups of 40 pregnant Crl:COBS CD rats were administered carbofuran (purity not reported) in the diet at concentrations of 0, 20, 60 or 160 ppm during GD 6 through 19. These dietary levels were equivalent to 0, 1, 3 and 8 mg/kg/day, respectively. On GD 20, at least 20 females from each group were sacrificed, and their uterus removed weighed and examined prior to examining the fetuses. The remaining females in each group were allowed to deliver normally, and the dams and pups were terminated on lactation Day (LD) 21. The dams were weighed daily during gestation, and dams and pups were observed daily of clinical signs of toxicity. On GD 20, fetuses were weighed, sexed, measured for crown to rump length, examined for external
malformations and variations, and prepared for skeletal examinations. For the dams that were allowed to deliver, the litter size, pup sex, number of still births and live births, and pup weights were determined on LD 0. Pup weights were also recorded on LD 4, 7, 14 and 21, and dam weights were recorded on LD 0, 7, 14 and 21. At termination on LD 21, the remaining pups were examined for gross external malformations and variation, and for skeletal malformations.

All females survived until study termination, and treatment-related signs of clinical toxicity were not observed in the dams or pups. During gestation, mean weights and weight gains for dams in the 20 ppm group were comparable to the control, but mean body weight and body weight gains were dose-dependently reduced in the 60 and 160 ppm groups (magnitude not reported in review). Dam body weights were also significantly reduced during the first week of lactation in the 160 ppm group. During treatment, food consumption in the 20 and 60 ppm groups was comparable to the control, but food consumption was slightly reduced in the 160 ppm group.

On GD 20, no significant changes were seen in the mean number of corpora lutea, total implantations, pre- or post-implantation loss, early or late resorptions, number fetuses/litter or fetal sex ratio. Fetal body weights and fetal malformations and variations were also comparable to the control group. For delivered pups, no treatment-related differences were observed in pup sex ratio or body length, and pup weights in the 20 ppm group were comparable to the control. However, pup body weights were slightly reduced (NS) in the 60 ppm group, and were significantly reduced in the 160 ppm group. On PND 21, malformations and variations in the treated pups were generally comparable to the control group. Carbofuran was not teratogenic at dietary concentrations up to 160 ppm (8 mg/kg/day). The LOAEL for offspring toxicity is 60 ppm (3 mg/kg/day), based on reduced pup body weights, and the NOAEL is 20 ppm (1 mg/kg/day).

**Developmental Toxicity (Rabbit, OCSPP 870.3700)**

In a developmental toxicity study (MRID 00076762), groups of 5 New Zealand White rabbits were exposed to carbofuran (purity not reported) in 0.5% aqueous Methocel via gavage at dose levels of 0, 0.12, 0.50 or 2.0 mg/kg/day on GD 6 through 18. The does were sacrifice on GD 29 and subjected to cesarean sections for examinations of the uterus and fetuses.

All does in the control, low- and mid-dose groups survived until study termination, and one doe in the high dose group died on GD 11, though the cause of death could not be determined. Maternal body weight gains were comparable in the control, low- and mid-dose groups. In the high dose group, body weight gain was reduced by 20% during treatment, but weight gains were comparable to the control thereafter. Matting and/or staining of the anogenital fur was noted in all groups during gestation, although the duration of the effect was longer at the high dose. Three does (one in each treatment group) aborted near the end of the gestation period.
No treatment-related changes were seen in the mean number of corpora lutea, implantations/litter, pre- or post-implantation loss, early or late resorptions, number of live fetuses/litter, fetal sex ratio, or soft tissue abnormalities. The maternal LOAEL was 2 mg/kg/day based on reduce body weight gains, and the NOAEL was 0.5 mg/kg/day. The developmental NOAEL was 2.0 mg/kg/day (highest dose tested).

**Developmental neurotoxicity (Rat; OCSPP 870.6300)**

In a developmental neurotoxicity study (MRID 43378101), groups of 24 impregnated Crl:CD (SD)BR rats (51 days old) were fed diets containing 0, 20, 75, or 300 ppm (1.70-1.73, 4.95-6.91, or 8.57-31.38 mg/kg/day) of carbofuran (99.1% purity) from GD 6 through PND 10. Dams were observed for clinical signs of toxicity and mortality daily, and body weight and food consumption were recorded weekly throughout gestation and lactation.

Pups were observed twice daily for mortality and morbidity, and were weighed on PND 0, 4, 11, 17 and 21. Litters were culled to 4/pups/sex on PND 4, and the remaining pups were necropsied. The following indices were calculated for each group: Mean pup live birth index (PND 0), pup viability index (PND 4; prior to culling), and the pup weaning index (PND 21). The selected pups were evaluated for pinna detachment, incisor eruption, eye opening, VO, and PPS. Motor activity, auditory startle response and swimming, learning and memory evaluations were measured on one pup/sex/litter. Other selected pups were sacrificed on PND 11 and 60, for measurement of body and brain weights. Six pups/sex/dose group were also sacrificed on PND 11 and 60 for microscopic evaluation the brain, spinal cord, sciatic nerve, and skeletal muscle nerves. ChE activity not measured.

No maternal deaths or abortions were noted, and there were no treatment-related signs of clinical toxicity in the dams. The length of gestation was similar between treated and control groups. Dam body weights were decreased (p<0.01) in the high-dose group by 17, 10 and 10% on GDs 10, 15 and 20, compared to the control, and body weight gains were reduced (p<0.01) in the mid and high-dose groups from GD 6-10, and in the high-dose group from GD 10-15 and 15-20. Food consumption in the mid and high-dose groups was reduced (p<0.05 or <0.01) from GD 6-10, 10-15, and 15-20.

At the mid and high dose, there was increased pup mortality during the first 4 days of lactation resulting in decreased (p<0.01) viability indices (83.4 and 33.8%, respectively) compared to the control (98.5%). Live birth indices were comparable between control (97.7%) and treated groups (91.9-99.4%), and although the mid and high dose groups had lower weaning indices (72.9 and 69.6%, respectively) than controls (87%), the differences were not significant. In the mid and high dose groups, pup body weight was decreased (p<0.01) at birth by 6 and 16%, respectively, and during the entire lactation period from 6-25% and 16-38%, respectively. The developmental landmarks for pinna detachment, lower incisor eruption and eye opening were
delayed (p<0.01) in all dose groups, but were generally not dose-dependent. However, the delays noted in VO and PPS were dose-dependent. In the mid- and high-dose groups VO was delayed (p<0.01) by 0.9 and 3.0 days, respectively, and PPS was delayed (p<0.01) by 3.0 and 3.9 days, respectively. Although not reported by sex and not always significant, pup body weights during early development (PND 28-49) were decreased by 12-16% at the mid dose and 21-37% at the high dose, indicating that pup body weight may have impacted VO and PPS.

No treatment-related effect was seen on mean motor activity or auditory startle values, but treatment-related effects were seen in the mid and high-dose groups on learning and memory in the water “y” maze time trails.

At PND 11, there were decreases (p<0.01 or <0.05) in both body and brain weights in the mid-dose group (↓23 and 12%, respectively) and high-dose group (↓43 and 25%, respectively). At PND 60, only body weights were significantly decreased in the mid-dose (↓15%) and high-dose (↓17%) groups. There were no treatment-related gross necropsy findings or histopathological findings in pups on PND 11 or 60.

The maternal LOAEL was 75 ppm (6 mg/kg/day) based on decreased body weight gain and decreased food consumption during GD 6-10. The maternal toxicity NOAEL was 20 ppm (1.7 mg/kg/day).

The LOAEL for developmental toxicity is 75 ppm (6 mg/kg/day) based on increased pup mortality, decreased pup body weight, and delayed PPS and VO. However, the Agency notes that the delay and VO and PPS were likely attributable to decreased body weight gains. The NOAEL for developmental neurotoxicity in pups was 20 ppm (1.7 mg/kg/day).

### Three-Generation Reproduction in Rodents (Rat, OCSPP 870.3800)

In a 3-generation reproduction study in rats (MRID 00030514), Crl:CD BR rats were exposed to carbofuran (95.6% purity) in the diet at 0, 20 or 100 ppm through 3 generations (F0, F1, and F2); these dietary levels were equivalent to overall doses of 0, 1 and 5 mg/kg/day, respectively. Parental animals were mated at a 1:2 ratio (male:female), and each treatment group consisted for either 10 males and 20 females (F0 and F1 parents) or 12 males and 24 females (F2 parents). Parental animals were bred twice, beginning at PND 100 to produce the following litters in each generation: F1a, F1b, F2a, F2b, F3a and F3b. In the F1 and F2 generations, litters were reduced to 10 pups/litter of equal sex ratio if possible. Parental animals for breeding of the F1 and F2 generations were selected from the second mating of the F0 and F1 generations, respectively. Parents and pups were observed daily for mortality, clinical signs of toxicity, and general behavior and appearance. Individual body weights and food consumption of parental animals were recorded on a weekly basis, and female body weights were determined on GD 0, 7, 14 and 21, and LD 0, 4, 14 and 21. Each generation was evaluated for male and female fertility,
duration of gestation, litter size, number of pups/sex/litter. The growth, viability and survival of pups were evaluated through weaning, and pups weights were determined by litter on LD 0, 4, and 14, and individual by sex on LD 21. Pups not utilized as parents were sacrificed at weaning (LD 21), and parental animals were sacrificed following weaning of the second litter. At sacrifice, animals were weighed and examined for pathological lesions or abnormalities, and selected organs were weighed, including the liver, kidneys, heart, spleen, adrenals, thyroid, testes and ovaries.

No treatment-related changes were noted in mortality, clinical signs or behavior of parents or pups in any generation. For the parental animals, body weights and food consumption in the 20 ppm group were comparable to the control, but body weights and food consumption values for the 100 ppm group (including during gestation and lactation) were consistently lower than the control (magnitude not reported) and the body weight differences attained significance in each generation.

No treatment-related effects were observed on male or female mating indices or fertility, gestation index or duration, number of pups/litter, or pup sex ratio. Pup viability and mean body weights in the 20 ppm group were similar to the control. However, pup viability at LD 4 was slightly reduced in each generation at 100 ppm, and litter weights were lower on LD 0, 4, 14 and 21 at 100 ppm. Combined male and female pup weights at 100 ppm were significantly lower than the control in each generation. Significant (p<0.05 or 0.01) increases and/or decreases in absolute and/or relative organ weights were noted in males and/or females from the F2 parents and F3b weaning rats. However, these organ weight changes were not consistent or necessarily dose-dependent, with exception of the spleen and liver weights which were significantly decreased in males and females in the 100 ppm group. No treatment-related lesions or abnormalities were noted in any treatment group at necropsy.

The parental and reproductive LOAEL is 100 ppm (5 mg/kg/day), based on reduced body weight and food consumption and NOAEL is 20 ppm (1 mg/kg/day).

**Chronic Toxicity/Carcinogenicity (Rat, OCSPP 870.4300)**

In a combined chronic toxicity/carcinogenicity study (MRID 00043745), groups of 90 Crl:CD BR rats/sex were fed diets containing carbofuran (95.6% purity) at levels of 0, 10, 20 or 100 ppm for up to 104 weeks; these dietary levels are approximately equivalent to doses of 0, 0.5, 1.0 or 5 mg/kg/day. Ten rats/sex from each group were randomly allocated for interim sacrifices at 6, 12 and 18 months, and the surviving animals were sacrificed at 24 months. Rats were observed daily for mortality and clinical signs of toxicity. Body weights and food consumption were determined weekly, and ophthalmoscopic examinations were performed on all rats at 12 and 24 months. Hematology, clinical chemistry analyses (including ChE) and urinalysis were conducted using 10 rats/sex/group at 6, 12, 18 and 24 months. All animals that died or were
sacrificed were subjected to a necropsy and examined for gross and microscopic pathological lesions. The following organs were weighed at necropsy: spleen, liver, kidneys, testes, ovaries, heart, brain, lungs, adrenals, thyroid and pituitary glands. Histopathological examinations were conducted on organs/tissues, including the pituitary, thyroid, adrenals, liver, kidneys, testes with epididymides, prostate, seminal vesicles, ovaries, uterus and mammary glands.

There were no treatment-related effects on mortality to clinical signs of toxicity. Food consumption was similar between the control and treated groups, and mean body weights for both sexes in the low- and mid-dose groups were comparable to the control. However, body weights were reduced in the high dose group, with the reductions attaining significant for the high-dose males at most intervals. At 18 and 24 months, body weights in the high-dose group were decreased by 11 and 5%, respectively, in males and by 4 and 9% in females. Ophthalmoscopic examinations shown no treatment-related changes, and hematological and urinalysis parameters were similar between treated and control groups. Clinical chemistry parameters were also similar between the treated and control groups, with the exception of ChE levels. Although plasma, RBC and brain ChE levels were similar between the control and low- and mid-dose groups, ChE activity as inhibited in all three compartments in high-dose males and females, attaining significance (p<0.01 or <0.05) at most sampling intervals. At 12, 18 and 24 months, ChE activity was decreased compared to the control in high-dose males by 25-37% in plasma, 18-24% in RBC, and 11-25% in brain. At the same intervals, ChE activity was decreased in high-dose females by 10-26% in plasma, 11-19% in RBC, and 18-43% in brain. Compared to the control, significant (p<0.01 or <0.05) changes in various tissues/organ weights were observed in males and females from all three dose groups at all four sampling intervals. However, the weight changes were not consistent in their direction (increase/decrease) or in terms of their occurrence in sex, time or organ, and were not dose-dependent. In particular, no treatment-related changes were seen in absolute or relative weights of the testes, ovaries, thyroid, adrenal or pituitary glands at any dose level. In addition, no treatment-related, histopathological changes were noted in the occurrence of neoplastic and non-neoplastic lesions in any organs/tissues, including the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, mammary, thyroid, adrenal or pituitary glands.

Carbofuran did not have oncogenic potential in the chronic rat study; and the LOAEL for systemic effects and ChE inhibition is 100 ppm (5 mg/kg/day) based on ChE inhibition and decreased body weight. The NOAEL is 20 ppm (1 mg/kg/day).

**Oncogenicity (Mouse, OCSPP 870.4200)**

In a mouse carcinogenicity study (MRID 00030512), groups of 100 CD-1 mice/sex were fed diets containing carbofuran (95.6% purity) at levels of 0, 20, 125 or 500 ppm for up to 104 weeks; these dietary levels are approximately equivalent to doses of 0, 2.9, 7 and 71 mg/kg/day. Ten mice/sex from each group were randomly allocated for interim sacrifices at 6, 12 and 18
months, and the surviving animals were sacrificed at 24 months. Mice were observed 2-3 times daily for mortality, morbidity and clinical signs of toxicity, and body weights and food consumption were determined weekly. Hematology, clinical chemistry analyses (including ChE) and urinalysis were conducted at 6, 12, 18 and 24 months using 5 mice/sex/group for urinalysis at each interval, and 5 mice/sex/group for clinical chemistry and hematology. All animals that died or were sacrificed were subjected to a necropsy and examined for gross and microscopic pathological lesions. The following organs were weighed at necropsy: heart, lungs, spleen, liver, kidneys, testes, ovaries, brain, adrenals, thyroid/parathyroids and pituitary gland. Histopathological examinations were conducted on organs/tissues, including the pituitary, thyroid, adrenals, liver, kidneys, testes, epididymides, prostate, seminal vesicles, ovaries, uterus and mammary glands.

There were no treatment-related effects on mortality to clinical signs of toxicity. Mean body weights for both sexes in the low- and mid-dose groups were comparable to the control. Body weights for the high-dose group were generally lower than the control through the first 78 weeks, but significance was attained on only 5 times for males and four times for females. By Week 104, mean body weights were reduced by only 5% (NS) in high-dose males and females. Hematological and urinalysis parameters were similar between treated and control groups. Clinical chemistry parameters were also similar between the treated and control groups, with the exception of ChE levels. Brain ChE levels in males and females from the mid- and high-dose groups were significant lower (magnitude not specified) than the control at all sampling intervals. Compared to the control, significant changes in various tissues/organ weights were observed at the different, but the changes were determined to be not biological significant. In particular, no treatment-related changes were seen in absolute or relative weights of the testes, ovaries, thyroid, adrenal or pituitary glands at any dose level. In addition, no treatment-related, histopathological changes were noted in the occurrence of neoplastic and non-neoplastic lesions in any organs/tissues, including the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, mammary, thyroid, adrenal or pituitary glands at any dose level.

Carbofuran did not have oncogenic potential in the mouse carcinogenicity study; and the systemic LOAEL is 500 ppm (71 mg/kg/day) based on body weight and food consumption decreases, and the NOAEL is 125 ppm (18 mg/kg/day). For ChE inhibition, the LOAEL is 125 ppm (18 mg/kg/day) and the NOAEL is 20 ppm (2.9 mg/kg/day).

**Chronic Toxicity in Non-Rodents (Dog, OCSPP 870.4100)**

In a chronic dog toxicity study (MRID 00129507), groups of 6- to 8-month old Beagle dogs (6/sex/group) were fed diets containing carbofuran (96.1% purity) at levels of 0, 10, 20 or 500 ppm for up to 52 weeks, equivalent to doses of 0, 0.5, 1.0 and 12.5 mg/kg/day, respectively. The dogs were observed daily for mortality, morbidity and clinical signs of toxicity. Individual body weights and food consumption were determined weekly, and ophthalmoscopic examinations
were performed at 6 and 12 months. Routine hematology and clinical chemistry analyses were conducted every month (including ChE levels in plasma and RBC), and urinalyses were conducted every two months. At study termination, ChE levels in brain were also determined. All animals that died or were sacrificed were subjected to a necropsy and examined for gross and microscopic pathological lesions. Routine organ weights were determined at necropsy, including liver, kidneys, testes, ovaries, adrenals, thyroid and pituitary glands. Histopathological examinations were conducted on all organs/tissues, including the pituitary, thyroid, adrenals, liver, kidneys, testes, epididymides, prostate, ovaries, uterus and mammary glands. Due to treatment-related malnutrition in the high-dose group, the high-dose animals were supplemented intermittently with control diet being at Week 29.

No signs of clinical toxicity were reported. One male in the high-dose group died during Week 28; the death was determined to be treatment-related as findings at necropsy were consistent with metabolite disease due to carbofuran exposure. Food consumption was similar between the control and treated groups, with the exception of the high-dose group on Week 44. Food consumption on this week was decreased (p<0.01 or <0.05) in both high-dose males (↓36%) and females (↓41%). Mean body weights for both sexes in the low- and mid-dose groups were comparable to the control. However, body weights were reduced in the high dose group compared to the control from Week 10 until study termination. Compared to the control, mean body weights from Week 10 through 52 were decrease in high-dose males by 18-52% (p<0.01 or <0.05) and in high-dose females by 7-21% (NS). Over the course of the study, mean body weight gain was negative (p<0.01) for both high-dose males (-0.9 kg) and females (-0.4 kg). Urinalysis parameters were similar between treated and control groups, and clinical chemistry parameters were also similar between the control and low- and mid-dose groups and the high-dose females. Most significant changes in clinical chemistry were observed in the high-dose males at 12 months and included reductions in total protein (↓18%), calcium (↓14%) and sodium (↓7%). Plasma ChE levels at the low-dose and for mid-dose females were similar to the control, except for initial decreases (↓18-19%) in low-dose males during the first month of treatment. Plasma ChE levels were decreased (p<0.01) by 24-31% in mid-dose males, 78-87% in high-dose males and 77-84% in high-dose females. Cholinesterase levels in the RBC of all treated groups were similar to the control, except in high-dose males at 6 months (↓27%, p<0.01). No significant reductions in brain ChE were noted in any dose group, although brain ChE levels were decreased by 24% (NS) in high-dose males. Hematology also indicated anemia in the high-dose males beginning at Month 5; hematocrit, hemoglobin and total erythrocytes were reduced by 20-22% (p<0.01) at study termination.

At study termination, the only significant changes noted in organ weights were decreases (p<0.05) in absolute heart (↓15%) and brain (↓15%) weights in high-dose males; however, relative (to body) heart and brain weights were not significantly reduced. In addition, no treatment-related changes were seen in absolute or relative weights of the testes, ovaries, thyroid,
adrenal or pituitary glands at any dose level. Gross examination at necropsy revealed the lack of body fat in 2/5 high-dose males and alopecia in 1/5 high-dose males and 1/6 high-dose females, which were considered treatment-related. Microscopic finding considered to be treatment-related included: (i) increased incidence of hepatocellular cytoplasmic atypia (unspecified) with a centrilobular pattern of distribution in 9/12 dogs at the low-dose, 7/12 at the mid-dose and 6/11 at the high dose (vs. 2/12 in control); (ii) lung inflammation in 3/12 dogs at the low-dose, 1/12 at the mid-dose, and 7/11 at the high dose (vs. 0/12 in control); (iii) degeneration and giant cell formation in seminiferous tubules and aspermia in the testes of 1/6 males in the low- and mid-dose groups and 4/5 males at the high dose (vs. 0/6 control), and (iv) uterine hyperplasia and hydrometria in 3/6 low-dose females, 2/6 mid-dose females, and 1/6 high-dose females (vs. 0/6 in control). Although possibly treatment related, the biological significance of the changes noted in the uterus is questionable as it was not dose-dependent. In addition, the effects noted on testes at the high dose are likely secondary to the poor nutritional status and severe systemic toxicity of carbofuran in high-dose males (decreased body weight and anemia). No other treatment-related, histopathological changes were noted in any organs/tissues, including the epididymides, prostate, ovaries, mammary, thyroid, adrenal or pituitary glands.

The systemic LOAEL is 500 ppm (12.5mg/kg/day; LDT) based on toxic signs, decreased body weight and food consumption in both sexes, anemia in males, decreased absolute brain and heart weight in males, and lung inflammation in 5/5 males and 2/6 females; the NOAEL is 20 ppm (0.5 mg/kg/day). The LOAEL for ChE is 20 ppm (0.5 mg/kg/day) based on plasma ChE inhibition.

**Avian Reproduction Toxicity (Quail, OCSPP 850.2300)**

Avian reproduction studies were performed for carbofuran on two species: mallard duck and northern bobwhite quail. Results of these tests showed parental mortality at all the concentrations tested, with LOAEL of 2.0 ppm in the mallard study (MRID 00129500) and 15.0 ppm for the northern bobwhite study (MRID 00129501). The studies did not establish a NOAEC when the mallards were exposed to the lower concentration. The critical observed effect was adult survival at all exposure levels tested.

For the mallard study, onset of mortality was observed between days 8 and 15 for all exposure levels and continued for the duration of the study. Some birds that died during the period before egg-laying were replaced. This replacement resulted in different exposure durations and different exposure levels, complicating the evaluation of the results. The initial starting number for the four treatment levels (controls, 2.0 ppm, 5.0 ppm, and 10.0 ppm) was 35 birds. At 2.0 ppm, 8 birds were replaced. At 5.0 ppm, 6 birds were replaced and at 10.0 ppm, 11 birds were replaced. This resulted in a total of 43 birds exposed at 2.0 ppm, 41 at 5.0 ppm, and 46 at 10.0 ppm. By the end of the period before egg production (12 weeks), 16 of the 43 birds at 2.0 ppm were dead, 18 of the 41 birds at 5.0 ppm were dead, and 26 of the 46 at 10.0 ppm were dead. By
the end of the study, 24 of the 43 birds exposed to 2.0 ppm carbofuran had died (56%). A total of 31 of 41 birds exposed to 5.0 ppm had died (75%), and 43 of the 46 birds exposed to 10.0 ppm had died (93%).

Similar results were observed in the northern bobwhite study, however the lowest test concentration in this study was 15 ppm. There were effects on mortality (14% of birds dead) in both the middle and high (53 and 180 ppm) treatment groups. Clinical signs of toxicity included subdued behavior, unsteadiness, and ruffled appearance. Bodyweights of adult birds (both male and female) at the highest treatment group were significantly lower than controls.

Because of the mortality that occurred in these studies, reproductive physiology or developmental impairment, if it occurred in the tests, was obscured. While the total number of eggs produced in all treatment groups was significantly lower than in the control group, the authors concluded, after mortality levels were taken into account, that there did not appear to be any dose response with regard to egg production in surviving female birds. Further, there were no reported marked differences between treatments in overall development and hatching of eggs laid or the survivability of the ducklings.

The overall egg production of the treatment groups was significantly reduced in comparison to controls. The net effect of the parental mortality was a proportionate reduction in egg production or survivability of offspring. Substantially fewer eggs were produced and the number of surviving ducklings reduced. However, in the absence of a NOAEC for either parental mortality, or reproductive physiology or developmental impairment, the level at which avian survival would not be adversely effected from chronic exposure has not been established, and these studies do not provide insight into carbofuran’s potential effects on avian reproduction. However, the studies do show that chronic exposure to carbofuran has the potential to substantially reduce avian survival at levels that can occur following carbofuran applications.

**Fish Early Life Stage Toxicity (Rainbow trout, OCSPP 850.1400)**

In a fish early life stage toxicity study (MRID 00126862, groups of Rainbow trout (*Oncorhynchus mykiss*) embryos (~20 day old) were exposed to carbofuran (99.6% purity) at nominal concentrations of 5, 9, 19, 38 or 75 μg/L from egg stage up to 90 days post-hatch (101-day exposure) in a flow-through system. The study included a negative control group (dilution water) and solvent control group (acetone, percent no reported). Mean measured concentrations of carbofuran were <0.3, 7.16, 12.3, 24.8, 56.7 and 88.7 μg/L. Each group consisted of 200 embryos (50 embryos/replicate), and the fry were reduced to 40 per treatment (10/replicate) on exposure Day 21. The embryos were evaluated for percent hatch (Day 14) and time to hatch, and the fry were evaluated for clinical signs, survival, and growth. Fry body length was determined at 30 and 60 days post-hatch, and body length and wet weight were determined at 75 and 90 days post-hatch (101 day exposure).
Hatchability of rainbow trout eggs was not significantly lower in treated groups (96-99%) relative to controls (96%). Fry exposed to 56.7 µg/L exhibited rapid respiration by Day 20 (larval stage) and scoliosis by Day 90, and both symptoms continued until test termination. Fry exposed to 88.7 µg/L exhibited the same symptoms, in addition to excitability by Day 20 through to test termination.

Fry survival in the treated groups was not affected at 30 days post-hatch, but survival was significantly reduce at concentrations of 56.7 and 88.7 µg/L at 60, 75 and 90 days post-hatch. Body length at 30 days post-hatch was significantly reduced in the 88.7 µg/L group, compared the control, and body length was also significantly reduced in the 56.7 and 88.7 ug/L groups at 60, 75 and 90 days post-hatch. Body weight was significantly reduced at 56.7 and 88.7 ug/L at 75 days, but only in fish at 88.7 ug/L by 90 days post-hatch. The overall LOAEC and NOAEC were 56.7 and 24.8 µg/L, based on decreased survival and growth of fry.

**Fish Early Life Stage Toxicity (Sheepshead minnow, OCSPP 850.1400)**

In a fish early life stage toxicity study (MRID 40818401), groups of Sheepshead minnow (Cyprinodon variegatus) embryos (<48 h old) were exposed to carbofuran (96.9% purity) at nominal concentrations of 17.6, 43.4, 68.0, 120.0, and 256.0 µg a.i/L. A negative control group and solvent control (20 µl/L DMF) were included in the study. Mean measured concentrations were not provided. The embryos were evaluated for percent hatch and time to hatch, and fry were evaluated for clinical signs, and survival and growth (length and wet weight) at study termination (35 days).

Hatching of sheepshead minnow embryos was significantly (p<0.05) reduced at test concentrations ≥68.0 µg a.i/L. Mortality of juvenile fish was significantly (p<0.05) increased at test concentrations ≥120.0 µg a.i/L. Sheepshead minnow exposed to concentrations of carbofuran of ≥17.6 µg a.i/L (the lowest concentration tested) was significantly (p<0.05) smaller than the solvent control fish on both a length and wet weight basis. Difference in growth between the negative control and the solvent control were attributed to the presence of the solvent as an organic substrate for microorganisms which were utilized by the solvent control fish as a diet supplemental. The LOAEC for this study was 17.6 µg a.i/L and the NOAEC was < 17.6 µg a.i/L based on significant reductions of length, and wet weight at the lowest treatment concentration.

In a fish early life stage toxicity study (MRID 43250501), groups of Sheepshead minnows (Cyprinodon variegatus) embryos (<24 h old) were exposed to carbofuran (98% purity) at nominal concentrations of 3.8, 7.7,16, 32, or 64 µg/L from egg stage to 35 days post-hatch in a flow-through system. A negative control group (dilution water) and solvent control group (0.1 mL DMF/L) were included in the study. Mean measured concentrations were <0.80, 2.6, 6.0, 16, 26 and 51 µg/L µg/L. Groups consisted of 200 embryos/treatment (40 embryos/replicate; 20
per cage). The embryos were evaluated for percent hatch and time to hatch, and fry were evaluated for clinical signs, and survival and growth (length and wet weight) at study termination (35 days).

No embryos hatched in the 51 µg/L group, and the percent hatch was reduced to 91, 24 and 14% for the 6.0, 16, and 26 µg/L groups, respectively, compared to 100% in the controls. Time to hatch was 5 days at 6.0 µg/L and 8 days at 16 and 26 µg/L, compared to 3 days for controls. Day 35 survival was reduced to 19 and 9% in the 16 and 26 µg/L groups. No significant differences were noted in body length and weight at 35 post-hatch, and no sub-lethal effects were observed. The overall LOAEC and NOEAC were 6.0 and 2.6 µg/L, respectively, based on percent hatch.
APPENDIX 3: References Not Utilized in the Carbofuran WoE Analysis

In 2009, after public review and comment, a final list of 67 chemicals and schedule for issuing Test Orders for the EDSP Tier 1 screening battery was made available in a Federal Register Notice issued October 21, 2009 (74 FR 54422). The agency’s review of the initial data submitted as “other scientifically relevant information (OSRI) was provided in the Report of the Endocrine Disruptor Review Team (USEPA, 2010).

Beginning in 2011, the agency has reviewed data cited as “OSRI which included Part 158 studies previously submitted to the agency for registration/reregistration, published literature articles and/or Tier 1 assays. The agency also conducted a more recent search (2009 to 2014) of available scientific literature for any additional relevant information for their weight of evidence (WoE) evaluations. These articles were evaluated in accordance with the agencies Evaluation Guidelines for Ecological Toxicity Data in Open Literature, May 2011 (http://www.epa.gov/pesticides/science/efed/policy_guidance/team_authors/endangered_species_reregistration_workgroup/PDF_rot/esa_evaluation_open_literature.pdf) and the 2012 Guidance for considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (http://www.epa.gov/pesticides/science/lit-studies.pdf).

The following published and unpublished references were considered for use in the WoE analysis for Carbofuran but were not utilized due to one or more of the following reasons: 1) the article was not available in English; 2) the compound of interest was not used in the study; 3) the test material was not adequately described; 4) a formulated end-use product or mixture of chemicals was utilized as the test material; 5) only acute mortality toxicity data were provided; 6) the experimental conditions were not adequately described; 7) only an abstract of the study was available; 8) the reference is a review article or book chapter and does not contain primary study data; 9) insufficient information was available to adequately assess the validity of the study results; 10) the 40 CFR Part 158 guideline study was classified as unacceptable/inadequate; 11) the study dealt only with non-EDSP assay development; 12) no specific endocrine-related endpoints were assessed in the study; and 13) the study contained only data on invertebrates.


Bio-efficacy of some insecticides for the management of early shoot borer, *Chilo infescatellus* (Snellen) in Sugarcane

Arun K Choudhary, Pawan K Amrate and Animesh Chatterjee

**Abstract**

Two year (2015-16 and 2016-17) field experiment was conducted in Randomized block design with eight treatments (seven insecticidal treatments and untreated control) and three replications to know the bio-efficacy of some insecticides for the management of early shoot bore, *Chilo infescatellus* (Snellen) in Sugarcane. All the insecticidal treatments recorded significantly less ESB infestation (3.02 to 10.62 %); more NMC (77.62 to 81.17 thousand/ha) and cane yield (87.58 to 98.38 t/ha) as compared to the untreated control (17.05%, 74.69 thousand/ha and 81.25 t/ha, respectively). The chlorantraniliprole 0.4 % GR 22.5 kg/ha followed by fipronil 0.3% GR @ 25 kg/ha both at planting and 60 days after planting found to be the statistically at par and best as they recorded least ESB infestation (3.02 and 3.22 %, respectively) as well as maximum NMC (81.17 and 80.25 thousand/ha) and cane yield (98.38 and 96.84 t/ha). The flubendiamide 39.35% SC @ 125 ml/ha and chlorantraniliorile 18.5% SC @ 375 ml/ha both at 30 and 60 days after planting were statistically at par with each other and the next better treatment in reducing the Early shoot infestation, NMC and cane yield.

**Keywords:** bio-efficacy, insecticides, chlorantraniliprole, fipronil, early shoot borer, sugarcane

**Introduction**

Sugarcane (*Saccharum officinarum* L.) is also termed as “Wonder cane” have versatile utility and vast capability to grow in almost all agro-ecological situations, except the extreme ecological conditions. It chiefly utilized in manufacturing of white sugar, bio-fuels, ethanol and cogeneration of electricity and played an important role in socio economical development in rural India. It occupied about 2.57 per cent (5 M ha.) of total cropped area, contributes nearly 10 per cent of agricultural GDP and provides lively hood to about 6 million growers (Pathak *et al* 2017) [1]. The crop is affected significantly by biotic factors, it is estimated that losses incurred to be in the tune of 20 per cent in cane yield and 15 per cent in sugar recovery. Although pests are more devastating in subtropics, they often reach serious levels in tropics necessitating development of strategies to maintain them below threshold levels (Ramaraju, 2017) [3]. Early shoot borer, *Chilo infescatellus* *Snellen* is key pest of sugarcane, distributed all sugarcane growing areas of India. It infests the crop during early growth phase from February to June. If its infestation occur during germination stage, kills the mother shoot resulting drying of whole plant creating gap in the field, while if the attack coincide with the tillering phase the clump does not killed, but the crop stand affected due to mortality of tillers. It is estimated that it can kill mother shoots up to 26 to 95 per cent, primary/secondary tillers up to 6.4 to 27 per cent and tertiary tillers up to 75.0 per cent. Considering the importance of pest and capacity to incurred losses, an experiment was conducted to study the bio-efficacy of some insecticides to have a chemical, which effectively reduces the early shoot borer in sugarcane.

**Material and Methods**

The bio-efficacy of some insecticides were tested consecutively for two years 2015-16 and 2016-17 against early shoot bore, *Chilo infescatellus* (Snellen) of sugarcane at All India Coordinated Research Project on Sugarcane, Zonal Agricultural Research Station, Powarkheda, Hoshangabad (MP). The trial encompassed eight treatments i.e., soil application of fipronil 0.3% GR @ 25 kg/ha, chlorantraniliprole 0.4 % GR 22.5 kg/ha, phorate 10% CG @ 15 kg/ha, carbafuran 3% CG @ 33 kg/ha, all at planting and 60 days after planting; spraying of
chlorantraniliprole 18.5% SC @ 375 ml/ha, spinosad 45% SC @ 90 ml/ha and flubendiamide 39.35% M/M SC @ 125 ml/ha at 30 and 60 days after planting and untreated control. Sugarcane variety namely CoC 671 was planted and grown with all recommended packages and practices except plant protection measures. The treatments were replicated thrice in plots measured 6.0 x 5.4 m. with a inter row space of 90 cm. The granular insecticides applied at planting and 60 days after planting, while the spray of insecticides (SC formulation) was done at 30 and 60 days after planting. The observations on germination at 45 days after planting, the dead heart counts/meter at 30, 60, 90, and 120 DAP, the diameter (mm), height (cm) and number of millable canes at full grown stage and cane yield were recorded at harvest. The per cent germination, per cent infestation at 30, 60 90 120 DAP and cumulative per cent infestation, NMC/ha, cane yield t/ha calculated. The data so obtained were subjected to randomized block design statistical procedure and presented in table1 and figure 1.

Results and Discussion
The perusal of pooled data for 2015-16 and 2016-17 (table no. 1) revealed that initially at 30 days after planting (DAP) the per cent infestation of ESB is less (0.79 to 2.77%) in treatments of granular application at planting and 60 DAP as compared to other (7.95 to 8.67%). At 60, 90 and 120 DAP; soil application of chlorantraniliprole 0.4 G @ 22.5 kg /ha and fipronil 0.3 G @ 25 kg /ha both at 0 and 60 DAP were the best and significantly at par in reducing the ESB infestation, followed by spray flubendiamide 39.35 % SC @ 125 ml/ha and chlorantraniliprole 18.5 SC 375 ml/ha both at 30 and 60 DAP, while the soil application of phorate 10 G @ 15 kg/ha at 0 and 60 DAP, spray of spinosad 45 SC @ 90 ml/ha at 30 and 60 DAP and soil application of carbofuran 3 G @ 33 kg/ha at planting and 60 DAP were intermediate in this respect. As per the cumulative per cent infestation of ESB (overall effect), all the insecticidal treatments significantly reduced the ESB infestation (3.02 to 10.62 %) as compared to the untreated control (17.05%). The soil application of chlorantraniliprole 0.4 G @ 22.5 kg /ha (3.02%) followed by soil application of fipronil 0.3 G @ 25 kg /ha both at planting and 60 DAP (3.22%) found to be the significantly best in reducing the ESB infestation and were significantly at par with each other. The spray of flubendiamide 39.35 % SC @ 125 ml/ha at 0 and 60 DAP and spray of chlorantraniliprole 18.5 SC 375 ml/ha both at 30 and 60 DAP (5.33%) were significantly at par with each other and the next better treatment in reducing the ESB. Although, the soil application of phorate 10 G @ 15 kg/ha at 0 and 60 DAP (7.79%) was significantly inferior to the previous treatments but was significantly superior to the spray of spinosad 45 SC @ 90 ml/ha at 30 and 60 DAP (10.10%) and soil application of carbofuran 3 G @ 33 kg/ha at 0 and 60 DAP (10.62%). In different treatments, the cane diameter (mm) and cane height (cm) ranged in between 25.47 to 26.40 mm and 201.90 to 206.67 cm respectively and differed non-significantly. The insecticidal treatments showed slight numerical superiority in this respect over untreated control. The insecticidal treatments recorded significantly more NMC (77.62 to 80.25 thousand/ha) and cane yield (87.58 to 98.38 t/ha) as compared to untreated control (74.69 thousand/ha and 81.25 t/ha, respectively). The soil application of chlorantraniliprole 0.4 G @ 22.5 kg /ha (81.17) followed by soil application of fipronil 0.3 G @ 25 kg /ha both at planting and 60 DAP (80.25) registered significantly maximum NMC, both were significantly at par with each other. The spray of chlorantraniliprole 18.5 SC 375 ml/ha (78.71), spray of flubendiamide 39.35 % SC @ 125 ml/ha both at 30 and 60 DAP (78.40) and soil application of phorate 10 G @ 15 kg/ha at planting and 60 DAP (78.24) were next better performing treatments in this respect; all were significantly at par with each other. While the spray of spinosad 45 SC @ 90 ml/ha at 30 and 60 DAP (77.63) and soil application of carbofuran 3 G @ 33 kg/ha at planting and 60 DAP (77.62) were significantly at par with each other and to the prior three also. In respect of cane yield, the treatments showed the same trend and significances as in NMC. The soil application of chlorantraniliprole 0.4 G @ 22.5 kg /ha (98.38 t/ha) followed by soil application of fipronil 0.3 G @ 25 kg /ha both at 0 and 60 DAP (96.84 t/ha) recorded significantly the maximum cane yield, while the spray of chlorantraniliprole 18.5 SC 375 ml/ha (93.36 t/ha), spray of flubendiamide 39.35 % SC @ 125 ml/ha both at 30 and 60 DAP (93.06 t/ha) and soil application of phorate 10 G @ 15 kg/ha at planting and 60 DAP (90.05 t/ha) were intermediate in respect of cane yield recorded. Our results are in confirmation with the findings of Bhavani et al (2017) [4], Badgajar (2017) [5] and Bhavani et al. (2017) [4], who found that the soil application of chlorantraniliprole 0.4 G @ 22.5 kg /ha at 0 and 60 DAP is best in reducing the ESB infestation and increasing the cane yield in sugarcane. Similarly as ours, the Bhavani et al. (2017) [4] also postulated that fipronil 0.3 G @ 25 kg /ha at 0 and 60 DAP is next better insecticidal treatment in controlling the ESB and increasing the cane yield in sugarcane, while Mann et al. (2009) [7] found the fipronil was highly toxic against Chilo infuscatellus as it reduces the dead hearts by 65%. Sardana (2001 also found fipronil 0.3 G as most effective insecticide in reducing the borer pest in sugarcane and increasing the yield.

Table 1: Bio-efficacy of some insecticides for the effective management of early shoot borer, Chilo infuscatellus Snellen in Sugarcane, Powarkheda (M.P.) (pooled of 2015-16 & 2016-17)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatments</th>
<th>ESB (%)</th>
<th>Diameter (cm)</th>
<th>Height (cm)</th>
<th>NMC (000/ha)</th>
<th>Cane Yield (t/ha)</th>
<th>Per cent increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Germin. (%)</td>
<td>30 DAP</td>
<td>60 DAP</td>
<td>90 DAP</td>
<td>120 DAP</td>
<td>Cumulative</td>
</tr>
<tr>
<td>T1</td>
<td>Soil Application of fipronil 0.3 G @ 25 kg /ha at the time of planting and 60 DAP</td>
<td>76.30</td>
<td>0.87</td>
<td>1.75</td>
<td>1.44</td>
<td>1.46</td>
<td>3.22</td>
</tr>
<tr>
<td>T2</td>
<td>Soil Application of Chlorantraniliprole 0.4 G @ 22.5 kg /ha at the time of planting and 60 DAP</td>
<td>76.69</td>
<td>0.79</td>
<td>1.59</td>
<td>1.30</td>
<td>1.45</td>
<td>3.02</td>
</tr>
<tr>
<td>T3</td>
<td>Spray of Chlorantraniliprole 18.5 SC 375 ml/ha at 30 and 60 DAP</td>
<td>75.52</td>
<td>8.67</td>
<td>2.18</td>
<td>2.13</td>
<td>1.68</td>
<td>5.53</td>
</tr>
<tr>
<td>T4</td>
<td>Spray of spinosad 45 SC @ 90 ml/ha at 30 and 60 DAP</td>
<td>79.17</td>
<td>7.95</td>
<td>3.82</td>
<td>4.57</td>
<td>4.61</td>
<td>10.10</td>
</tr>
<tr>
<td>T5</td>
<td>Spray of flubendiamide 39.35 % SC @</td>
<td>76.82</td>
<td>8.19</td>
<td>3.73</td>
<td>1.38</td>
<td>1.53</td>
<td>5.33</td>
</tr>
<tr>
<td></td>
<td>125 ml/ha at 30 and 60 DAP (50 g a.i./ha)</td>
<td>250 ml/ha at 30 and 60 DAP (100 g a.i./ha)</td>
<td>500 ml/ha at 30 and 60 DAP (200 g a.i./ha)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td>---------------------------------</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>T6 Soil Application of phorate 10 G @ 15 kg/ha at the time of planting and 60 DAP</td>
<td>77.54</td>
<td>1.74</td>
<td>3.46</td>
<td>2.73</td>
<td>4.54</td>
<td>7.79</td>
<td>25.95</td>
</tr>
<tr>
<td>T7 Soil Application of carbofuran 3 G @ 33 kg/ha at the time of planting and 60 DAP</td>
<td>76.30</td>
<td>2.77</td>
<td>5.00</td>
<td>5.45</td>
<td>5.05</td>
<td>10.62</td>
<td>25.74</td>
</tr>
<tr>
<td>T8 Un-treated control</td>
<td>74.74</td>
<td>8.63</td>
<td>10.11</td>
<td>10.34</td>
<td>6.70</td>
<td>17.05</td>
<td>25.47</td>
</tr>
<tr>
<td>S Em ±</td>
<td>1.22</td>
<td>0.34</td>
<td>0.17</td>
<td>0.14</td>
<td>0.13</td>
<td>0.18</td>
<td>0.31</td>
</tr>
<tr>
<td>CD (p=0.05)</td>
<td>NS</td>
<td>0.98</td>
<td>0.49</td>
<td>0.41</td>
<td>0.38</td>
<td>0.52</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Fig 1:** Bio-efficacy of new insecticides for the control of sugarcane early shoot borer, chilo infuscatus Snellen, Powarkheda (M.P)

**Conclusion**
This can be concluded that the soil application of chlorantraniliprole 0.4 G @ 22.5 kg /ha or fipronil 0.3 G @ 25 kg /ha at planting and 60 DAP can recommend for the effective management of ESB in sugarcane.

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5. Badgugar MP, Chaudhari PM, Ajotikar MV, Solanke AV. “Evaluation of new chemical molecules against sugarcane early shoot bore (Chilo infuscatus Snellen),” in Proceedings International Symposium on “Sugarcane research since Co 2016: 100 years and beyond (SucroSym 2017), at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, 2017, 374-376.


Carbofuran-Induced Endocrine Disruption in Adult Male Rats

Ryan T. Goad, John T. Goad, Bassam H. Atieh & Ramesh C. Gupta

Pages 233-239 | Published online: 19 Oct 2008

Abstract

The objective of this investigation was to determine the acute toxic effects of the carbamate insecticide carbofuran on the levels of endocrine hormones in the serum of male Sprague-Dawley rats. Using chemiluminescent immunoassay, the hormones determined were progesterone, cortisol, estradiol, testosterone, triiodothyronine (T3), total thyroxine (total T4), and non-protein-bound thyroxine (free T4). Rats exposed to an acute dose of carbofuran (1.5 mg/kg, s.c.) showed the onset of cholinergic signs (salivation, chewing, and fine tremors) within 5–7 min. With increasing intensity, toxic signs of maximal severity (severe convulsions and fasciculations) were observed within 30–60 min, and lasted for about 2 to 3 h. Time courses of hormones for 24 h revealed significant alterations in hormone levels during 0.5 to 3 h, with the exception of estradiol at 6 h. The levels of progesterone, cortisol, and estradiol were significantly increased (1279%, 202%, and 150%, respectively), while the levels of testosterone were decreased by 88%. No significant change occurred in thyroid hormones (T3, total T4, and free T4) at any time during the time course, despite the fact that body temperature was significantly low at 1 to 2 h after carbofuran injection. Carbofuran caused a >2-fold increase in glucose during early hours of toxicity. The results suggest that an acute exposure to carbofuran may cause transient endocrine disruption, which may consequently lead to serious reproductive problems following repeated exposure.
### Search active substances

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EVALUATION OF DIFFERENT INSECTICIDES AGAINST RICE STEM BORER AND RICE LEAF FOLDER

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EVALUATION OF DIFFERENT INSECTICIDES AGAINST RICE STEM BORER AND RICE LEAF FOLDER

Waqas Wakil, Mansoor-ul-Hasan, Rizwan Akbar & Assam Gulzar
Department of Agri. Entomology, University of Agriculture, Faisalabad

To achieve the objective of effective control over insect pests of rice and enhancing its yield economically, a study was undertaken to evaluate different sprayable and granular insecticides for the control of rice stem borer and leaf folder on the rice variety Basmati-385 by the application of 5 insecticides. Of the granular insecticides, furadan application proved to be the best both in controlling the attack of stem borer and leaf folder as well as better yield per acre, while among sprayable insecticides, nurelle-D proved better for the control of these pests. The furadan application gave the maximum yield (1527 kg acre⁻¹) with cost benefit ratio of 1:6.67. In sprayables, nurelle-D gave the maximum yield (1263 kg acre⁻¹) with cost benefit ratio of 1:5.99. The monetary benefit with granulars and sprayables comes to Rs. 8250.66 and Rs. 5874.66 respectively, the difference being significantly high.

INTRODUCTION

Among other factors, low yields of rice in Pakistan due to damages by insect pests are the major constraints. About 128 species of insects have been reported attacking the rice crop. Of these, 15 to 20 insect species are known to be the pests of paramount importance and are regularly noticed in tropical Asia. Rice stem borers play havoc with rice crop every year. Leaf and plant hoppers have also attained the status of regular pests of this crop throughout the rice belt of the Punjab province (Majid et al., 1979). Recent addition of the rie leaf folder, Cnaphalocrocis medinalis (Gn.) to the list also poses a threat to economic production of rice in the Punjab. Damage due to rice leaf folder may sometimes go as high as 60% (Kushwaha and Singh, 1984). For the control of the insect pests of rice, the insecticides like ekalux, kilvil, lannate, padan and diazinon have been tried and recommended by Panda and Shi (1989), Khan and Khaliq (1989), Mustafa et al., (1990), Mustafa and Razzaq (1991), Biswas and Mandal (1992), Prasad et al., (1995), Sharma and Singh (1995), Singh et al., (1995 a, b) during the last two decades. The present studies have been undertaken to evaluate the efficacy of lorsban, decis, nurelle-D, thimet and furadan with a view to find out the efficacy of new entrants and economic control of rice insect pests.

MATERIALS AND METHODS

The experiment was laid out following randomised complete block design having six treatments and three repeats each. The plot size was 33' x 13'. The detail of treatments is as under:

<table>
<thead>
<tr>
<th>Treatment Code</th>
<th>Insecticide</th>
<th>Application Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Lorsban 40 EC (chlordimetion)</td>
<td>@ 500 ml/acre</td>
</tr>
<tr>
<td>T2</td>
<td>Decis-D 12.5 + 300 EC (deltamethrin + dimethoate)</td>
<td>@ 500 ml/acre</td>
</tr>
<tr>
<td>T3</td>
<td>Nurelle-D 505 EC (cypermethrin + chlorpyrifos)</td>
<td>@ 500 ml/acre</td>
</tr>
<tr>
<td>T4</td>
<td>Thimet 5 CT (phorate)</td>
<td>@ 9 kg/acre</td>
</tr>
<tr>
<td>T5</td>
<td>Furadan 3 CT (carbofuran)</td>
<td>@ 9 kg/acre</td>
</tr>
<tr>
<td>T6</td>
<td>Untreated control</td>
<td></td>
</tr>
</tbody>
</table>

Population density of insect pest complex was recorded for deciding the appropriate time of insecticide application. The insecticides were applied twice. The first application of insecticides was made after 45 days of transplanting of nursery and 2nd 35 days after 1st application. The observations on percent infestation of stem borer (dead heart (OH) and white head (WH) and folded leaves were recorded 168 hr after each application.

RESULTS AND DISCUSSION

It is apparent from Table 1 that the results with various treatments were significantly different from the untreated check, which exhibited the highest damage (1.713% OH, 3.033% WH and 3.627% folded leaves). Application of furadan gave the best results where the pest infestation was significantly the lowest (0.22% OH, 0.10% WH and 0.31% folded leaves) and the yield significantly the highest (1908 g/100 hills) of all the treatments. It was followed by nurelle-D which was significantly better than lornban, decis-D and thimet. Thimet proved statistically similar to lornban and decis-D with respect to OH. WH percentage...
and percentage of folded leaves and yield components were significantly higher under thimet than lornsan and decis-D. These results are similar to those of the findings of Khan and Khaliq (1989), Mustafa et al. (1990), Mustafa and Razzaq (1991), Prasad et al. (1995) and Singh et al. (1995a, b).

Paddy yield as a result of treatment with lornsan, decis-D, nurelle-D, thimet and furadan was 904.0, 823.7, 1262.6, 1048.0 and 1526.6 kg/acre respectively, against 609.86 kg/acre in case of control which showed an increase in yield of 294.14, 213.84, 652.74, 438.14 and 916.74 kg/acre respectively over control. Maximum yield (1908 g/100 hills) was obtained in furadan treated plots which was significantly different from all other treatments having the yield of 1578, 1310, 1130, 1030 and 762.3 g/100 hills respectively in case of nurelle-D, thimet, lornsan, decis-D and control. These findings are similar to those of Khan and Khaliq (1989), Prasad et al. (1995) and Singh et al. (1995a).

The statistics of correlation matrix revealed negative correlation between dead heart/white head/leaf folder and yield with coefficient values of -0.846, -0.807 and 0.752 respectively.

### Table 1. Comparison of mean percentage infestation of stem borer and leaf folder and yield

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dead hearts (kg)</th>
<th>White head (kg)</th>
<th>Folded leaves (kg)</th>
<th>Yield (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Lornsan</td>
<td>0.883 b</td>
<td>1.270 b</td>
<td>1.603 c</td>
<td>1130 d</td>
</tr>
<tr>
<td>T2 Decis-D</td>
<td>0.790 b</td>
<td>1.063 b</td>
<td>1.610 c</td>
<td>1030 d</td>
</tr>
<tr>
<td>T3 Nurelle-D</td>
<td>0.310 c</td>
<td>0.586 c</td>
<td>0.893 d</td>
<td>1578 b</td>
</tr>
<tr>
<td>T4 Thimet</td>
<td>0.803 b</td>
<td>1.143 b</td>
<td>2.677 b</td>
<td>1310 c</td>
</tr>
<tr>
<td>T5 Furadan</td>
<td>0.223 d</td>
<td>0.103 d</td>
<td>0.323 c</td>
<td>1908 a</td>
</tr>
<tr>
<td>T6 Control</td>
<td>1.713 a</td>
<td>3.033 a</td>
<td>3.627 a</td>
<td>762.3 c</td>
</tr>
</tbody>
</table>

### Table 2. A correlation matrix between dead hearts, white head, leaf folder and yield

<table>
<thead>
<tr>
<th>Characters</th>
<th>Dead hearts</th>
<th>White head</th>
<th>Folded leaves</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dead hearts</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. White heads</td>
<td>0.967</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Folded leaf</td>
<td>0.918</td>
<td>0.887</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>4. Yield</td>
<td>-0.846</td>
<td>-0.807</td>
<td>-0.752</td>
<td>1.000</td>
</tr>
</tbody>
</table>

### Table 3. Yield increase over control, benefit ac", expenses ac" and C:B ratio in Basmati-385

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield ac&quot; (kg)</th>
<th>Increase over control (kg)</th>
<th>Benefit ac&quot; (Rs.)</th>
<th>Expenses ac&quot; (Rs.)</th>
<th>C:B ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1'1 Lornsan</td>
<td>904.0</td>
<td>294.14</td>
<td>2647.26</td>
<td>575</td>
<td>1:4.60</td>
</tr>
<tr>
<td>1'2 Decis-D</td>
<td>823.7</td>
<td>213.84</td>
<td>1924.56</td>
<td>1100</td>
<td>1:1.75</td>
</tr>
<tr>
<td>T3 Nurelle-D</td>
<td>1262.6</td>
<td>652.74</td>
<td>5874.66</td>
<td>980</td>
<td>1:5.94</td>
</tr>
<tr>
<td>T4 Thimet</td>
<td>1048.0</td>
<td>438.14</td>
<td>3943.26</td>
<td>1000</td>
<td>1:3.94</td>
</tr>
<tr>
<td>T5 Furadan</td>
<td>1526.6</td>
<td>916.74</td>
<td>8250.66</td>
<td>1237.5</td>
<td>1:6.67</td>
</tr>
<tr>
<td>T6 Control</td>
<td>609.86</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Rate of Basmati-385 (Paddy) = Rs. 9.00 kg 

Furadan has the highest cost than all other test insecticides, but it gave the maximum reduction in rice borer and leaf folder infestation with maximum cost benefit ratio 1:6.67, while out of sprayable insecticides, nurelle-D caused the maximum reduction in infestation with 1:5.94 cost benefit ratio.

### REFERENCES


HAZARD ASSESSMENT OF THE INSECTICIDE CARBOFURAN TO AQUATIC ORGANISMS IN THE SACRAMENTO RIVER SYSTEM
PREFACE

The California Department of Fish and Game (CDFG) is responsible for fish and wildlife management programs and for the protection of fish and wildlife. The CDFG protects fish and wildlife from damage caused by pesticides through consultation as a member of the mandated California Department of Pesticide Regulation (DPR) Pesticide Registration and Evaluation Committee and Pesticide Advisory Committee. Through consultation with CDFG, the Regional Water Quality Control Boards also protect fish and wildlife by promulgating and enforcing water quality standards for pesticides and other toxic materials. In recognition of the need for applicable environmental standards for fish and wildlife, DPR contracted with CDFG for the assessment of the effects of pesticides on fish and wildlife and to facilitate the development of water quality criteria which will protect fish and wildlife.

This document is the third in a series of hazard assessments for pesticides used on rice which recommends studies and conditions necessary for the protection of fish and wildlife. Hazard assessments have also been prepared for the herbicides molinate and thiobencarb and the insecticide methyl parathion.
Hazard Assessment of the Insecticide Carbofuran to Aquatic Organisms in the Sacramento River System

by

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Pesticide Investigations Unit
1701 Nimbus Road, Suite F
Rancho Cordova, California  95670

SUMMARY

An interim Water Quality Criterion (WQC) for protection of sensitive aquatic organisms from the insecticide carbofuran (Furadan®) was developed for California's Sacramento River system. The discharge of rice pesticides into the Sacramento-San Joaquin Estuary lasts for 45 to 60 days from May through June. Although focused on effects from rice tailwater in the Sacramento Valley, this assessment may be useful for other crops and environments.

Sixty-one tests on the acute and chronic effects of carbofuran to aquatic plants and animals were evaluated. Insufficient data were available to calculate a Final Acute Value (FAV) for carbofuran according to Environmental Protection Agency (EPA) procedures; data on insects and amphipods were lacking. The most sensitive species tested in acute toxicity tests was the dungeness crab Cancer magister, with a 96-h EC₅₀ value of 1.5 µg/L. Because of the data deficiency, an interim FAV, equal to the acute toxicity value of carbofuran for the dungeness crab, was proposed.

Similarly, sufficient reliable data were not available to calculate a Final Chronic Value (FCV) from either chronic values or using a Final Acute-to-Chronic Ratio (FACR). A chronic study with the cladoceran Ceriodaphnia dubia did not measure exposure levels, and a chronic study with dungeness crab used widely separated (ten-fold) exposure levels. A 28-day chronic test with the nonnative marine mysid Mysidopsis bahia did result in a No Observable Effect Concentration (NOEC) of 0.4 µg/L and a Lowest Observable Effect Concentration (LOEC) of 0.98 µg/L; however, there is no LC₅₀ value for this species. These conditions produced imprecise ACR values. Because of these concerns, an interim FCV equal to the 70-d NOEC value of 0.5 µg/L carbofuran for the dungeness crab was proposed. Thus, an interim WQC of 0.5 µg/L carbofuran is proposed.

Carbofuran exhibits additive acute toxicity with malathion and methyl parathion, two insecticides used on rice and found concurrently in agricultural drain water. Thus, an acceptable level of carbofuran in the presence of these other two insecticides may be lower than that proposed here. Additional study is needed to further characterize additive toxicity among all pesticides used on rice taking into consideration both lethal...
Concentrations of carbofuran were first measured in the agricultural drains in 1987. Maximum concentrations of carbofuran in drains have declined from 13 µg/L in 1987 to 0.6 µg/L in 1991. Concentrations of carbofuran up to 2.1 µg/L have been detected in the Sacramento River near the city of Sacramento. These monitoring data indicate that a hazard to sensitive aquatic invertebrates may have existed in the agricultural drains, especially prior to 1991. Maximum concentrations (in µg/L) of carbofuran in the Colusa Basin Drain exceeded the criterion in 1987 (13), 1988 (4.4), 1989 (1.5), 1990 (1.1) and 1991 (0.6). Similar data for the Sacramento River suggest that a hazard may have existed prior to 1988 but no or little hazard to aquatic invertebrates currently exists; maximum concentrations were 2.1 µg/L carbofuran in 1987 but <1.0 µg/L carbofuran since 1988.

The hazard assessment procedure is a reiterative process by which new data are evaluated to refine water quality criteria. A new criterion will be generated when the necessary data become available. Acceptable acute and chronic tests are needed to better define the WQC and the effects of carbofuran on the environment. Acute toxicity tests with an insect and an amphipod are required to calculate a FAV. It is also required that the chronic test with the cladoceran *Ceriodaphnia dubia* be repeated with measured exposure levels. It is recommended that a chronic test with the estuarine mysid *Neomysis mercedis* be completed because of their sensitivity to carbofuran and importance in the aquatic environment of the Sacramento-San Joaquin Estuary.
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This assessment was funded by a reimbursable contract (FG 1005) with the Department of Pesticide Regulation. We appreciate the constructive comments received from the California Department of Pesticide Regulation and the California Regional Water Quality Control Board.
INTRODUCTION

Carbofuran (Furadan®) is an insecticide registered for use in California on a variety of crops, including rice, sugar beets, alfalfa, and field corn. In a study of carbofuran use in Colusa, Glenn, and Yolo counties conducted by the California Department of Pesticide Regulation (DPR), researchers concluded that the major portion of carbofuran residues found in agricultural drain water of the Sacramento Valley probably originated from rice. This was concluded because carbofuran use on rice was much greater than on any other crop, and the volume of runoff water from rice fields was greater (Nicosia et al. 1990). Use between 1980 and 1990 has varied from approximately 30,000 to 50,000 kilograms (kg) carbofuran (active ingredient [a.i.]) on 40,000 to 80,000 hectares of rice in California (Table 1).

For the past decade there has been concern over the hazards of rice pesticides to aquatic organisms in the Sacramento-San Joaquin Estuary. The discharge of rice pesticides including carbofuran into the Sacramento-San Joaquin Estuary lasts for 45 to 60 days from May through June. Assessments of rice pesticides have identified hazards to aquatic organisms in the agricultural drains and the Sacramento-San Joaquin Estuary (Cornacchia et al. 1984; Finlayson and Faggella 1986; Harrington 1990; State Water Resources Control Board 1990, Menconi and Harrington 1992). The Central Valley Regional Water Quality Control Board (CVRWQCB) found toxicity of Colusa Basin Drain water to aquatic invertebrates in 1988 and 1989 (CVRWQCB 1988, 1989), and the California Department of Fish and Game (CDFG) found toxicity in 1990 (Finlayson et al. 1991). Norberg-King et al. (1991) identified carbofuran and methyl parathion as possible causes of toxicity to cladocerans in Colusa Basin Drain water, and Finlayson et al. (1991) identified methyl parathion toxicity in the Colusa Basin Drain water. The insecticides carbofuran, methyl parathion, and malathion used on rice have also demonstrated additive acute toxicity (Fujimura et al. 1991a).
Table 1. Carbofuran use on rice in California, 1979-1990. Data from Department of Pesticide Regulation Pesticide Use Report Database.

<table>
<thead>
<tr>
<th>Year</th>
<th>Kg a</th>
<th>Ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990b</td>
<td>28,393</td>
<td>54,002</td>
</tr>
<tr>
<td>1989</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>1988</td>
<td>26,770</td>
<td>53,418</td>
</tr>
<tr>
<td>1987</td>
<td>26,126</td>
<td>50,759</td>
</tr>
<tr>
<td>1986</td>
<td>25,728</td>
<td>41,039</td>
</tr>
<tr>
<td>1985</td>
<td>26,483</td>
<td>39,394</td>
</tr>
<tr>
<td>1984</td>
<td>40,167</td>
<td>53,821</td>
</tr>
<tr>
<td>1983</td>
<td>33,090</td>
<td>45,681</td>
</tr>
<tr>
<td>1982</td>
<td>52,490</td>
<td>80,706</td>
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<tr>
<td>1981</td>
<td>49,208</td>
<td>83,519</td>
</tr>
<tr>
<td>1980</td>
<td>37,234</td>
<td>62,458</td>
</tr>
<tr>
<td>1979</td>
<td>13,188</td>
<td>23,202</td>
</tr>
</tbody>
</table>

a active ingredient (a.i.)
b January to June 1990
c not available

The CVRWQCB (1990) developed a performance goal of 0.4 µg/L for carbofuran in rice return water. Performance goals are intended to bring surface water pesticide concentrations down to levels that approach water quality objectives. The CVRWQCB reviews new information as it becomes available and may revise performance goals (CVRWQCB 1992).

The CDFG first measured concentrations of carbofuran in the agricultural drains in 1987. From 1987 to 1990, maximum concentrations of carbofuran detected by CDFG in the Colusa Basin Drain have declined from 13 to 0.6 µg/L, and maximum concentrations in the Sacramento River at Village Marina have declined from 2.1 to 0.4 µg/L (Table 2). In 1990 and 1991, carbofuran was present in the Colusa Basin Drain for a period of more than two months, a sufficient length of time to result in chronic exposures for aquatic organisms.
The hazard assessment procedure compares measured environmental concentrations with toxic effects likely to result from those exposures. Environmental fate data including studies on hydrolysis and photodegradation in soil, water, and air; aerobic and anaerobic soil and aquatic metabolism; volatility; leaching; sorption; and uptake by plants and animals were also reviewed. These data were used to determine pesticide degradation rate, environmental transport, and potential to reach nontarget organisms.

Toxic effects of carbofuran to aquatic animals were determined by evaluating tests listed in the published literature and public and corporate laboratory reports. Sources of published literature included CDFG Pesticide Investigations Unit library, the State of California Resource Agency library, and college and university libraries. CDFG also obtained corporate laboratory reports from confidential files which were submitted to DPR in support of pesticide registration.

Available data on carbofuran were evaluated for conformance with specific criteria, outlined by Harrington (1990). Each study was screened for compliance of test methods used with procedures adapted by the EPA (1985) and the American Society of Testing and Materials (ASTM 1980, 1987a, 1987b, 1988a, 1988b, 1989). While tests did not have to comply with all requirements, tests were rejected if they did not observe certain fundamental protocols, such as maintaining proper organism survival in a control treatment, testing only with healthy, unstressed organisms, and using appropriate testing procedures. Test descriptions that did not contain sufficient information for proper evaluation were rejected if attempts to obtain the necessary data from the original researcher failed.

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Maximum concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colusa Basin Drain</td>
<td>1991</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>1990</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>1989</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>1988</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>1987</td>
<td>13.0</td>
</tr>
<tr>
<td>Sacramento River</td>
<td>1991</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td></td>
<td>1990</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>1989</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>1988</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>1987</td>
<td>2.1</td>
</tr>
</tbody>
</table>

ENVIRONMENTAL FATE

Hydrolysis is the primary breakdown mechanism for carbofuran. Carbofuran is stable in water at low and neutral pH but rate of hydrolysis increases rapidly with increasing pH. Carbofuran has a hydrolysis t₁/₂ of 35 days at pH 7.0 and 350 days at pH 6.0. Carbofuran is also degraded through photolysis and is not likely to accumulate in water exposed to sunlight. The t₁/₂ of carbofuran in sediment is between one and two months. Carbofuran does not bioaccumulate (National Research Council of Canada 1979). There have been no detectable residues of carbofuran in catfish collected from the Colusa Basin Drain during several rice growing seasons (Harrington and Lew 1992, 1989).
Based on certain physicochemical properties specified by the California Pesticide Contamination Prevention Act (Stats. 1985, Ch. 1298, Sec. 1), carbofuran is mobile in water and soil. These properties include low soil adsorption coefficient, relatively long hydrolysis and anaerobic soil metabolism half-lives, and high water solubility (DPR 1991). These properties also account for the season-long occurrence of carbofuran in agricultural drain waters containing rice tailwater (Finlayson et al. 1991).

Certain agronomic practices and conditions increase the persistence of carbofuran in soil, including soil incorporation, use of granular formulations, high soil organic matter, low soil pH, and low soil temperature and moisture (DPR 1990). Numerous waterfowl poisonings have shown that carbofuran granules applied during the spring to rice field berms or other areas that remain dry during the rice growing season can cause wildlife mortality the following fall (E.E. Littrell, CDFG, personal communication, 1992).

**ACUTE TOXICITY TO AQUATIC ANIMALS**

The EPA (1985) guidelines recommend eight categories of freshwater organisms from which data should be available for deriving a freshwater Final Acute Value (FAV), and eight categories of saltwater organisms for deriving a saltwater FAV (Table 3). The EPA (1985) document does not discuss deriving an estuarine FAV. Because the Sacramento River system includes the Sacramento-San Joaquin Estuary, the FAV must protect both fresh and saltwater species. Previous hazard assessments on the pesticides molinate and thiobencarb (Harrington 1990) and methyl parathion (Menconi and Harrington 1992) combined values for freshwater, saltwater, and estuarine organisms. The EPA freshwater and saltwater lists of recommended categories of organisms were combined into a list of nine species categories (Table 4). Although a deviation from EPA (1985) guidelines, the
combined list meets EPA taxa requirements, both freshwater and saltwater species are represented, and the combined list represents a broader spectrum of sensitivity to carbofuran.

Fifty tests on the acute toxicity of carbofuran to aquatic animals were evaluated for use in deriving the FAV using CDFG guidelines described by Harrington (1990, 1991). Twenty-eight of these tests were determined to be acceptable for use in deriving the FAV (Appendix A). Values from accepted tests are tabulated in Table A-1; values from unaccepted tests are tabulated in Table A-2. Acute toxicity values ranged from 1.5 µg/L, the 96-h EC\textsubscript{50} value for dungeness crab *Cancer magister* to >10,000 µg/L, the 48-h EC\textsubscript{50} value for eastern oyster *Crassostrea virginica*.

Acceptable acute toxicity tests were available for organisms from seven of the nine combined freshwater and saltwater species categories adapted from EPA (1985) recommendations. To fill the two remaining categories, acceptable acute tests are needed for at least one insect and either a second insect or a second species in a phylum other than Arthropoda or Chordata.

Acute toxicity values from accepted tests were ranked (Table 5). The lowest four values are the most significant determinants of the FAV (Appendix D). EPA (1985) procedures specify that the range between the highest and lowest of the four lowest Genus Mean Acute Values (GMAVs) should not be greater than a factor of 10. The GMAV for bluegill (88 µg/L) is greater than the next lowest GMAV (mysid, 3.6 µg/L) by a factor greater than 20. The filling of the existing data gaps will likely result in the range of four lowest values being within a factor of 10. Therefore, the lowest GMAV (1.5 µg/L) will be used as the interim FAV until the two data gaps have been filled.
Table 3. Eight species categories recommended by EPA (1985) for deriving freshwater and saltwater Final Acute Values (FAV).

<table>
<thead>
<tr>
<th>Freshwater FAV</th>
<th>Saltwater FAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. One Salmonid</td>
<td>1, 2. Two families in phylum Chordata</td>
</tr>
<tr>
<td>2. Another family in class Osteichthyes</td>
<td></td>
</tr>
<tr>
<td>3. Another family in phylum Chordata</td>
<td>3. One family not in Arthropoda or Chordata</td>
</tr>
<tr>
<td>4. One family not in phylum Arthropoda or Chordata</td>
<td>4, 5, 6. Three other families not in Chordata</td>
</tr>
<tr>
<td>5. One insect family or any phylum not already represented</td>
<td></td>
</tr>
<tr>
<td>6. One planktonic crustacean</td>
<td>7. Mysidae or penaeidae family</td>
</tr>
<tr>
<td>7. One benthic crustacean</td>
<td>8. One other family not already represented</td>
</tr>
<tr>
<td>8. One insect</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Nine species categories for deriving a Final Acute Value for the Sacramento-San Joaquin Estuary and corresponding animal used for carbofuran.

<table>
<thead>
<tr>
<th>Category of Species</th>
<th>Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Family Salmonidae</td>
<td>Rainbow trout</td>
</tr>
<tr>
<td>2. Another family in class Osteichthyes</td>
<td>Bluegill</td>
</tr>
</tbody>
</table>
3. Another family in phylum Chordata | Yellow perch  
4. Family not in phylum Arthropoda or Chordata | Eastern Oyster  

5. Family Mysidae or Penaeidae | Mysid  
6. One planktonic crustacean | Cladoceran  
7. Benthic crustacean | Dungeness crab  

8. An insect  
9. Another insect, or a phylum not represented  

*a acceptable test not available for this category*
Table 5. Ranked Genus Mean Acute Values (GMAV) from accepted acute toxicity tests on carbofuran.

<table>
<thead>
<tr>
<th>Rank</th>
<th>GMAV (µg/L)</th>
<th>Species</th>
</tr>
</thead>
</table>
| 1    | 1.5         | Dungeness crab$^{a,b}$  
                        Cancer magister |
| 2    | 2.6         | Cladoceran$^b$  
                        Ceriodaphnia dubia |
| 3    | 3.6$^c$     | Mysid$^{a,b}$  
                        Neomysis mercedis |
| 4    | 88          | Bluegill$^a$  
                        Lepomis macrochirus |
| 5    | 164         | Lake trout  
                        Salvelinus namaycush |
| 6    | 193$^c$     | Striped bass$^{a,b}$  
                        Morone saxatilis |
| 7    | 226$^c$     | Yellow perch  
                        Perca flavescens |
| 8    | 248$^c$     | Channel catfish$^a$  
                        Ictalurus punctatus |
| 9    | 386         | Sheepshead minnow  
                        Cyprinodon variegatus |
| 10   | 396$^c$     | Brown trout  
                        Salmo trutta |
| 11   | 536$^d$     | Genus: Oncorhynchus  
                        Rainbow trout (477)$^a$  
                        Oncorhynchus mykiss |
| 12   | 1,270       | Fathead minnow  
                        Pimephales promelas |
| 13   | $>$10,000   | Eastern oyster  
                        Crassostrea virginica |

$^a$ species occurs in Sacramento-San Joaquin Estuary.  
$^b$ derived from most sensitive life stage toxicity value(s).  
$^c$ geometric mean of values from several toxicity tests on this species.
geometric mean of values from several toxicity tests on this genus.
CHRONIC TOXICITY TO AQUATIC ANIMALS

Eleven chronic toxicity tests on carbofuran were evaluated for acceptability for use in deriving the Final Chronic Value (FCV). Values from the seven accepted tests and four unaccepted tests are tabulated in Tables B-1 and B-2, respectively.

The No Observable Effects Concentration (NOEC) values from acceptable tests ranged from 0.4 µg/L to 25.0 µg/L for 28-d exposure for Mysidopsis bahia and 90-d exposure to dungeness crab C. magister adults, respectively. The Lowest Observable Effects Concentration (LOEC) values ranged from 0.98 µg/L to 250 µg/L for M. bahia and dungeness crab adults, respectively. Maximum Acceptable Toxicant Concentration (MATC) values (NOEC X LOEC)\(^{1/2}\) ranged from 0.63 to 79 µg/L for M. bahia and dungeness crab adults, respectively.

The EPA (1985) specifies two methods for calculating a Final Chronic Value (FCV). If chronic toxicity data are available for all the categories of organisms specified for deriving the FAV, the same method used for calculating the FAV may be used for the FCV. If insufficient data are available, the FCV is obtained by dividing the FAV by a Final Acute-to-Chronic Ratio (FACR). The FACR is usually calculated as the geometric mean of ACR values from a minimum of three species, including a fish, an invertebrate, and an acutely sensitive species. Acceptable chronic toxicity tests were available for the three species categories. However, there are complete (acute and chronic values) data for only one invertebrate species, the dungeness crab. There is no acute value for the mysid M. bahia. Invertebrates are the most sensitive to carbofuran. Additionally, the NOEC and the LOEC values for the dungeness crab were separated by an order of magnitude, resulting in imprecise MATC and ACR values. The LOEC is normally only twice the NOEC.
As with other organophosphate and carbamate insecticides, the ACR values appear to increase with increasing acute values (Table 6). This is consistent with other observations on insecticides which show acute and chronic toxicity to invertebrates to be similar (Norberg-King et al. 1991). EPA (1985) procedures specify that if ACR values increase or decrease with acute toxicity values, then the FACR should be calculated as the geometric mean of the ACR values for only those species whose acute toxicity values are close (within a factor of 10) to the FAV. The ACR values from dungeness crab test results were used to estimate a FACR value of 1.5 ([0.9 x 2.4]^{1/2}). However EPA (1985) procedures recommend against using a FACR lower than two, so two was used as the FACR.

The FCV could be derived by dividing the interim FAV by the FACR, resulting in a value of 0.75 µg/L (1.5/2). However, this value is higher than the NOEC values of 0.4 µg/L for the marine mysid M. bahia and 0.5 µg/L for the dungeness crab zoeae. We propose to lower the FCV to a level demonstrated to not have an adverse effect on an important indigenous species, the dungeness crab. Therefore, in lieu of additional data, the NOEC for dungeness crab zoeae (0.5 µg/L) was used as the interim FCV.
Table 6. Acute-to-Chronic Ratio (ACR) Values for invertebrates and fish exposed to carbofuran.

<table>
<thead>
<tr>
<th>Species</th>
<th>LC$_{50}$ (µg/L)</th>
<th>MATC (µg/L)</th>
<th>Reference(s)</th>
<th>ACR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dungeness crab zoeae</td>
<td>1.5</td>
<td>1.6</td>
<td>Caldwell 1977</td>
<td>0.9$^a$</td>
</tr>
<tr>
<td>Cancer magister</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dungeness crab adults</td>
<td>190</td>
<td>79</td>
<td>Caldwell 1977</td>
<td>2.4$^a$</td>
</tr>
<tr>
<td>Cancer magister</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sheepshead minnow</td>
<td>386</td>
<td>33.6</td>
<td>Parrish et al. 1977</td>
<td>11.5</td>
</tr>
<tr>
<td>Cyprinodon variegatus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinook salmon</td>
<td>610</td>
<td>12.5</td>
<td>Faggella et al. 1990</td>
<td>48.8</td>
</tr>
<tr>
<td>Oncorhynchus tshawytscha</td>
<td></td>
<td></td>
<td>Fujimura et al. 1990</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ These ACRs were used to derive the calculated ACR
TOXICITY TO AQUATIC PLANTS

Four tests on carbofuran toxicity to aquatic plants were evaluated for acceptability in deriving the Final Plant Value (FPV). The FPV is the lowest toxicity value demonstrated in tests with biologically important endpoints (EPA 1985). None of the tests indicated that carbofuran was more toxic to aquatic plants than to aquatic animals; LOEC values were >100 µg/L. Thus, levels protective for aquatic animals will also be protective for aquatic plants.

HAZARD ASSESSMENT

Water Quality Criterion

The most sensitive species tested for acute toxicity were the dungeness crab *C. magister* with a GMAV of 1.5 µg/L, the cladoceran *Ceriodaphnia dubia* with a GMAV of 2.6 µg/L, and the mysid *Neomysis mercedis* with a GMAV of 3.6 µg/L. Mysids, cladocerans, and the dungeness crab all occur in the Sacramento-San Joaquin Estuary, and the CDFG WQC guidelines are intended to provide full protection to sensitive resident species (Harrington 1990). The interim FCV of 0.5 µg/L carbofuran would provide protection for these species and is proposed as the interim WQC. The WQC of 0.5 µg/L proposed here approximates the performance goal for carbofuran of 0.4 µg/L established by the CVRWQCB (1990).

The WQC represents maximum rather than average concentrations. Organisms normally respond to average concentrations. Maximum concentrations of rice pesticides including carbofuran have been shown to be approximately twice the average concentration (Finlayson et al. 1991) and therefore, the WQC provides an inherent two-fold safety margin.
The interim WQC is based on the toxicity of carbofuran alone. Because carbofuran exhibits additive toxicity with malathion and methyl parathion (Fujimura et al. 1991a), two other rice insecticides frequently found in Sacramento Valley waterways during the time that carbofuran is present, reevaluation of the WQC may be necessary. An acceptable level of carbofuran in the presence of the other two insecticides would be less than the WQC proposed here because of additive toxicity. Additive toxicity occurs when the observed toxicity for a mixture is equal to the sum of the potential toxicity of the individual components. Acceptable levels (AL) in surface water can be represented by the equation: \[
\text{AL/WQC (carbofuran)} + \text{AL/WQC (methyl parathion)} + \text{AL/WQC (malathion)} = 1.
\] This approach was used by Harrington (1990) in assessing rice herbicide toxicity and by CVRWQCB (1990) in determining deleterious levels of pesticides in surface waters. No information exists on the influence rice herbicides have on the toxicity of rice insecticides and vice versa. Reevaluation of the interim WQC will be necessary when the acute and chronic toxicity data gaps (see below) are filled.

**Hazard to Aquatic Animals**

The species demonstrating the greatest sensitivity in acute toxicity tests were the saltwater dungeness crab *C. magister* with a 96-h EC$_{50}$ value of 1.5 µg/L and the freshwater cladoceran *C. dubia* with a 48-h EC$_{50}$ value of 2.6 µg/L (Table A-1). *M. bahia* demonstrated the greatest sensitivity in chronic toxicity tests, with a 28-d NOEC value of 0.4 µg/L (Table B-1).

Carbofuran has been used on rice since 1979, with use ranging between 13,188 kg on 23,202 ha in 1979 to 52,490 kg on 83,519 ha in 1982. CDFG first measured concentrations of carbofuran in the agricultural drains in 1987. Concentrations of carbofuran were detected (>0.1 µg/L) in the Colusa Basin Drain in 1990 for a period of more than two months during mid-April
through late June (Finlayson et al. 1991). Maximum concentrations of carbofuran in the Colusa Basin Drain have declined from 13.0 µg/L in 1987, to 4.4 µg/L in 1988 (Harrington and Lew 1989), to 1.5 µg/L in 1989, to 1.1 µg/L in 1990 (Harrington and Lew 1992), and to 0.6 µg/L in 1991 (CDFG unpublished data 1991). Maximum concentrations of carbofuran in the Sacramento River have also declined from 2.1 µg/L in 1987 to <1.0 µg/L in 1988 (Harrington and Lew 1989) and 1989, to 0.6 µg/L in 1990 (Harrington and Lew 1992) and to <0.4 in 1991 (DPR 1992).

Although carbofuran levels in the agricultural drains and the Sacramento River have clearly declined since 1987, the data indicate that a hazard to sensitive aquatic invertebrates in the agricultural drains may have existed because environmental levels had exceeded acute toxicity levels for the cladoceran *C. dubia* and the mysid *N. mercedis*, especially in 1987 and 1988. Some of the agricultural drains are contiguous with the Sacramento River and contain water and organisms originating from the Sacramento River. A similar hazard to sensitive aquatic invertebrates in the Sacramento River does not appear to have existed, especially since 1987. Concentrations of carbofuran in the Sacramento River have generally been below detection levels (<0.1 µg/L to <1.0 µg/L) since 1987. Many sensitive aquatic invertebrates inhabit the upper Sacramento River including mysids and several species of cladocerans and copepods (L. Mecum, Bay- Delta Special Projects Division, California Department of Fish and Game, unpublished data, December 7, 1992).

**Data Requirements**

Data were available for seven of the nine species categories adapted from EPA (1985) recommendations for use in deriving an FAV (Table 3). Acceptable acute toxicity tests are necessary for one insect and a second insect or a species in a phylum other than Arthropoda or Chordata (Table 7). Acute toxicity tests should be performed with the stonefly *Pteronarcys*
californica and the amphipod Gammarus sp., species resident in the Sacramento River drainage and Sacramento-San Joaquin Estuary. A chronic toxicity test with the cladoceran C. dubia using measured concentrations of carbofuran is necessary to better define the FACR. An acute toxicity test with the mysid M. bahia, or a chronic toxicity test with the mysid N. mercedis are recommended to further refine the FACR and WQC.
Table 7. Minimum required and suggested data for a complete hazard assessment of carbofuran to aquatic animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Acute Test</th>
<th>Chronic Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stonefly</td>
<td>required</td>
<td>-----</td>
</tr>
<tr>
<td><em>Pteronarcys californica</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scud</td>
<td>required</td>
<td>-----</td>
</tr>
<tr>
<td><em>Gammarus sp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cladoceran</td>
<td>-----</td>
<td>required</td>
</tr>
<tr>
<td><em>Ceriodaphnia dubia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mysid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>suggested</td>
<td>-----</td>
</tr>
<tr>
<td><em>Mysisidopsis bahia</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mysid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-----</td>
<td>suggested</td>
</tr>
<tr>
<td><em>Neomysis mercedis</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> These are the most desirable species to test, but other resident species that fulfill EPA data recommendations would also be acceptable.

<sup>b</sup> Either an acute toxicity test with *M. bahia* or a chronic toxicity test with *N. mercedis* is suggested.
LITERATURE CITED


Brandt, O., R. W. Fujimura, and B. J. Finlayson. 1992. The use of Neomysis mercedis (Crustacea: Mysidacea) for estuarine toxicity tests. California Department of Fish and Game, Aquatic Toxicology Laboratory, Elk Grove, California.


Harrington, J. 1991. Procedures used by the California Department of Fish and Game to assess the hazard of pesticides on the State's aquatic resources. Proceedings of the 43rd Annual California Weed Conference. Santa Barbara, California.


APPENDIX A. Abstracts of accepted and unaccepted acute toxicity tests reviewed for hazard assessment.

**Accepted acute toxicity tests** - The following tests used accepted test methods.

**Brandt et al. (1992)** - In 1988 and 1990, 96-h flow-through acute toxicity tests were conducted by the California Department of Fish and Game (CDFG) on carbofuran technical (98.1% active ingredient) on juvenile and neonate mysid *Neomysis mercedis* (two tests for each life stage). Testing standards from ASTM (1988b) were generally used. Five concentrations of carbofuran were tested in replicate and solvent controls were used. Water quality parameters during the tests were: temperature of 17 ± 0.5°C, dissolved oxygen of 90-100% of saturation, pH of 8.2, and hardness of 250-400 mg/L as CaCO$_3$. Carbofuran exposure levels were measured twice with an average measured concentration of 92% of nominal. Control survival was greater than 90% in all cases. The 96-h LC$_{50}$ values for juveniles were 21 and 27 µg/L. The 96-h LC$_{50}$ values for neonates were 4.7 and 2.7 µg/L.

**Caldwell (1977), Caldwell (pers. comm.)** - In 1973 and 1974, 96-h static toxicity tests were conducted by U.S. Environmental Protection Agency (EPA) on carbofuran technical (% active ingredient not given) with first instar zoeae and adult dungeness crabs *Cancer magister*. Six concentrations of carbofuran were tested and there were acetone and water controls. Water quality parameters during the tests were: temperature of 13 °C, pH of 7.8 (zoeae) and 7.5 (adult), dissolved oxygen of >7.0 mg/L (zoeae) and 6.0 mg/L (adult), and salinity of 25 °/oo. Control survival and the measurement of carbofuran exposure levels were not mentioned. The 96-h LC$_{50}$ values were 2.5 µg/L for zoeae and 190 µg/L for adults and the 96-h EC$_{50}$ value based on inhibition of swimming as effect criterion was 1.5 µg/L for zoeae.
Faggella et al. (1990) - In 1988, a 96-h flow-through toxicity test was conducted by CDFG on carbofuran technical (98.1% active ingredient) with chinook salmon Oncorhynchus tshawytscha. ASTM (1988b) test methods were used. Five concentrations of carbofuran were tested in duplicate and a solvent control was included. Water quality parameters during the test were: temperature of 10.3 °C, Ph of 8.05, dissolved oxygen of 10.1 mg/L, hardness of 130 mg/L, and alkalinity of 111 mg/L. Control survival was >90%. Carbofuran exposure levels were measured at 24 and 72 hours. The 96-h LC₅₀ value was 610 µg/L for carbofuran.

FMC (1985c) - In 1985, a 48-h static toxicity test was conducted by Environmental Science and Engineering, Inc. on carbofuran technical (96.1%) with Eastern oyster Crassostrea virginica larvae. Internal lab testing procedures were used. A geometric series of six concentrations of carbofuran (dilution factor of 0.6) were tested in replicate and solvent and seawater controls were used. Water quality parameters during the tests were: temperature of 22 ±1°C, pH of 8.0-8.1, dissolved oxygen of 5.3-6.6 mg/L, and salinity of 21 °/oo. Control survival was 100% in both controls. The measurement of carbofuran exposure levels was not mentioned. The 48-h EC₅₀ values based on a reduction in the number of normal larvae as effect criterion was >5.0 mg/L, the highest concentration tested.

Fujimura et al. (1991b) - In 1988 and 1989, four 96-h acute toxicity tests were conducted by CDFG on carbofuran technical (98.1% active ingredient) with larval and juvenile striped bass Morone saxatilis. Test methods recommended by ASTM (1988b) were generally followed. Five concentrations of carbofuran were tested in replicate and solvent controls were used. Water quality parameters during the tests were: temperature of 17-19°C, Ph of 7.8-8.2, and hardness of 367-470 mg/L as CaCO₃. Carbofuran exposure levels were measured at 24 and 72 hour
intervals during the 96-h tests. Control survival varied from 90 to 100%. The 96-h LC$_{50}$ values varied between 130 to 180 µg/L for juveniles and 370 µg/L for larvae.

Mayer and Ellersieck (1986), Dwyer and Sappington (pers. comm.) - From 1965 to 1985, 96-h static and flow-through toxicity tests were conducted by the Columbia National Fisheries Laboratories of the U.S. Fish and Wildlife Service on carbofuran technical (99-100%) with coho salmon Oncorhynchus kisutch, steelhead (or rainbow) trout Oncorhynchus mykiss, brown trout Salmo trutta, fathead minnow Pimephales promelas, bluegill Lepomis macrochirus, lake trout Salvelinus namaycush, and yellow perch Perca flavescens. ASTM and EPA testing procedures were generally followed. Four or more concentrations of carbofuran were tested in replicate and a solvent (acetone) control was used. Water quality parameters during the tests averaged: pH of 7.1-9.5 and hardness of 40-314 mg/L CaCO$_3$. Carbofuran exposure concentrations were measured at 24-h intervals. The 96-h LC$_{50}$ values (in µg/L) were: coho salmon 530 (static), rainbow trout 380 and 600 (both static), brown trout 560 (static) and 280 (flow-through), lake trout 164 (flow-through), fathead minnow 1,990 and 872 (both static) and 1,180 (flow-through), channel catfish 248 (static), bluegill 88 (static), and yellow perch 240, 120 and 400 (all static). Although dissolved oxygen levels were not given, these tests were accepted because ASTM and EPA procedures were used and because of the reputation of the laboratories.

Norberg-King et al. (1991), Norberg-King (pers. comm.) - In 1990, a renewal acute toxicity test was performed on technical carbofuran (98.1% active ingredient) with cladoceran Ceriodaphnia dubia. EPA (1989) test methods were used. Five concentrations of carbofuran were tested (dilution factor 0.5) and a water control was included. Water quality parameters were: temperature of 25± 1°C, dissolved oxygen levels "adequate", pH of
7.9 and hardness of 45–50 mg/L as CaCO$_3$. Carbofuran exposure levels were not measured. Control survival was 100%. The 48-h LC$_{50}$ value was 2.6 µg/L. None of the test concentrations induced partial mortality, and therefore confidence limits were not calculated.

Parrish (1977) – In 1974 and 1975, a 96-h intermittent flow toxicity test was conducted by Bionomics, Inc. (under contract to EPA) on carbofuran technical (99%) with sheepshead minnow Cyprinodon variegatus. The bioassay methodology of APHA (1976) and EPA (1975) were used. Five concentrations of carbofuran were tested in replicate and a solvent control was used. Water temperature was 30 ±1°C. Carbofuran exposure levels ranged from 18–24% of nominal. Control survival was 100%. The 96-h LC$_{50}$ value was 386 µg/L.
**Unaccepted acute toxicity tests** - The following tests did not use accepted test methods and/or produce accepted results.

**Brown et al. (1979)** - In 1978, 96-h static and intermittent flow toxicity tests were conducted at Texas A & M University on carbofuran technical (99%) with 6-week old catfish *Ictalurus punctatus*. APHA (1971) testing procedures were followed. Tap and rice paddy water were used for dilution water (two paddy water tests for static conditions). Number of carbofuran concentrations, measuring carbofuran concentrations, and control survival were not mentioned. Concentrations of carbofuran were tested in replicate. Water quality parameters during the tests were: temperature of 23 ºC, salinity of 0.5 ‰ and pH of 8.5 (tap water) and 6.4 (paddy water), and "adequate" dissolved oxygen levels. The 96-h LC$_{50}$ values under static conditions were: 1,420 µg/L (tap water), 130 µg/L (paddy water) and 370 µg/L (paddy water). The 96-h LC$_{50}$ values under flow-through conditions were: 510 µg/L (tap water) and 480 µg/L (paddy water). These tests were not used due to lack of information on test procedures and results, including control survival, number and range of toxicants tested, and dissolved oxygen levels. Only the test results from tap water are listed in Table A-2.

**Carter and Graves (1973)** - In 1973, 96-h and 24-h static tests were conducted on carbofuran (formulation not given) with White River crawfish *Procambarus acutus*, bluegill *Lepomis macrochirus*, mosquitofish *Gambusia affinis*, channel catfish *Ictalurus punctatus*, and bullfrog *Rana catesbeiana* tadpoles. APHA testing procedures were used. Tests were performed on 5 replicate concentrations of carbofuran for crawfish and 2 replicate concentrations for the other species. Water quality parameters during the test were: temperature of 23-26 ºC, and dissolved oxygen of 7-10 mg/L. Controls, control survival, exposure regime, and carbofuran exposure measurements were not mentioned. The 96-h LC$_{50}$ values ranged from 80 µg/L for bluegill to 2,700
µg/L for bullfrog tadpoles. These tests was not used because information on control survival and the number and range of toxicant levels tested was not given.

Cheah et al (1980), Graves (pers. comm.) - In 1980, 96-h static tests were performed at Louisiana State University on carbofuran technical with juvenile red swamp crawfish Procambarus clarkii. EPA testing procedures were used. Four to seven concentrations of carbofuran were tested in replicate three times. Three solution controls were used. Water quality parameters were: temperature of 30±3°C, dissolved oxygen maintained by aeration, pH of 8.4, and hardness of 100 mg/L as CaCO₃. Carbofuran exposure levels were apparently not measured. Control survival averaged 95% (lowest was 86.7%). The 96-h LC₅₀ value for the crawfish was 500 µg/L. This data was not used because temperature variation was too great.

Dad et al. (1982) - In 1982, 96-h static tests were conducted on Furadan® (3% carbofuran formulation) with tubificid worms Tubifex tubifex and Limnodrilus hoffmeisteri. APHA (1971) testing procedures were used. Ten concentrations of carbofuran were tested in replicate. A control was also tested (addition of solvent in control was not mentioned). Water quality parameters during the test were: temperature of 18± 0.3°C, dissolved oxygen of 8.5 ± 0.3 mg/L, pH of 8.15± 0.3, and hardness of 165 ± 5 mg/L as CaCO₃. Carbofuran exposure levels were apparently not measured and control survivals were not mentioned. The 96-h EC₅₀ values for T. tubifex and L. hoffmeisteri were: 14,000 µg/L and 11,000 µg/L, respectively. These data were not used because of no control survival data and a formulation with a low percentage of carbofuran was used.
Davey et al. (1976) – From 1973 to 1975, 72-h static toxicity tests were conducted at the University of Arkansas on carbofuran (3%) with adult mosquitofish Gambusia affinis and juvenile green sunfish Lepomis cyanellus. Concentrations of carbofuran were tested in replicate three times, and solvent controls were included. Water quality parameters, number of treatments, carbofuran exposure level measurements, and control survival were not mentioned. The 72-h LC₅₀ value was 0.52 mg/L for mosquitofish and 0.16 mg/L for green sunfish. These data were not used because the exposures weren't 96 hours and essential information on control survival, water quality parameters, and exposure levels was not given.

FMC (1985a) – In 1985, a 96-h flow-through toxicity test was conducted by Environmental Science and Engineering, Inc. on carbofuran technical (96.1%) with Atlantic silverside Menidia menidia. ASTM (1980) testing procedures were used. A geometric series of six concentrations of carbofuran (dilution factor of 0.5) were tested in replicate. Natural seawater and acetone controls were included. Water quality parameters during the test were: temperature of 22-24 °C, pH of 7.5-8.3, dissolved oxygen of 52% saturation, and salinity of 20 o/oo. Control survival was 100% in both the solvent control and seawater control. Carbofuran exposure levels were measured on day 0 and day 4 and averaged 72% of nominal concentrations. The 96-h LC₅₀ value was 49 µg/L. This test was not accepted because dissolved oxygen levels were too low.

FMC (1985b) – In 1985, a 96-h flow-through toxicity test was conducted by Environmental Science and Engineering, Inc. on technical carbofuran (96.1%) with pink shrimp Penaenus duorarum. Internal lab testing procedures were used. Five concentrations of carbofuran (dilution factor of 0.5) were tested in replicate. Acetone and seawater controls were included. Water quality parameters during the tests were: temperature of 24 °C, pH of
7.7-8.0, dissolved oxygen of \( \$ \) 65% saturation, and salinity of 22 °/\( \circ \). Carbofuran exposure levels were measured at 0 and 96 hours during the test and averaged 85% of nominal (range:80-90%). Survival was 100% for both controls. The 96-h LC\(_{50}\) value was 12 µg/L. This value was not used because the temperature dropped to 15 °C during the first 24 hours of the test, thus possibly putting stress on the test organism.

Hartman and Martin (1985) - In 1984, 48-h static toxicity tests were conducted by the U.S. Fish and Wildlife Service on Furadan 4 (40.6% carbofuran) with adult cladoceran Daphnia pulex. EPA (1975) testing procedures were followed. Three concentrations of carbofuran (dilution factor 0.1) were tested in replicate and one water control was used. Water quality parameters during the test were: temperature of 15 °C, pH of 7.6, and hardness of 282 mg/L CaCO\(_3\). The measurement of carbofuran exposure level and control survival were not mentioned. The 48-h EC\(_{50}\) values based on mortality were 35 µg/L without suspended sediment and 45 µg/L with suspended sediment. These values were not used because the test description failed to report control survival and dissolved oxygen levels, only three concentrations of carbofuran were tested, and a formulation with a low percentage of carbofuran was used.

Johnson (1986), Johnson (pers. comm.) - In 1986, 48-h static toxicity tests were conducted by the U.S. Fish and Wildlife Service on technical carbofuran (99% active ingredient) with first instar cladoceran Daphnia magna and fourth instar midge Chironomus riparius. EPA (1975) testing procedures were used for testing. Three concentrations of carbofuran were tested with four replicates and a control was used. Water quality parameters for both tests were: temperature of 20±1°C, pH of 7.2, hardness of approximately 30 mg/L as CaCO\(_3\) and alkalinity of 35 mg/L as CaCO\(_3\). Survival of cladoceran controls was 100%. Survival of midge controls was not mentioned. Measurement of carbofuran
exposure levels were not mentioned. The 48-h EC\textsubscript{50} values for cladoceran and midge were 48 and 56 µg/L, respectively. These tests were not used because only three concentrations of carbofuran were tested.

**Kornak and Collins (1974)** - In 1974, a 24-h static toxicity test was performed on technical carbofuran (>90% active ingredient) with larvae midge *Chironomus tentans*. No recognized testing procedures (i.e., ASTM, EPA, APHA) were used. Concentrations of carbofuran were tested in triplicate and a control was included. Water temperature was maintained at 22°C. Dissolved oxygen was maintained by "gentle aeration". Measurement of carbofuran exposure levels and survival of controls were not mentioned. The 24-h LC\textsubscript{50} value was 1.6 µg/L (based on no response to prodding) and 0.7 µg/L (based on non-responsive and moribund individual counts). These values were not used because the number of concentrations, dilution factor, and control survival were not mentioned and the test was only 24 hours in duration.

**Mayer and Ellersieck (1986), Dwyer and Sappington (pers. comm.)** - Between 1965 and 1985, a 96-h static toxicity test was conducted by the Columbia National Fisheries Laboratories of the U.S. Fish and Wildlife Service on carbofuran formulation (50% active ingredient) with the bluegill *Lepomis macrochirus*. ASTM and EPA testing procedures were generally followed. Concentrations of carbofuran were tested in replicate and there was a solvent (acetone) control. Water quality parameters during the test were pH 7.1-9.5, and hardness 40-44 mg/L CaCO\textsubscript{3}. Toxicant concentrations were measured at 24-h intervals. The 96-h LC\textsubscript{50} value was 240 µg/L. This test was not accepted because a formulation with a low percentage of carbofuran was used.

**Pawar and Katdare (1984)** - In 1983, 96-h static toxicity tests were conducted at University of Poona, India, on carbofuran technical (75% active ingredient) with frog *Microhyla ornata*
embryos and tadpoles. No recognized testing procedures (i.e., ASTM, APHA, EPA) were used. Six concentrations of carbofuran were tested in replicate four times and a solvent control was used. Measurements of water quality parameters and carbofuran exposure levels were not mentioned. Control survival was 97.5%. The 96-h LC₅₀ values for embryos and tadpoles were 44,230 and 13,470 µg/L, respectively. These values were not used because dissolved oxygen and temperature levels were not reported, and a formulation with a low percentage of carbofuran was used.

Verma et al. (1980) - In 1979, 96-h toxicity tests were performed at the Pollution Relevant Research Laboratory of D.A.V. College in India on Furadan® (75% active ingredient) with catfish Mystus vittatus. A previous test done by the same researcher was used as a method guide. The number and levels of carbofuran concentrations tested, water quality parameters, control survival, and carbofuran exposure level measurements were not mentioned. The 96-h LC₅₀ value was 310 µg/L. This value was not used because a formulation with a low percentage of carbofuran was used, most of the test parameters were not reported, and this species is not found in the United States.

Verma et al. (1982) - In 1982, 96-h static toxicity tests were performed at the D.A.V. college in India on Furadan® (75% active ingredient) with the freshwater fish Saccobranchus fossilis. APHA (1971) testing methods were used. A logarithmic series of carbofuran concentrations were tested in replicate, and a solvent control was used. Water quality parameters during the test were: temperature of 18.2 ± 2°C, pH of 7.2 ± 0.2, dissolved oxygen of 4.84 mg/L (approximately 50% saturation), hardness of approximately 50 mg/L as CaCO₃, and of alkalinity 59 mg/L as CaCO₃. Measurements of carbofuran exposure levels were not mentioned. Control survival was 100%. The 96-h LC₅₀ value was 547 µg/L. This value was not used because a formulation with a low percentage of carbofuran was used, and dissolved oxygen
levels were below 60%.
Table A-1. Values (µg/L) from accepted tests on the acute toxicity of carbofuran to aquatic animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
<th>Method*</th>
<th>Formulation</th>
<th>Salinity/ Hardness</th>
<th>Test Length</th>
<th>Effect</th>
<th>Values (95% C.L. b)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluegill Lepomis macrochirus</td>
<td>Juv S,M</td>
<td>Technical (99%)</td>
<td>40 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>88 (75-104)</td>
<td>Mayer and Ellersieck 1986</td>
<td></td>
</tr>
<tr>
<td>Brown trout Salmo trutta</td>
<td>Juv S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>560 (475-660)</td>
<td>Mayer and Ellersieck 1986</td>
<td></td>
</tr>
<tr>
<td>Brown trout Salmo trutta</td>
<td>Juv F,M</td>
<td>Technical (99%)</td>
<td>314 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>280 (204-382)</td>
<td>Mayer and Ellersieck 1986</td>
<td></td>
</tr>
<tr>
<td>Channel catfish Ictalurus punctatus</td>
<td>Juv S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>248 (94-649)</td>
<td>Mayer and Ellersieck 1986</td>
<td></td>
</tr>
<tr>
<td>Chinook salmon Oncorhynchus tshawytscha</td>
<td>Juv F,M</td>
<td>Technical (98.1%)</td>
<td>130 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>610 (490-800)</td>
<td>Faggella et al. 1990</td>
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</tr>
<tr>
<td>Cladoceran Ceriodaphnia dubia</td>
<td>Neonate S,U</td>
<td>Technical (98.1%)</td>
<td>45-50 mg/L as CaCO₃</td>
<td>48-h</td>
<td>LC₅₀</td>
<td>2.6 (---) c</td>
<td>Norberg-King et al. 1991</td>
<td></td>
</tr>
<tr>
<td>Coho salmon Oncorhynchus kisutch</td>
<td>Juv S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>530 (432-650)</td>
<td>Mayer and Ellersieck 1986</td>
<td></td>
</tr>
<tr>
<td>Dungeness crab Cancer magister</td>
<td>Zoeae S,U</td>
<td>Technical (96.1%)</td>
<td>25 °/oo</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>2.5 (---) b</td>
<td>Caldwell 1977</td>
<td></td>
</tr>
<tr>
<td>Dungeness crab Cancer magister</td>
<td>Adult S,U</td>
<td>Technical (96.1%)</td>
<td>25 °/oo</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>190 (---) b</td>
<td>Caldwell 1977</td>
<td></td>
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<tr>
<td>Eastern oyster Crassostrea virginica</td>
<td>Embryo, Larvae S,U</td>
<td>Technical (96.1%)</td>
<td>21 °/oo</td>
<td>48-h</td>
<td>EC₅₀</td>
<td>&gt;5,000 (--)</td>
<td>FMC 1985c</td>
<td></td>
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<tr>
<td>Fathead minnow Pimephales promelas</td>
<td>Juv S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>1,990 (1385-2859)</td>
<td>Mayer and Ellersieck 1986</td>
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<tr>
<td>Fathead minnow Pimephales promelas</td>
<td>Juv S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>872 (479-1590)</td>
<td>Mayer and Ellersieck 1986</td>
<td></td>
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<tr>
<td>Species</td>
<td>Life Stage</td>
<td>Method*</td>
<td>Formulation</td>
<td>Salinity/ Hardness</td>
<td>Test Length</td>
<td>Effect</td>
<td>Values (µg/L) (95% C.L.)</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
<td>---------</td>
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<td>-------------</td>
<td>--------</td>
<td>--------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Fathead minnow</td>
<td>Juv</td>
<td>F,M</td>
<td>Technical (99%)</td>
<td>314 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>1,180 (813-1711)</td>
<td>Mayer and Ellersieck 1986</td>
</tr>
<tr>
<td>Lake trout</td>
<td>Juv</td>
<td>F,M</td>
<td>Technical (99%)</td>
<td>314 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>164 (119-226)</td>
<td>Mayer and Ellersieck 1986</td>
</tr>
<tr>
<td>Neomysis mercedis</td>
<td>Juv</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>250-400 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>21 (17-25)</td>
<td>Brandt et al. 1992</td>
</tr>
<tr>
<td>Neomysis mercedis</td>
<td>Juv</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>250-400 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>27 (22-33)</td>
<td>Brandt et al. 1992</td>
</tr>
<tr>
<td>Neomysis mercedis</td>
<td>Neo-</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>250-400 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>4.7 (4.2-5.1)</td>
<td>Brandt et al. 1992</td>
</tr>
<tr>
<td>Neomysis mercedis</td>
<td>Neo-</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>250-400 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>2.7 (2.5-5.2)</td>
<td>Brandt et al. 1992</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Juv</td>
<td>S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>380 (272-531)</td>
<td>Mayer and Ellersieck 1986</td>
</tr>
<tr>
<td>Sheepshead minnow</td>
<td>---</td>
<td>F,M</td>
<td>Technical (99%)</td>
<td>21 °/oo</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>386 (311-480)</td>
<td>Parrish 1977</td>
</tr>
<tr>
<td>Steelhead</td>
<td>Juv</td>
<td>S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>600 (436-826)</td>
<td>Mayer and Ellersieck 1986</td>
</tr>
<tr>
<td>Striped bass</td>
<td>Juv</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>381 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>130 (110-150)</td>
<td>Fujimura et al. 1991b</td>
</tr>
<tr>
<td>Striped bass</td>
<td>Juv</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>367 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>160 (130-200)</td>
<td>Fujimura et al. 1991b</td>
</tr>
<tr>
<td>Striped bass</td>
<td>Larvae</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>470 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>370 (320-440)</td>
<td>Fujimura et al. 1991b</td>
</tr>
<tr>
<td>Striped bass</td>
<td>Juv</td>
<td>F,M</td>
<td>Technical (98.1%)</td>
<td>390 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>180 (160-200)</td>
<td>Fujimura et al. 1991b</td>
</tr>
</tbody>
</table>
Table A-1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
<th>Method</th>
<th>Formulation</th>
<th>Salinity/Hardness</th>
<th>Test Length</th>
<th>Effect</th>
<th>Values (µg/L) (95% C.L.)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Perca flavescens</em> (Juvenile)</td>
<td>Juv</td>
<td>S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>240 (208-277)</td>
<td>Mayer and Ellersieck 1986</td>
</tr>
<tr>
<td><em>Perca flavescens</em> (Juvenile)</td>
<td>Juv</td>
<td>S,M</td>
<td>Technical (99%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>120 (82-176)</td>
<td>Mayer and Ellersieck 1986</td>
</tr>
<tr>
<td><em>Perca flavescens</em> (Juvenile)</td>
<td>Juv</td>
<td>S,M</td>
<td>Technical (99%)</td>
<td>42 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>400 (289-553)</td>
<td>Mayer and Ellersieck 1986</td>
</tr>
</tbody>
</table>

- **Species:** "Perca flavescens"
- **Method:** S = Static, F = Flow-through, M = Measured concentrations, U = Unmeasured concentrations
- **Formulation:** Technical (99%)
- **Salinity/Hardness:** 44 mg/L as CaCO₃
- **Test Length:** 96-h
- **Effect:** LC₅₀
- **Values (µg/L) (95% C.L.)**
- **Reference:** Mayer and Ellersieck 1986

*a* S = Static  
F = Flow-through  
M = Measured concentrations  
U = Unmeasured concentrations

*b* Confidence limits in parenthesis

*c* Not generated (see abstracts in Appendix A)
<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
<th>Method</th>
<th>Formulation</th>
<th>Salinity/Hardness</th>
<th>Test Length</th>
<th>Effect</th>
<th>Values (µg/L) (95% C.L.)</th>
<th>Reference</th>
<th>Test Deficiencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic silverside Juv</td>
<td>F,M</td>
<td>Technical</td>
<td>(96.1%)</td>
<td>18-19°/oo</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>49 (38-72)</td>
<td>FMC 1985a</td>
<td>1</td>
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<tr>
<td>Menidia menidia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Bluegill Juv</td>
<td>S,U</td>
<td>Furadan</td>
<td>(99%)</td>
<td>2-5 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>80 (--)</td>
<td>Carter and Graves 1973</td>
<td>2</td>
</tr>
<tr>
<td>Lepomis macrochirus</td>
<td>S,M</td>
<td>Technical</td>
<td>(50%)</td>
<td>44 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>240 (186-310)</td>
<td>Mayer and Ellersieck 1986</td>
<td>3</td>
</tr>
<tr>
<td>Bullfrog</td>
<td>S,U</td>
<td>Furadan</td>
<td>(--)</td>
<td>2-5 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>2,700 (--)</td>
<td>Carter and Graves 1973</td>
<td>2</td>
</tr>
<tr>
<td>Rana catesbeiana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catfish</td>
<td>---</td>
<td>F,U</td>
<td>Furadan</td>
<td>(75%)</td>
<td></td>
<td>96-h</td>
<td>LC₅₀ 310 (--)</td>
<td>Verma et al. 1980</td>
<td>2,3</td>
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<tr>
<td>Mystus vittatus</td>
<td>---</td>
<td>Technical</td>
<td>(--)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Channel catfish Juv</td>
<td>S,U</td>
<td>Furadan</td>
<td>(--)</td>
<td>2-5 mg/L as CaCO₃</td>
<td>24-h</td>
<td>LC₅₀</td>
<td>2,030 (--)</td>
<td>Carter and Graves 1973</td>
<td>2</td>
</tr>
<tr>
<td>Ictalurus punctatus</td>
<td>F,U</td>
<td>Technical</td>
<td>(99%)</td>
<td></td>
<td></td>
<td>96-h</td>
<td>LC₅₀ 510 (460-560)</td>
<td>Brown et al. 1979</td>
<td>2</td>
</tr>
<tr>
<td>Channel catfish</td>
<td>S,U</td>
<td>Technical</td>
<td>(99%)</td>
<td></td>
<td></td>
<td>96-h</td>
<td>LC₅₀ 1420 (1,290-1,700)</td>
<td>Brown et al. 1979</td>
<td>2</td>
</tr>
<tr>
<td>Channel catfish Juv</td>
<td>S,U</td>
<td>Technical</td>
<td>(99%)</td>
<td></td>
<td></td>
<td>96-h</td>
<td>LC₅₀ 1420 (1,290-1,700)</td>
<td>Brown et al. 1979</td>
<td>2</td>
</tr>
<tr>
<td>Cladoceran Adult Cladoceran pulex</td>
<td>Adult</td>
<td>S,U</td>
<td>Furadan</td>
<td>(40.6%)</td>
<td>282 mg/L as CaCO₃</td>
<td>48-h</td>
<td>EC₅₀ 35 (26.8-45.8)²d 45 (33.1-61.1)²e</td>
<td>Hartman and Martin 1985</td>
<td>2,3,4</td>
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<tr>
<td>Daphnia magna</td>
<td>1st instar</td>
<td>S,U</td>
<td>Technical</td>
<td>(99%)</td>
<td>30 mg/L as CaCO₃</td>
<td>48-h</td>
<td>EC₅₀ 48 (35-64)</td>
<td>Johnson 1986</td>
<td>4</td>
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<tr>
<td>Cladoceran Daphnia magna</td>
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<td></td>
<td></td>
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<tr>
<td>Frog</td>
<td>---</td>
<td>S,U</td>
<td>Technical</td>
<td>(--)</td>
<td></td>
<td>96-h</td>
<td>LC₅₀ embryo 44,230</td>
<td>Pawar and Katdare 1984</td>
<td>1,5</td>
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<tr>
<td>Microhyla ornata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tadpole 13,470</td>
<td></td>
<td></td>
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<tr>
<td>Green sunfish Juv</td>
<td>S,U</td>
<td>Carbofuran</td>
<td>(3%)</td>
<td></td>
<td>72-h</td>
<td>LC₅₀</td>
<td>160 (100-210)</td>
<td>Davey et al. 1976</td>
<td>3,6</td>
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<tr>
<td>Lepomis cyanellus</td>
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<tr>
<td>Midge Chironomus riparius</td>
<td>4th instar</td>
<td>S,U</td>
<td>Technical</td>
<td>(99%)</td>
<td>30 mg/L as CaCO₃</td>
<td>48-h</td>
<td>EC₅₀ 56 (31-99)</td>
<td>Johnson 1986</td>
<td>4</td>
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Table A-2. Values (µg/L) from unaccepted tests on acute toxicity of carbofuran to aquatic animals.
<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
<th>Method</th>
<th>Formulation</th>
<th>Salinity/Hardness</th>
<th>Test Length</th>
<th>Effect</th>
<th>Values (µg/L) (95% C.L.)</th>
<th>Reference</th>
<th>Test Deficiencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midge Chironomus tentans</td>
<td>Larvae</td>
<td>S,U</td>
<td>Technical (90%)</td>
<td>---</td>
<td>24-h</td>
<td>LC₅₀</td>
<td>1.6 (--0 0.7f (--))</td>
<td>Kornak and Collins 1974</td>
<td>2,6</td>
</tr>
<tr>
<td>Mosquitofish Gambusia affinis</td>
<td>Juv</td>
<td>S,U</td>
<td>Furadan (--)</td>
<td>2-5 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>300 (-- --)</td>
<td>Carter and Graves 1973</td>
<td>2</td>
</tr>
<tr>
<td>Mosquitofish Gambusia affinis</td>
<td>Adult</td>
<td>S,U</td>
<td>Carbofuran (3%)</td>
<td>---</td>
<td>72-h</td>
<td>LC₅₀</td>
<td>520 (450-570)</td>
<td>Davey et al. 1976</td>
<td>2,6</td>
</tr>
<tr>
<td>Pink shrimp Penaeus duorarum</td>
<td>Adult</td>
<td>F,M</td>
<td>Technical (96.1%)</td>
<td>22 °/oo</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>12 (9.4-14.0)</td>
<td>FMC 1985b</td>
<td>5</td>
</tr>
<tr>
<td>Red swamp crawfish Juv</td>
<td>Procambarus clarkii</td>
<td>S,U</td>
<td>Technical (--)</td>
<td>100 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>500 (-- --)</td>
<td>Cheah et al. 1980</td>
<td>5</td>
</tr>
<tr>
<td>Saccobranchus fossilis</td>
<td>---</td>
<td>S,U</td>
<td>Furadan (75%)</td>
<td>50 mg/L as CaCO₃</td>
<td>96-h</td>
<td>LC₅₀</td>
<td>547 (470-637)</td>
<td>Verma et al. 1982</td>
<td>3,1</td>
</tr>
<tr>
<td>Tubificid worm Tubifex tubifex</td>
<td>---</td>
<td>S,U</td>
<td>Furadan (3%)</td>
<td>165 mg/L as CaCO₃</td>
<td>96-h</td>
<td>EC₅₀</td>
<td>14,000 (-- --)</td>
<td>Dad et al. 1982</td>
<td>2,3</td>
</tr>
<tr>
<td>Tubificid worm Limnodrilus hoffmeisteri</td>
<td>---</td>
<td>S,U</td>
<td>Furadan (3%)</td>
<td>165 mg/L as CaCO₃</td>
<td>96-h</td>
<td>EC₅₀</td>
<td>11,000 (-- --)</td>
<td>Dad et al. 1982</td>
<td>2,3</td>
</tr>
<tr>
<td>White River crawfish Juv</td>
<td>Procambarus actetus</td>
<td>S,U</td>
<td>Furadan (--)</td>
<td>2-5 mg/L as CaCO₃</td>
<td>96-h</td>
<td>EC₅₀</td>
<td>500 (-- --)</td>
<td>Carter and Graves 1973</td>
<td>2</td>
</tr>
</tbody>
</table>

| a S = Static                  | c 1 = Dissolved oxygen level too low |
| F = Flow-through              | 2 = Essential test information not given |
| M = Measured concentrations   | 3 = Formulation tested <80% a.i. |
| U = Unmeasured concentrations | 4 = Insufficient number of concentrations tested |
| b Confidence limits in parenthesis | 5 = Temperature variation too wide |
| d Without sediment            | 6 = Test duration <96-h |
| e With sediment               | f Based on combined |
|                             | moribund and death counts |

---

Table A-2.
APPENDIX B. Abstracts of accepted and unaccepted chronic toxicity tests reviewed for hazard assessment.

Accepted chronic toxicity tests – The following tests used accepted test methods.

Caldwell (1977) – In 1974, 70-d and 90-d flow-through toxicity tests were conducted by EPA on carbofuran technical (% not given) with zoeae (70-d) and adult (90-d) dungeness crabs Cancer magister. Four replicates of three concentrations were tested with zoeae. Two replicates of four concentrations were tested with adults. The dilution factor of carbofuran in both tests was 0.1. Solvent and dilution water controls were included. Water quality parameters during the tests were: temperature 12.3 °C (zoeae) and 10.0 °C (adult), pH 8.1 (zoeae) and 7.9 (adult), dissolved oxygen 8.7 mg/L (zoeae) and 8.2 mg/L (adult), and salinity 28.8 °/oo (zoeae) and 25.4 °/oo (adult). Control survival with and without solvent was >80% for adults and <60% for zoeae. Measured carbofuran concentrations averaged 81.3% of nominal concentrations for tests with adults (range: 68-94%) and 136% of nominal concentrations for tests with zoeae (range: 126-146%). Although survival of zoeae controls declined dramatically after 50 days of testing, the dose response relationship was very clear. Complete (100%) mortality occurred at the LOEC exposure level (5 µg/L) and test species survival at the NOEC exposure level (0.5 µg/L) approximated that of control survival. The NOEC and LOEC values, based on survival, were 0.5 and 5 µg/L for zoeae and 25 and 250 µg/L for adults.

FMC (1981b) – In 1981, a 101-d flow-through embryos-to-fry toxicity test was conducted by Analytical Biochemistry Laboratories, Inc. on carbofuran technical (96.1%) with rainbow trout Oncorhynchus mykiss. ASTM (1979) and EPA (1972) testing procedures were followed. Five concentrations of carbofuran (dilution factor of 0.5) were tested with four replicates.
Acetone and water controls were included. Water quality parameters during the test were: temperature of 10 °C (eggs) and 12 °C (fry), pH of 8.1, dissolved oxygen of 7.8 mg/L, and ammonia of 0.53 mg/L. Control survival was 100% for both controls. Concentrations of carbofuran were measured on days 0, 1, 5, 10, and every 10 days after and averaged 136% of nominal concentrations (range: 114-144%). The NOEC and LOEC values, based on percentage of eggs hatched was 24.8 and 56.7 µg/L.

FMC (1982) - In 1982, a 21-d flow-through life-cycle toxicity test was conducted by Analytical Biochemistry Laboratories, Inc. on carbofuran technical (95.6%) with first instar of the cladoceran Daphnia magna. ASTM (1979, 1987), EPA (1975), and U.S. Federal Register (1978) testing procedures were followed. Five concentrations of carbofuran (dilution factor 0.5) were tested with four replicates. A solvent control was also included. Water quality parameters during the test were: temperature of 20 °C, pH of 8.1-8.7, and dissolved oxygen of 6.5-7.9 mg/L. Control survival averaged 93%. Toxicant concentrations were measured on days 0, 4, 7, 14, and 21 but were not compared with nominal concentrations. The NOEC and LOEC values based on survival were 9.8 and 27 µg/L, respectively.

FMC (1987) - In 1987, a 28-d flow-through life-cycle toxicity test was conducted by Enseco, Inc. on carbofuran technical (98.6%) with <24-hr old Mysidopsis bahia. ASTM (1987) testing procedures were followed. Five concentrations of carbofuran were tested with two replicates. A water control was also included. Water quality parameters during the test were: temperature of 27 ±1°C (on days 14 and 15 temperature was ranged from 25 to 27.6°C), pH of 8.0, and average dissolved oxygen of 4.9 mg/L. The average dissolved oxygen was above 60% of saturation 94% of the time. Dissolved oxygen concentration was at least 56% the other 6% of the time. Control survival averaged 77.5%. Toxicant concentrations were measured on days 4, 7, 10, 14, 17, 21, and
28. The NOEC and LOEC values based on survival were 0.4 and 0.98 µg/L, respectively. Although temperature varied more than 2° C and dissolved oxygen concentrations dropped below 60% this test was accepted because the temperature and dissolved oxygen deviations were slight and of short duration.

Fujimura et al. (1990) - In 1989, a 75-d flow-through embryos-to-fry toxicity test was performed by CDFG on carbofuran technical (98.1%) with chinook salmon Oncorhynchus tshawtscha. ASTM (1988a) bioassay standards were generally followed. Five concentrations of carbofuran were tested in replicate. Water and solvent controls were used. Water quality parameters during the test were: temperature of 10.2 °C, pH of 8.3, dissolved oxygen of 9.75 mg/L, and hardness of 83 mg/L as CaCO₃. Carbofuran concentrations were measured twice weekly during the test and averaged 97% of nominal (range: 92-104%). Control survival was 99%. The 75-d NOEC and LOEC values based on growth were 9.2 and 17 µg/L, respectively.

Parrish et al. (1977) - In 1974 and 1975, a 131-d flow-through embryos-to-fry toxicity test was conducted by Bionomics, Inc. (under contract to EPA) on carbofuran technical (99%) with sheepshead minnows Cyprinodon variegatus. APHA (1976) and EPA (1975) testing procedures were followed. Five concentrations of carbofuran (dilution factor of 0.5) were tested in replicate. Acetone and water controls were used. Water temperature was 30 °C. Control survival was 93% for both controls. Carbofuran exposure levels were measured and ranged from 18 to 24% of nominal concentrations. The NOEC and LOEC values, based on survival and hatching success were 23 and 49 µg/L, respectively.
**Unaccepted chronic toxicity tests** - The following tests used unaccepted test methods and/or produced unaccepted results.

**FMC (1981a)** - In 1981, a 14-d flow-through toxicity test was conducted by Analytical Biochemistry Laboratories, Inc. on carbofuran technical (96.1%) with juvenile rainbow trout *Oncorhynchus mykiss*. APHA (1976) and EPA (1975) testing procedures were used. Five concentrations of carbofuran (dilution factor 0.5) were tested. A dilution water control was included. The number of replicates tested for each concentration was not mentioned. Water quality parameters during the test were: temperature of 12 °C, pH of 7.8, dissolved oxygen of 9.2 mg/L, and ammonia of < 0.6 mg/L. Control survival was 100%. Toxicant concentrations averaged 87% of nominal concentrations (range: 78-110%). The NOEC and LOEC values based on the loss of equilibrium, were 56 and 98 µg/L, respectively. This test was not used because the test duration was not a significant portion of the rainbow trout life-cycle.

**Isensee and Tayaputch (1986)** - In 1985, a 60-d chronic microecosystem toxicity test was conducted on carbofuran technical (97%) which included mosquito fish *Gambusia affinis*. Two concentrations of carbofuran were tested in replicate three times. Two controls were included. Water quality parameters were not mentioned. Carbofuran exposure levels were measured on 5 occasions resulting in values which were 39 to 98% of nominal. No NOEC or LOEC values were given and unexplained mass mortality occurred in the control and treatment groups during the test.

**Johnson 1986** - In 1986, a 30-d microcosm test was conducted by the U.S. Fish and Wildlife Service with technical carbofuran (99% active ingredient) which included first star instar *Daphnia magna*. EPA (1975) testing procedures were used. Four replicates of three concentrations of carbofuran were tested with a control. Water quality parameters were: temperature 20± 1°C, pH 7.2, and
alkalinity 35 mg/L as CaCO$_3$. The researcher (contacted by phone) was unable to obtain information on control survival and NOEC and LOEC values.

Norberg-King et al. (1991), Norberg-King (pers. comm.) - In 1990, 7-d static chronic tests were performed on technical carbofuran (98.1% active ingredient) with cladoceran Ceriodaphnia dubia. Five concentrations of carbofuran were tested (dilution factor 0.5) and a water control was included. Water quality parameters were: temperature of 25± 1°C, dissolved oxygen levels "adequate", pH of 7.9 and hardness of 45-50 mg/L as CaCO$_3$. Carbofuran exposure levels were not measured. Control survival was 100%. The 7-d NOEC and LOEC values based on survival were 2.6 and 1.3 µg/L, respectively. This test was not accepted because carbofuran exposure levels were not measured.
Table B-1. Values (µg/L) from accepted tests on chronic toxicity of carbofuran to aquatic animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
<th>Method</th>
<th>Formulation</th>
<th>Salinity/Hardness</th>
<th>Test Length</th>
<th>Effect</th>
<th>Values (95% C.L.)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladoceran <em>Daphnia magna</em></td>
<td>1st instar</td>
<td>F,M</td>
<td>Technical (95.6%)</td>
<td>255 mg/L as CaCO³</td>
<td>21-d</td>
<td>NOEC</td>
<td>9.8</td>
<td>FMC 1982</td>
</tr>
<tr>
<td>Chinook salmon <em>Onchorhyncus tshawytscha</em></td>
<td>Embryos- Fry</td>
<td>F,M</td>
<td>Technical (98.1%) as CaCO₃</td>
<td>83 mg/L</td>
<td>75-d</td>
<td>NOEC</td>
<td>9.2</td>
<td>Fujimura et al. 1990</td>
</tr>
<tr>
<td>Dungeness crab <em>Cancer magister</em></td>
<td>Adult</td>
<td>F,M</td>
<td>Technical (96.1%)</td>
<td>25.4 %/oo</td>
<td>90-d</td>
<td>NOEC</td>
<td>25</td>
<td>Caldwell 1977</td>
</tr>
<tr>
<td>Dungeness crab <em>Cancer magister</em></td>
<td>Zoeae</td>
<td>F,M</td>
<td>Technical (96.1%)</td>
<td>28.8±1.4 %/oo</td>
<td>70-d</td>
<td>NOEC</td>
<td>0.5</td>
<td>Caldwell 1977</td>
</tr>
<tr>
<td>Mysid <em>Mysidopsis bahia</em></td>
<td>24-h</td>
<td>F,M</td>
<td>Technical (98.6%)</td>
<td>28-30 %/oo</td>
<td>28-d</td>
<td>NOEC</td>
<td>0.4</td>
<td>FMC 1987</td>
</tr>
<tr>
<td>Rainbow trout <em>Oncorhyncus mykiss</em></td>
<td>Embryos- Fry</td>
<td>F,M</td>
<td>Technical (96.1%) as CaCO₃</td>
<td>255 mg/L</td>
<td>101-d</td>
<td>NOEC</td>
<td>24.8</td>
<td>FMC 1981b</td>
</tr>
<tr>
<td>Sheepshead minnow <em>Cyprinodon variegatus</em></td>
<td>Embryos- Parish</td>
<td>F,M</td>
<td>Technical (99%)</td>
<td>--</td>
<td>131-d</td>
<td>NOEC</td>
<td>23.0</td>
<td>Parrish et al. 1977</td>
</tr>
</tbody>
</table>

*S = Static  
F = Flow-through  
M = Measured concentration  
U = Unmeasured concentration

Confidence limits in parenthesis.
Table B-2. Values (µg/L) from unaccepted tests on chronic toxicity of carbofuran to aquatic animals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
<th>Method&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Formulation</th>
<th>Salinity/ Hardness</th>
<th>Test Length</th>
<th>Effect</th>
<th>Values (95% C.L.&lt;sup&gt;b&lt;/sup&gt;)</th>
<th>Test Deficiencies&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cladoceran <em>Daphnia magna</em></td>
<td>1st instar</td>
<td>C, U</td>
<td>Technical (99% a.i.)</td>
<td>---</td>
<td>30-d</td>
<td>NOEC/LOEC</td>
<td>none given</td>
<td>Johnson 1986</td>
<td></td>
</tr>
<tr>
<td>Daphnia magna instar</td>
<td>---</td>
<td>C, U</td>
<td>Technical (99% a.i.)</td>
<td>---</td>
<td>60-d</td>
<td>NOEC/LOEC</td>
<td>none given</td>
<td>Isensee and Tayaputch 1986</td>
<td></td>
</tr>
<tr>
<td>Rainbow trout <em>Oncorhynchus mykiss</em></td>
<td>Juv</td>
<td>F, M</td>
<td>Technical (96.1%)</td>
<td>255 mg/L as CaCO₃</td>
<td>14-d</td>
<td>NOEC</td>
<td>56.0</td>
<td>FMC 1981a</td>
<td></td>
</tr>
<tr>
<td>Cladoceran <em>Ceriodaphnia dubia</em></td>
<td>Neonate</td>
<td>S, U</td>
<td>Technical (98.1%)</td>
<td>45-50 mg/L as CaCO₃</td>
<td>7-d</td>
<td>NOEC</td>
<td>1.3</td>
<td>Norberg-King et al. 1991</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> C = Microcosm  
S = Static  
F = Flow through  
M = Measured concentrations  
U = Unmeasured concentrations

<sup>b</sup> Confidence limits in parenthesis

<sup>c</sup> 1 = Essential information not given  
2 = Unexplainable mass mortality in control  
3 = Test length unacceptable
APPENDIX C. Abstracts of aquatic plant toxicity tests reviewed for hazard assessment.

Hartman and Martin (1985) - In 1984, 14-d and 48-h static tests were conducted by the U.S. Fish and Wildlife Service on Furadan® (40.6% active ingredient) with sago pondweed Potamogeton pectinatus and little duckweed Lemna minor, respectively. Little duckweed was tested with and without sediment. No major recognized testing standards were used. Three carbofuran concentrations were tested in replicate and a control was used with pondweed tests. Six concentrations of carbofuran and a control were tested with and without sediment with duckweed tests. Water temperature was maintained at 22°C. Measurement of carbofuran exposure levels was not mentioned. Neither the 14-d nor the 48-h tests showed significant growth inhibition at any of the carbofuran levels (< 10 mg/L) tested. Thus, the effect values for both plants were > 10 mg/L carbofuran.

Johnson (1986) - In 1986, a 30-d microcosm test was conducted by the U.S. Fish and Wildlife Service with technical carbofuran (99% active ingredient) which included green alga Selenastrium capricornutum. EPA (1975) testing procedures were used. Four replicates of three concentrations of carbofuran were tested with a control. Water quality parameters were: temperature of 20±1°C, pH of 7.2, and alkalinity of 35 mg/L as CaCO₃. Treatments with higher concentrations of carbofuran exhibited higher growth rates.

Kar and Singh (1978) - In 1978, a 10-d static toxicity test was conducted by the Central Rice Research Institute, India on Furadan® (3% active ingredient) with blue-green alga Nostoc muscorum. No recognized testing procedures were used. Nine concentrations of carbofuran were tested. Water temperature was 24±2°C. Measurement of carbofuran exposure levels was not mentioned. The 10-d NOEC and LOEC values were approximated as 50
and 100 mg/L, respectively. A formulation with a low percentage of carbofuran was used.

Megharaj et al. (1989) - In 1989, 32-d toxicity tests were conducted on technical carbofuran (75% active ingredient) with green alga *Scenedesmus mijugatus*. Bioassay procedures of Goulding and Ellis (1981) were used. Seven concentrations of carbofuran (1, 2, 5, 10, 20, 50 and 100 mg/L) were tested in triplicate. A water control was included. Water temperature was 28 ± 4°C and dissolved oxygen levels were maintained by shaking the test containers four times a day. Measurement of carbofuran exposure levels was not mentioned. The 6-d NOEC and LOEC values based on growth, were 5 and 10 mg/L, respectively. The 32-d NOEC and LOEC values were 10 and 20 mg/L, respectively.
Table C-1. Values (µg/L) from tests on toxicity of carbofuran to aquatic plants.

<table>
<thead>
<tr>
<th>Species</th>
<th>Life Stage</th>
<th>Method</th>
<th>Formulation</th>
<th>Salinity/Hardness</th>
<th>Test Length</th>
<th>Effect</th>
<th>Values (µg/L) (95% C.L.(^b))</th>
<th>Reference</th>
<th>Test Deficiencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue-green alga</td>
<td>---</td>
<td>S, U</td>
<td>Furadan(^a)</td>
<td>---</td>
<td>10-d</td>
<td>NOEC</td>
<td>50</td>
<td>Kar and Singh 1978</td>
<td>1</td>
</tr>
<tr>
<td><em>Nostoc muscorum</em></td>
<td></td>
<td></td>
<td>(3% a.i.)</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green alga</td>
<td>---</td>
<td>S, U</td>
<td>Technical</td>
<td>---</td>
<td>6-d</td>
<td>NOEC</td>
<td>5,000</td>
<td>Megharaj et al 1989</td>
<td>2</td>
</tr>
<tr>
<td><em>Scenedesmus bijugatus</em></td>
<td></td>
<td></td>
<td>(75% a.i.)</td>
<td></td>
<td>32-d</td>
<td>NOEC</td>
<td>10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LOEC 50,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green alga</td>
<td>---</td>
<td>S, U</td>
<td>Technical</td>
<td>---</td>
<td>30-d</td>
<td></td>
<td></td>
<td>Johnson 1986</td>
<td>3</td>
</tr>
<tr>
<td><em>Selenastrium capricornutum</em></td>
<td></td>
<td></td>
<td>(99% a.i.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Little duckweed</td>
<td>---</td>
<td>S, U</td>
<td>Furadan(^a)</td>
<td>282 mg/L</td>
<td>48-h</td>
<td>EC(_{50})</td>
<td>&gt;10,000</td>
<td>Hartman and Martin 1985</td>
<td>1,3</td>
</tr>
<tr>
<td><em>Lemna minor</em></td>
<td></td>
<td></td>
<td>(40.6% a.i.)</td>
<td>as CaCO(_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sago pondweed</td>
<td>---</td>
<td>S, U</td>
<td>Furadan(^a)</td>
<td>282 mg/L</td>
<td>14-d</td>
<td>LOEC</td>
<td>&gt;10,000</td>
<td>Hartman and Martin 1985</td>
<td>1,3</td>
</tr>
<tr>
<td><em>Potamogeton pecinatus</em></td>
<td></td>
<td></td>
<td>(40.6% a.i.)</td>
<td>as CaCO(_3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) S = Static  
\(^b\) F = Flow through  
\(^b\) M = Measured concentrations  
\(^U\) U = Unmeasured concentrations  
\(^b\) Confidence limits in parenthesis  
\(^b\) 1 = Formulation with a low percentage of carbofuran was used in testing  
\(^b\) 2 = Test duration unacceptable  
\(^b\) 3 = No detrimental effect observed.
APPENDIX D. Procedures used by the California Department of Fish and Game to assess pesticide hazards to aquatic resources.

The California Department of Fish and Game's (CDFG) Pesticide Investigations Unit (PIU) assesses the hazards of pesticides to California's aquatic resources. An important element of CDFG's hazard assessment procedure is establishing water quality criteria (WQC) for specific waters of the state using a method modified from guidelines developed by the U.S. Environmental Protection Agency (EPA 1985). The hazard assessment procedure also evaluates toxicity studies and includes only toxicity data generated in tests using accepted procedures to generate the WQC. Finally, hazard assessments evaluate the effectiveness of the WQC in protecting sensitive aquatic organisms.

Toxicity test data are obtained from the scientific literature, and from confidential laboratory reports submitted to the EPA and the California Department of Pesticide Regulation (DPR). CDFG evaluates the acceptability of the test methods used in these toxicity tests by examining the following aspects of both acute and chronic tests: 1) test method, 2) test type, 3) test species, 4) water quality maintenance and monitoring, 5) toxicant maintenance, and 6) test design. Within each of these categories as many as nine elements are used to evaluate test procedures. Studies are not required to comply with every element, but tests are rejected if they do not observe certain fundamental procedures such as maintaining proper survival of organisms in a control treatment or testing only with healthy, unstressed organisms. Studies are also rejected if they contain insufficient information to properly evaluate the tests or if the study did not follow standard testing procedures (ASTM 1980, 1987a, 1987b, 1988a, 1988b, 1989).

Data from acceptable acute and chronic toxicity studies on freshwater and saltwater organisms are used in determining a
Final Acute Value (FAV), Final Chronic Value (FCV) and Final Plant Value (FPV). The FAV is derived using the following procedure:

1. The Species Mean Acute Value (SMAV) is calculated for each species for which at least one acute value is available. The SMAV is the geometric mean of the results of all acceptable toxicity tests. When one or more life stages are available for the same species, the data for the more sensitive life stages are used to calculate the SMAV. Acute values that appeared to be questionable (i.e., that differ by more than a factor of 10 in comparison with other acute data for the same species and for other species in the same genus) are not used in calculating the SMAV.

2. The Genus Mean Acute Value (GMAV) is calculated for each genus for which one or more SMAVs are available. The GMAV is the geometric mean of the SMAVs available for the genus.

3. The GMAVs are ranked (R) from "1" for the lowest to "N" for the highest. GMAVs are arbitrarily assigned successive ranks when two or more are identical.

4. The cumulative probability (P) is calculated for each GMAV as R/(N+1).

5. The four GMAVs having cumulative probabilities closest to 0.05 are selected. If fewer than 59 GMAVs are available, these four will always be the four lowest GMAVs.

6. The FAV is calculated using the selected GMAVs and Ps, as:

\[
S^2 = \frac{3((\ln \text{GMAV})^2) - (3(\ln \text{GMAV})^2/4)}{3(P) - (3(\%P)^2/4)}
\]
\[ L = \left(3 \ln (\text{GMAV}) - S(3\%)) \right)/4 \]

\[ A = S(\%0.05) + L \]

\[ \text{FAV} = e^A \]

If sufficient data are available, the FCV is calculated using the same procedure as described for the FAV. If sufficient data are not available, the following procedure is used:

1. Chronic values are obtained by calculating the geometric mean of the NOEC and the LOEC from an acceptable chronic toxicity test.

2. Acute-Chronic Ratios (ACR) are calculated for each chronic value for which at least one corresponding appropriate acute value is available using for the numerator the geometric mean of the results of all acceptable acute tests. Whenever possible, the acute test(s) should be part of the same study as the chronic test.

3. The species mean ACR is calculated for each species as the geometric mean of all ACRs available for that species.

4. The Final ACR is calculated as the geometric mean of all the species mean ACRs available for both freshwater and saltwater species.

5. The FCV is then calculated by dividing the FAV by the Final ACR.

If no chronic toxicity data are available, the FCV can be estimated by applying a conversion factor of 0.1 to the lowest acute value.

The FPV is derived using the following procedure:
1. A plant value is the result of a 96-hour test conducted with an algae or a chronic test conducted with an aquatic vascular plant. Because standardized testing procedure have not been established for algae or aquatic vascular plants, all test durations are considered.

2. The FPV is obtained by selecting the lowest result from a test with an aquatic plant species in which the endpoint was biologically important.

   The lowest of these three values is used as the WQC. Separate WQCs can be generated for freshwater and saltwater species if toxicity differences are noted or if the specific water system is strictly saltwater or freshwater, i.e. if the water system does not include an estuary. The WQC can be lowered further to protect important sensitive species.

   Hazard assessments compare the WQC generated for specific waters with environmental concentrations determined through monitoring programs. If environmental concentrations are greater than the WQC, CDFG determines that aquatic resources are threatened and proposes hazard mitigation.

   The hazard assessment procedure is a reiterative process by which new data are evaluated to refine the WQC. Hazard assessments usually recommend additional toxicity tests with commonly used testing organisms and potentially sensitive native species.
Rotterdam Convention

Operation of the Prior Informed Consent Procedure for Banned or Severely Restricted Chemicals

Decision Guidance Document

Carbofuran

Secretariat of the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade

Food and Agriculture Organization of the United Nations

UNEP
Introduction

The objective of the Rotterdam Convention is to promote shared responsibility and cooperative efforts among Parties in the international trade of certain hazardous chemicals in order to protect human health and the environment from potential harm and to contribute to their environmentally sound use, by facilitating information exchange about their characteristics, by providing for a national decision-making process on their import and export and by disseminating these decisions to Parties. The Secretariat of the Convention is provided jointly by the United Nations Environment Programme (UNEP) and the Food and Agriculture Organization of the United Nations (FAO).

Candidate chemicals for inclusion in the prior informed consent (PIC) procedure under the Rotterdam Convention include those that have been banned or severely restricted by national regulatory actions in two or more Parties in two or more different regions. Inclusion of a chemical in the PIC procedure is based on regulatory actions taken by Parties that have addressed the risks associated with the chemical by banning or severely restricting it. Other ways might be available to control or reduce such risks. Inclusion does not, however, imply that all Parties to the Convention have banned or severely restricted the chemical. For each chemical included in Annex III of the Rotterdam Convention and subject to the PIC procedure, Parties are requested to make an informed decision whether they consent or not to the future import of the chemical.

At its eighth meeting, held in Geneva from 24 April to 5 May 2017, the Conference of the Parties agreed to list carbofuran in Annex III of the Convention and adopted the decision-guidance document with the effect that this group of chemicals became subject to the PIC procedure.

The present decision guidance document was communicated to designated national authorities on 15 September 2017 in accordance with Articles 7 and 10 of the Rotterdam Convention.

Purpose of the decision guidance document

For each chemical included in Annex III of the Rotterdam Convention, a decision guidance document has been approved by the Conference of the Parties. Decision guidance documents are sent to all Parties with a request that they make a decision regarding future import of the chemical.

Decision guidance documents are prepared by the Chemical Review Committee. The Committee is a group of government-designated experts established in line with Article 18 of the Convention, which evaluates candidate chemicals for possible inclusion in Annex III of the Convention. Decision guidance documents reflect the information provided by two or more Parties in support of their national regulatory actions to ban or severely restrict the chemical. They are not intended as the only source of information on a chemical nor are they updated or revised following their adoption by the Conference of the Parties.

There may be additional Parties that have taken regulatory actions to ban or severely restrict the chemical and others that have not banned or severely restricted it. Risk evaluations or information on alternative risk mitigation measures submitted by such Parties may be found on the Rotterdam Convention website (www.pic.int).

Under Article 14 of the Convention, Parties can exchange scientific, technical, economic and legal information concerning the chemicals under the scope of the Convention including toxicological, ecotoxicological and safety information. This information may be provided directly to other Parties or through the Secretariat. Information provided to the Secretariat will be posted on the Rotterdam Convention website.

Information on the chemical may also be available from other sources.

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1 According to the Convention, the term “chemical” means a substance, whether by itself or in a mixture or preparation and whether manufactured or obtained from nature, but does not include any living organism. It consists of the following categories: pesticide (including severely hazardous pesticide formulations) and industrial.

2 According to the Convention, the term “Party” means a State or regional economic integration organization that has consented to be bound by the Convention and for which the Convention is in force.
Disclaimer

The use of trade names in the present document is primarily intended to facilitate the correct identification of the chemical. It is not intended to imply any approval or disapproval of any particular company. As it is not possible to include all trade names presently in use, only a number of commonly used and published trade names have been included in the document.

While the information provided is believed to be accurate according to data available at the time of preparation of the present decision-guidance document, FAO and UNEP disclaim any responsibility for omissions or any consequences that may arise there from. Neither FAO nor UNEP shall be liable for any injury, loss, damage or prejudice of any kind that may be suffered as a result of importing or prohibiting the import of this chemical.

The designations employed and the presentation of material in this publication do not imply the expression of any opinion whatsoever on the part of FAO or UNEP concerning the legal status of any country, territory, city or area or of its authorities or concerning the delimitation of its frontiers or boundaries.
### Standard core set of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>&lt;</td>
<td>less than</td>
</tr>
<tr>
<td>≤</td>
<td>less than or equal to</td>
</tr>
<tr>
<td>&gt;</td>
<td>greater than</td>
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<tr>
<td>≥</td>
<td>greater than or equal to</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µm</td>
<td>micrometre</td>
</tr>
<tr>
<td>AR</td>
<td>applied radioactivity</td>
</tr>
<tr>
<td>ARfD</td>
<td>acute reference dose</td>
</tr>
<tr>
<td>a.i.</td>
<td>active ingredient</td>
</tr>
<tr>
<td>ADI</td>
<td>acceptable daily intake</td>
</tr>
<tr>
<td>AOEL</td>
<td>acceptable operator exposure level</td>
</tr>
<tr>
<td>b.p.</td>
<td>boiling point</td>
</tr>
<tr>
<td>bw</td>
<td>body weight</td>
</tr>
<tr>
<td>°C</td>
<td>degree Celsius (centigrade)</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstracts Service</td>
</tr>
<tr>
<td>cc</td>
<td>cubic centimetre</td>
</tr>
<tr>
<td>CILSS</td>
<td>Permanent Interstate Committee for Drought Control in the Sahel</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribose nucleic acid</td>
</tr>
<tr>
<td>DT₅₀</td>
<td>dissipation time 50%</td>
</tr>
<tr>
<td>EC</td>
<td>European Community</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>median effective concentration</td>
</tr>
<tr>
<td>ED₅₀</td>
<td>median effective dose</td>
</tr>
<tr>
<td>EEC</td>
<td>European Economic Community</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>EHC</td>
<td>Environmental Health Criteria</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GAP</td>
<td>Good Agricultural Practices</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>ha</td>
<td>hectare</td>
</tr>
<tr>
<td>i.m.</td>
<td>intramuscular</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>median inhibitory concentration</td>
</tr>
<tr>
<td>IFOAM</td>
<td>International Federation of Organic Movements</td>
</tr>
<tr>
<td>ILO</td>
<td>International Labour Organization</td>
</tr>
<tr>
<td>IPCS</td>
<td>International Programme on Chemical Safety</td>
</tr>
<tr>
<td>IPM</td>
<td>Integrated Pest Management</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>JMPR</td>
<td>Joint FAO/WHO Meeting on Pesticide Residues (Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues)</td>
</tr>
<tr>
<td>k</td>
<td>kilo- (x 1000)</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>Koc</td>
<td>soil organic partition coefficient</td>
</tr>
<tr>
<td>Kow</td>
<td>octanol–water partition coefficient</td>
</tr>
<tr>
<td>kPa</td>
<td>kilopascal</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LC₅₀</td>
<td>median lethal concentration</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>median lethal dose</td>
</tr>
<tr>
<td>LOAEL</td>
<td>lowest-observed-adverse-effect level</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>LOEL</td>
<td>lowest-observed-effect level</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>m</td>
<td>metre</td>
</tr>
<tr>
<td>m.p.</td>
<td>melting point</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mPa</td>
<td>millipascal</td>
</tr>
<tr>
<td>MRL</td>
<td>maximum residue limit</td>
</tr>
<tr>
<td>MTD</td>
<td>maximum tolerated dose</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>NOAEC</td>
<td>no-observed-adverse-effect concentration</td>
</tr>
<tr>
<td>NOAEL</td>
<td>no-observed-adverse-effect level</td>
</tr>
<tr>
<td>NOEC</td>
<td>no-observed-effect concentration</td>
</tr>
<tr>
<td>NOEL</td>
<td>no-observed-effect level</td>
</tr>
<tr>
<td>OECD</td>
<td>Organisation for Economic Co-operation and Development</td>
</tr>
<tr>
<td>PCPA</td>
<td>Pest Control Products Act</td>
</tr>
<tr>
<td>PEC</td>
<td>predicted environmental concentration</td>
</tr>
<tr>
<td>PHED</td>
<td>pesticide handler’s exposure database</td>
</tr>
<tr>
<td>PNEC</td>
<td>predicted no-effect concentration</td>
</tr>
<tr>
<td>Pow</td>
<td>octanol-water partition coefficient, also referred to as Kow</td>
</tr>
<tr>
<td>PMRA</td>
<td>Pest Management Regulatory Agency (Canada)</td>
</tr>
<tr>
<td>PPE</td>
<td>personal protective equipment</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million (used only with reference to the concentration of a pesticide in an experimental diet. In all other contexts the terms mg/kg or mg/L are used).</td>
</tr>
<tr>
<td>RfD</td>
<td>reference dose (for chronic oral exposure; comparable to ADI)</td>
</tr>
<tr>
<td>RMS</td>
<td>Rapporteur Member State</td>
</tr>
<tr>
<td>SMR</td>
<td>standard(ized) mortality ratio</td>
</tr>
<tr>
<td>SPC</td>
<td>Sahelian Pesticide Committee</td>
</tr>
<tr>
<td>STEL</td>
<td>short-term exposure limit</td>
</tr>
<tr>
<td>TER</td>
<td>toxicity exposure ratio</td>
</tr>
<tr>
<td>TLV</td>
<td>threshold limit value</td>
</tr>
<tr>
<td>TWA</td>
<td>time-weighted average</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environment Programme</td>
</tr>
<tr>
<td>US EPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VOC</td>
<td>volatile organic compound</td>
</tr>
<tr>
<td>w/w</td>
<td>weight for weight</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>wt</td>
<td>weight</td>
</tr>
</tbody>
</table>
Decision guidance document for a banned or severely restricted chemical

Carbofuran

Published: September 2017

1. Identification and uses (see Annex 1 for further details)

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Chemical name</td>
<td>IUPAC: 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate</td>
</tr>
<tr>
<td>and other names</td>
<td>CA: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate</td>
</tr>
<tr>
<td>or synonyms</td>
<td>PIN: 2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C_{12}H_{15}NO_{3}</td>
</tr>
<tr>
<td>Chemical structure</td>
<td></td>
</tr>
</tbody>
</table>

CAS-No.(s)          | 1563-66-2                                                                         |
Harmonized System   | 2932 99                                                                          |
Customs Code        | EINECS: 216-353-0                                                                 |
Other numbers       | CIPAC: 276                                                                        |
| Category           | Pesticide                                                                        |
Regulated category  | Pesticide                                                                        |
Use(s) in regulated category | According to the European Union (EU) notification, carbofuran was used as insecticide through incorporation into soil (at drilling) to control soil insects where maize, sugar beet or sunflowers are grown. Both references note that carbofuran can be used as acaricide, insecticide and nematicide, but during the peer review process only the insecticide use was evaluated. According to the Canadian notification carbofuran was applied using conventional ground equipment to canola, mustard, sunflower, corn (sweet, field and silage), sugar beet, green pepper, potato, raspberry, strawberry, turnip and rutabaga and could also be applied by aerial equipment to corn (field, silage and sweet), canola and mustard. According to the notifications from Cabo Verde, Chad, the Gambia, Mauritania, the Niger, Senegal and Togo\(^3\) (hereafter referred to as the CILSS countries carbofuran is used in agriculture to control a great variety of defoliators and wood boring insects which attack many fruit and vegetable crops, potatoes, corn and soybean, banana, coffee, sugar beet and rice. It is also stated to be used in forests. |
Trade names         | Trade names from the EU notification (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2006), p8): The representative formulated products for the EU evaluation were Furadan 5G, a granule (GR) and Diafurian 5G, a microgranule (MG). Trade names from the Canadian notification (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), p43): The registered carbofuran products at the

\(^3\) These seven parties share a common pesticide registration body, the Sahelian Pesticides Committee set up by the Permanent Interstate Committee for Drought Control in the Sahel (CILSS). As the CILSS member states take together decisions on the registration of pesticides at a regional level, the notifications submitted by the seven African parties refer to the same final regulatory action.
time of the risk assessment were Furadan 480 Flowable Systemic Insecticide and Furadan 480 F Systemic Liquid Insecticide.

Trade names from the notifications from the CILSS countries (UNEP/FAO/RC/CRC.11-INF-13.En, SPC (2012), p1): carbofuran is sold under the trade name of Furadan by Food Machinery Corporation (FMC Corporation), the main producer in the USA. Carbofuran is also sold under other trade names such as Carbodan, Carbosip, Chinofur, Curaterr, Furacarb, Kenafuran, Pillarfuron, Rampart, Nex, and Yaltox, Crisfuranc, and by Crystal Chemical Inter America.

**Formulation types**
The formulations in the EU notification are Furadan 5G, a granule (GR) and Diafuran 5G, a microgranule (MG, UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2006), p8). The content of carbofuran in the representative formulations is 50.5 g/kg (pure) and 50.27 g/kg (pure), respectively (EFSA (2006), p9).

The formulations in the Canadian notification (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), p43), Furadan 480 Flowable Systemic Insecticide and Furadan 480 F Systemic Liquid Insecticide, are both suspensions with a carbofuran content of 480 g/L.

The types of formulations mentioned in the CILSS notification (UNEP/FAO/RC/CRC.11-INF-13.En, SPC (2012), p1) and their content of carbofuran is not clear.

**Uses in other categories**
There is no reported use as an industrial chemical.

**Basic manufacturers**
There are two applicants mentioned in the EU notification, FMC and Dianica (EFSA (2006), p11), as well as two registrants in the Canadian notification, FMC Corporation and Bayer CropScience Inc. (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), p43). Two manufacturers are also mentioned in the CILSS notification (UNEP/FAO/RC/CRC.11-INF-13.En, SPC (2012), p1), the Food Machinery Corporation (FMC Corporation), the main producer in the USA, and Crystal Chemical Inter America.

## 2. Reasons for inclusion in the PIC procedure

Carbofuran is included in the PIC procedure as a pesticide. It is listed on the basis of the final regulatory actions taken by the European Union, Canada and the CILSS countries (for details see 2.1 below) to ban carbofuran as a pesticide.

It should be noted that the severely hazardous pesticide formulation, “Dustable powder formulations containing a combination of benomyl at or above 7 percent, carbofuran at or above 10 per cent and thiram at or above 15 percent”, is already listed in Annex III of the Convention.

No final regulatory actions relating to industrial chemical uses have been notified.

### 2.1 Final regulatory action (see Annex 2 for further details)

**European Union**

**Reason:** Human Health and the Environment

**Canada**
The CILSS countries involved are Cabo Verde, Chad, the Gambia, Mauritania, the Niger, Senegal and Togo. These seven parties share a common pesticide registration body, the Sahelian Pesticides Committee (SPC) set up by the Permanent Interstate Committee for Drought Control in the Sahel (CILSS). As the CILSS member states take together decisions on the registration of pesticides at a regional level, the notifications submitted by the seven African parties refer to the same final regulatory action.

On the recommendation of the SPC, carbofuran has been banned by the decision of CILSS Coordinating Minister N 008/MAE-MC/2015 of 08 April 2015. The decision was based on the reasons stated in Sahelian Pesticide Committee: Annex to the decision to ban Carbofuran; June 2012/reviewed in November 2014 (UNEP/FAO/RC/CRC.11/6, and UNEP/FAO/RC/CRC.11-INF-13.En, Sahelian Pesticide Committee: SPC (2012)).

### 2.2 Risk evaluation (see Annex 1 and 2 for further details)

#### European Union

**Human Health**

A risk assessment was carried out on the basis of Directive 91/414/EEC (replaced by Regulation (EC) 1107/2009). It concluded that carbofuran was not demonstrated to fulfil the safety requirements laid down in Article 5 (1) (a) and (b) of Directive 91/414/EEC (replaced by Regulation (EC) 1107/2009). The consumer risk assessment, which raised a concern about the acute exposure of vulnerable groups of consumers, in particular children, could not be finalised due to the lack of information as regards certain relevant residues (notification forms, section 2.4.2.1, p. 8) (UNEP/FAO/RC/CRC.11/6).

**Environment**

It was concluded that carbofuran was not demonstrated to fulfil the safety requirements laid down in Article 5 (1) (a) and (b) of Directive 91/414/EEC (replaced by Regulation (EC) 1107/2009). The environmental risk assessment identified a number of concerns with regard to ecotoxicology. The risk for ground water contamination was assessed to be high, but could not be concluded, in particular because the data did not provide sufficient information about a number of metabolites which have a hazardous profile. Furthermore, concerns remain as regards the risk assessment for birds and mammals, aquatic organisms, bees, non-target arthropods, earthworms, and soil non-target organisms (UNEP/FAO/RC/CRC.11/6).

#### Canada

**Human Health**

A risk assessment was carried out and published in two documents; Pest Management Regulatory Agency (PMRA) Health Canada (2010): Carbofuran – RVD2010-16 Re-evaluation Decision, 8 December 2010; Pest Management Regulatory Agency (PMRA) Health Canada (2009): Carbofuran – PRVD2009-11 Proposed Re-evaluation Decision, 31 July 2009. Based on the label directions of pesticide products containing carbofuran that were registered at the time of the review, use of the pesticide carbofuran posed an unacceptable risk to workers conducting certain mixing, loading, applying or post-application activities. An aggregate dietary risk assessment demonstrated that exposure to carbofuran from food and drinking water was unacceptable. Therefore it was concluded that carbofuran did not meet Health Canada’s current standards for human health protection (UNEP/FAO/RC/CRC.11/6).

**Environment**

In the above risk assessments, based on the label directions of pesticide products containing carbofuran that were registered at the time of the review, use of the pesticide carbofuran posed an unacceptable risk to terrestrial and aquatic organisms, and therefore did not meet Health Canada’s standards for environmental protection.

Additionally, thirty three environmental incident reports from the United States and Canada were considered during the review of carbofuran, and indicated that exposure to carbofuran under the registered use pattern resulted in avian, small wild mammal and bee mortality (UNEP/FAO/RC/CRC.11/6).
### CILSS countries

#### Human health and the environment

Carbofuran presents risks to human health and especially to non-target organisms in the environment, making it very difficult to handle it without risks for users in Sahelian countries. These risks have justified its ban in many countries of the world among which include the European Union member states.

A consultation mission conducted on behalf of the Sahelian Pesticide Committee (SPC) concluded that the SPC should stop the registration of the pesticides of toxicity class Ib since they are used by poorly trained small farmers who don’t respect the safety measures (CILSS countries supporting documentation p. 32 paragraph 4.2.4).

The Sahelian Pesticide Committee stopped the registration of carbofuran-based pesticides in CILSS countries in 2006 taking into account:

- The fragile ecology of CILSS countries already characterized by an imbalance of ecosystems and the disappearance of organisms useful to the environment;
- Non-compliance with recommended measures for a safe use of carbofuran by users in the context of CILSS countries;
- The presence of pesticide residues in harvested crops and the behaviour of local people make the risk unacceptable.

Further to the pollution of Sahel ground water which constitutes the main drinking water resource with open wells, several sources agree that carbofuran is highly toxic to birds. One single grain may kill a bird (oral LD₅₀ of 0.4 mg/kg body weight). Carbofuran is highly toxic to fresh water invertebrates and moderately to highly toxic to fresh water fish (UNEP/FAO/RC/CRC.11/6).

### 3. Protective measures that have been applied concerning the chemical

#### 3.1 Regulatory measures to reduce exposure

**European Union**
The complete entry into force of all provisions of Commission Decision 2007/416/EC of 13 June 2007 was 13 December 2008 since all uses of plant protection products containing carbofuran were prohibited as from that date at the latest (notification form).

**Canada**
Sale of pesticides products containing carbofuran was prohibited in Canada effective December 31, 2010. The use of pesticide products containing carbofuran was prohibited after December 31, 2012. Pesticide products containing carbofuran can no longer be used in Canada (notification form).

**CILSS countries**
On the recommendation of the Sahelian Pesticides Committee (SPC), carbofuran was banned by the decision of CILSS Coordinating Minister N 008/MAE-MC/2015 of 08 April 2015. Carbofuran products can no longer be used in the CILSS countries (notification form).

#### 3.2 Other measures to reduce exposure

**European Union**
None reported – none required since all uses of plant protection products containing carbofuran were prohibited in the EU.

**Canada**
None reported – none required since pesticide products containing carbofuran can no longer be used in Canada.

**CILSS countries**
None reported – none required since carbofuran products can no longer be used in the CILSS countries.
3.3 Alternatives

It is essential that before a country considers substituting alternatives, it ensures that the use is relevant to its national needs, and the anticipated local conditions of use. The hazards of the substitute materials and the controls needed for safe use should also be evaluated.

European Union
No information on alternatives was provided in the EU notification or in the supporting documentation.

Canada
At the time of the regulatory action, registered alternative products were available for some uses of carbofuran, however, for canola, mustard, raspberry, strawberry and sugar beet, there were no registered (or viable) alternative active ingredients to carbofuran for the control of certain pests (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada 2008 and 2010).

CILSS countries
Chemical alternatives: Several alternative pesticides to carbofuran were identified by CILSS countries. The Indian Committee of pesticide experts recommended the following pesticides on paddy rice and other crops: chlorantraniliprole, flubendiamide and quinalphos.

According to Jon Tollefson and Erin Hodgson, from the Department of Entomology of IOWA State University in the USA, the alternative for the protection of corn against root worms is to add seeds treated with a neonicotinoid pesticide like Poncho™ in the applicator. In case of post-emergence liquid treatment Lorsban™ 4E, an ethylchlorpyriphos-based formulation is an option. Currently five formulations authorized by the Sahelian Pesticide Committee under the name of Dursban are ethylchlorpyriphos-based.


Integrated Pest and production management (IPPM): The experience in IPPM launched by FAO in collaboration with the Ministries of Agriculture in several countries of the Sahel yielded important results in agricultural production and pest management. This initiative of Good Agricultural Practices (GAP) will improve the agricultural productivity and train several growers who are potential facilitators. According to the CILSS IPPM is based on the following principles:
- A sound and judicious use of pesticides ;
- The acquisition of knowledge and practical skills critical to pest control;
- The reinforcement of decision-making capacity of growers at a field level;
- The development of a better low-cost productivity which protects the environment (UNEP/FAO/RC/CRC.11-INF-12.En, SPC (2012), p1).

General
There are a number of alternative methods involving chemical and non-chemical strategies, including alternative technologies available, depending on the individual crop-pest complex under consideration. Countries should consider promoting, as appropriate, integrated pest management (IPM), agroecology and organic agriculture as a means of reducing or eliminating the use of hazardous pesticides.

Advice may be available through National IPM focal points, the FAO, International Federation of Organic Movements (IFOAM), and agricultural research or development agencies. Where it has been made available by governments, additional information on alternatives to carbofuran may be found on the Rotterdam Convention website www.pic.int.

3.4 Socio-economic effects

European Union
No information on socio-economic effects was reported.

Canada
No information on socio-economic effects was reported.

CILSS countries
No information on socio-economic effects was reported.
## 4. Hazards and Risks to human health and the environment

### 4.1 Hazard Classification

<table>
<thead>
<tr>
<th>WHO / IPCS</th>
<th>Highly hazardous (Class 1b) (UN classification)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IARC</td>
<td>Group 1 Acetylcholinesterase (AChE) inhibitors, 1A Carbamates (Canadian notification).</td>
</tr>
<tr>
<td><strong>European Union</strong></td>
<td>Classification of the EU in accordance with Council Directive 67/548/EEC</td>
</tr>
<tr>
<td></td>
<td>T+  - Very toxic.</td>
</tr>
<tr>
<td></td>
<td>R26 - Very toxic by inhalation.</td>
</tr>
<tr>
<td></td>
<td>R28 - Very toxic if swallowed.</td>
</tr>
<tr>
<td></td>
<td>N   - Dangerous for the environment.</td>
</tr>
<tr>
<td></td>
<td>R50/53 - Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.</td>
</tr>
<tr>
<td></td>
<td>Classification of the EU according to Regulation (EC) No 1272/2008, which implements the UN GHS in the European Union</td>
</tr>
<tr>
<td></td>
<td><strong>Acute Tox. 2 * - H330 - Fatal if inhaled.</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Acute Tox. 2 * - H300 - Fatal if swallowed.</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Aquatic Acute 1 - H400 - Very toxic to aquatic life.</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Aquatic Chronic 1 - H410 - Very toxic to aquatic life with long lasting effects.</strong></td>
</tr>
<tr>
<td></td>
<td>(* = This classification shall be considered as a minimum classification.)</td>
</tr>
<tr>
<td><strong>US EPA</strong></td>
<td>Classification of the USEPA according to the USEPA’s 2007 Reregistration Eligibility Decision for Carbofuran</td>
</tr>
<tr>
<td></td>
<td>Acute oral toxicity category I: Highly acutely toxic</td>
</tr>
<tr>
<td></td>
<td>Acute dermal toxicity category III: Slightly acutely toxic</td>
</tr>
<tr>
<td></td>
<td>Acute inhalation toxicity category I: Highlly acutely toxic</td>
</tr>
<tr>
<td></td>
<td>Acute eye irritation category III: Minimal irritation</td>
</tr>
<tr>
<td></td>
<td>Primary dermal irritation category IV: Mild or slight irritation</td>
</tr>
<tr>
<td></td>
<td>Skin sensitization: Non sensitizer</td>
</tr>
</tbody>
</table>
### 4.2 Exposure limits


<table>
<thead>
<tr>
<th>Commodity</th>
<th>MRL (mg/kg)</th>
<th>Year of adoption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana</td>
<td>0.1 mg/Kg</td>
<td>2013</td>
</tr>
<tr>
<td>Meat of cattle, goats, horses, pigs and sheep</td>
<td>0.05 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Horse fat</td>
<td>0.05 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Cattle fat</td>
<td>0.05 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Goat fat</td>
<td>0.05 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Maize</td>
<td>0.05 mg/Kg</td>
<td>2005</td>
</tr>
<tr>
<td>Rape seed</td>
<td>0.05 mg/Kg</td>
<td>2004</td>
</tr>
<tr>
<td>Sheep fat</td>
<td>0.05 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Pig fat</td>
<td>0.05 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td><strong>Edible offal of cattle, goats, horses, pigs &amp; sheep</strong></td>
<td>0.05 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Sorghum</td>
<td>0.1 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Sunflower seed</td>
<td>0.1 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Sugar cane</td>
<td>0.1 mg/Kg</td>
<td>2011</td>
</tr>
<tr>
<td>Spices, roots and rhizomes</td>
<td>0.1 mg/Kg</td>
<td>2004</td>
</tr>
<tr>
<td>Cotton seed</td>
<td>0.1 mg/Kg</td>
<td>2004</td>
</tr>
<tr>
<td>Rice, Husked</td>
<td>0.1 mg/Kg</td>
<td>2004</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>0.2 mg/Kg</td>
<td>2005</td>
</tr>
<tr>
<td>Mandarin</td>
<td>0.5 mg/Kg</td>
<td>2010</td>
</tr>
<tr>
<td><strong>Oranges, Sweet, Sour (including Orange-like hybrids): several cultivars</strong></td>
<td>0.5 mg/Kg</td>
<td>2010</td>
</tr>
<tr>
<td><strong>Sorghum straw and fodder, Dry</strong></td>
<td>0.5 mg/Kg</td>
<td>2001</td>
</tr>
<tr>
<td><strong>Rice straw and fodder, Dry</strong></td>
<td>1 mg/Kg</td>
<td>2004</td>
</tr>
<tr>
<td>Coffee beans</td>
<td>1 mg/Kg</td>
<td>1999</td>
</tr>
<tr>
<td>Citrus pulp, Dry</td>
<td>2 mg/Kg</td>
<td>2001</td>
</tr>
</tbody>
</table>

(*) At or about the limit of determination

(#) Based on the use of carbosulfan

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### Other information

The CODEX Pesticide Residues in Food Online database reference above also contains the following information:

- **Acceptable Daily Intake (ADI)/PTDI**: 0-0.001 mg/kg body weight (2008)
- **Residue definition**: Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant and animal commodities: carbofuran and 3-hydroxycarbofuran expressed as carbofuran. The residue is not fat-soluble


[Carbofuran.pdf](http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report09/Carbofuran.pdf) contains the following information on **Acceptable Daily Intake (ADI)/Acute Reference Dose (ARfD)**.

A periodic review of the toxicology of carbofuran was carried by the 1996 JMPR. An ADI of 0–0.002 mg/kg bw was established. In 2002, an ARfD of 0.009 mg/kg bw was established. The 2008 JMPR evaluated newly submitted studies on acute toxicity and re-examined relevant data which had been considered by previous Meetings. The 2008 Meeting established an **ARfD of 0.001 mg/kg bw**. The Meeting noted that this ARfD was lower than the current ADI of 0–0.002 mg/kg bw. The Meeting 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**.

A periodic review of the residue and analytical aspects of both carbofuran and carbosulfan was carried out by the 1997 JMPR. The carbofuran residue is defined as carbofuran + 3-
hydroxycarbofuran for compliance with MRLs. For the purposes of dietary intake, the residue definition for carbofuran arising from use of carbosulfan and carbofuran is carbofuran + free and conjugated 3-hydroxycarbofuran, expressed as carbofuran. The analytical methods include an acid hydrolysis step to release the conjugate.

**European Union**

**MRLs** The EU notification (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA 2006), p25, as well as EFSA(2009), p40 reports that MRLs for carbofuran residues, defined as sum of carbofuran and 3-hydroxycarbofuran expressed as carbofuran equivalents, have been proposed by the Rapporteur Member State (RMS) at the LOQ level. This results in different MRLs proposed by the RMS for the same crop, since the proposal is based on the respective LOQ reached in the residue trials submitted by the two different applicants.

Sugar beet 0.02* mg/kg (based on Dianica studies); 0.1* mg/kg (based on FMC studies)
Maize 0.02* mg/kg (based on Dianica studies); 0.1* mg/kg (based on FMC studies)
Sunflower seed 0.02* mg/kg (based on Dianica studies)

It was noted that the data base (per applicant) from which the MRL proposals are derived was not complete according to current requirements and consequently the MRL proposals should be considered as provisional.


**EU Risk Assessment Acceptable Daily Intake** (ADI) = 0.00015 mg/kg bw/day. This is based on the LOAEL of 0.03 mg/kg bw/day in pups on post-natal day 11 from the acute neurotoxicity study in rats for brain Acetylcholinesterase (AChE) inhibition. An uncertainty factor of 200 to account for inter- and intra-species variation, and to extrapolate to a NOAEL was applied.

**EU Risk Assessment Provisional Acceptable Operator Exposure Level** (AOEL) = 0.0003 mg/kg bw/day. This is based on the NOAEL of 0.03 mg/kg bw/day in adults from the acute neurotoxicity study in rats for brain AChE inhibition. The adult NOAEL was considered to be the most representative value for exposure to carbofuran for operators. An uncertainty factor of 100, to account for inter- and intra-species variation, was applied.

**EU Risk Assessment Provisional Acute Reference Dose** (ARfD) = 0.00015 mg/kg bw/day. This is based on the LOAEL of 0.03 mg/kg bw/day in pups on post-natal day 11 from the acute neurotoxicity study in rats for brain AChE inhibition. An uncertainty factor of 200 to account for inter- and intra-species variation, and to extrapolate to a NOAEL was applied.

**Canada** (the following has been taken from UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), p17-19)

**Determination of Acceptable Daily Intake** to estimate dietary risk from repeat exposure to carbofuran, the two acute oral cholinesterase activity studies in the rat (as discussed under 3.3.1 Determination of Acute Reference Dose) were selected for risk assessment. The quick-acting and reversible nature of carbamate inhibition is considered as justification to default to the acute LOAEL which is lower than the subchronic or chronic NOAELs. In the case of carbofuran, long-term daily exposures are considered as multiple daily exposures with each causing transient inhibition of cholinesterase with potential resulting toxicity. Standard uncertainty factors of 10-fold for interspecies extrapolation and 10-fold for intra-species variability were applied along with an additional 3-fold uncertainty factor because a NOAEL was not achieved in these studies. With respect to the Pest Control Products Act (PCPA) factor, all of the required studies relevant to assessing risks to infants and children were available for this assessment. Accordingly, the PCPA factor was reduced to 1-fold and the composite assessment factor was 300. ADI = 0.05 mg/kg bw/day/300 = 0.0002 mg/kg bw/day.

This ADI provides a margin of safety of >2,500 to the developmental NOAEL (decreased viability), >500 to the lowest NOAEL for testicular effects and >1,000 to the lowest LOAEL for maternal toxicity. It is thus considered protective of all populations including men, pregnant women, infants and children.

**Determination of Acute Reference Dose** To estimate acute dietary risk (1 day), the LOAEL of 0.05 mg/kg bw was selected from the two acute oral cholinesterase activity studies in the rat based on cholinesterase inhibition. Standard uncertainty factors of 10-fold for interspecies extrapolation and 10-fold for intra-species variability were applied along with an additional 3-fold uncertainty factor because a NOAEL was not achieved in these studies. With respect to the PCPA factor, all of the required studies relevant to assessing risks to infants and children were available for this assessment. Accordingly, the PCPA factor was reduced to 1-fold and the composite assessment factor was 300. ARfD = 0.05 mg/kg bw / 300 = 0.0002 mg/kg bw.

**WHO drinking water guideline**
On the basis of the JMPR ADI (2.2 µg/kg of body weight, if not rounded) and assuming a 60-kg body weight, drinking-water consumption of 2 litres/day and an allocation of 10% of the ADI to drinking-water, a guideline value of 7 µg/litre (rounded figure) can be calculated for carbofuran (WHO 2004, 2011).

<table>
<thead>
<tr>
<th>4.3 Packaging and labelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>The United Nations Committee of Experts on the Transportation of Dangerous Goods classifies the chemical in:</td>
</tr>
<tr>
<td>Hazard Class and Packing Group:</td>
</tr>
<tr>
<td>- Hazard Class: 6.1</td>
</tr>
<tr>
<td>- Packing Group: I, II and III- IMDG Code: UN No.2757</td>
</tr>
<tr>
<td>For further information on the classification of mixtures, special provisions and packing instructions see United Nations (2015).</td>
</tr>
<tr>
<td>It is recommended to follow the FAO Guidelines on good labelling practice for pesticides (FAO 2015)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>International Maritime Dangerous Goods (IMDG) Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>For carbofuran (pure substance):</td>
</tr>
<tr>
<td>UN No. 2757</td>
</tr>
<tr>
<td>Carbamate pesticide, solid, toxic (carbofuran)</td>
</tr>
<tr>
<td>Class 6.1</td>
</tr>
<tr>
<td>Marine pollutant, taken from the TEC (<a href="http://www.inchem.org/documents/icsc/icsc/eics0122.htm">http://www.inchem.org/documents/icsc/icsc/eics0122.htm</a>)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transport Emergency Card</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEC (R)-61GT7-I (<a href="http://www.inchem.org/documents/icsc/icsc/eics0122.htm">http://www.inchem.org/documents/icsc/icsc/eics0122.htm</a>)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4.4 First aid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NOTE: The following advice is based on information available from the World Health Organisation and the notifying countries and was correct at the time of publication. This advice is provided for information only and is not intended to supersede any national first aid protocols.</strong></td>
</tr>
</tbody>
</table>

The following has been taken from the FAO/WHO Data Sheets on Pesticides No 56 Carbofuran, published in an Annex to the DGD for the severely hazardous pesticide formulation, i.e. dustable powder formulations containing a combination of benomyl at or above 7 percent, carbofuran at or above 10 per cent and thiram at or above 15 percent (FAO/UNEP (2004/2005), also available at http://www.pic.int/Portals/5/DGDs/DGD_Dustable%20powder%20formulations_EN.pdf)

**EMERGENCY AID**

**General** - Carbofuran is a carbamate pesticide of very high toxicity. It is an acute poison, absorbed by inhalation of dust and spray mist; from the gastrointestinal tract; and, to a lesser extent, through the intact skin. Most formulations should be handled by trained personnel wearing suitable protective clothing.

**Early symptoms of poisoning** - Early symptoms of poisoning may include headache, weakness, giddiness and nausea. Later there may be perspiration, stomach pains, blurred vision, excessive salivation, slurred speech, and muscle twitching, tremor, diarrhoea and vomiting.

**Treatment before person is seen by a physician, if these symptoms appear following exposure** - The person should stop work immediately, remove contaminated clothing and wash the affected skin with soap and water, if available, and flush the area with large quantities of water. If swallowed, vomiting should be induced immediately if the person is conscious. In the event of collapse, artificial respiration should be given, preferably by mechanical means. If mouth-to-mouth resuscitation is used vomit may contain toxic amounts of carbofuran. If the eyes are contaminated, flush them with water for at least 15 minutes. If carbofuran is inhaled, remove victim to fresh air immediately (FAO/UNEP 2004/2005).

**MEDICAL DIAGNOSIS AND TREATMENT IN CASES OF POISONING**

**General information** - Carbofuran is a carbamate insecticide of very high toxicity. It is absorbed from the gastrointestinal tract and by inhalation, and only to a limited extent through the intact skin. Its mode of action is by reversible inhibition of acetyl cholinesterase. Erythrocyte cholinesterase is more inhibited than plasma cholinesterase. Symptoms of mild poisoning are short lasting and in case of occupational over-exposure occur without delay and at doses well below the fatal dose. Because of its rapid metabolism and excretion it does not accumulate in the tissues.
Symptoms and signs - Symptoms of poisoning include excessive sweating, headache, chest tightness, weakness, giddiness, nausea, vomiting, stomach pains, salivation, blurred vision, slurred speech and muscle twitching. Paraesthesia and mild skin reactions have also been reported. Diagnosis can be based on a recent history of activities and non-reactive pupils of the eyes.

Laboratory - Because carbofuran is a reversible inhibitor of cholinesterase, measurements of cholinesterase activity should be made by a method which minimizes the reactivation of inhibited enzyme. Erythrocyte cholinesterase determination is more informative than either plasma or whole blood cholinesterase, but the enzyme will only be inhibited for a short time (few hours) after exposure. The presence of metabolites of carbofuran in urine is also indicative of exposure.

Treatment - If the pesticide has been ingested, unless the patient is vomiting, rapid gastric lavage should be performed using 5% sodium bicarbonate, if available. For skin contact, the skin should be washed with soap and water. If the compound has entered the eyes, they should be washed with isotonic saline or water. Since the symptoms of poisoning with carbofuran are of short duration, atropine treatment is usually not necessary by the time the patient reaches a place where this antidote is available. Where there are manifest symptoms 1-2 mg of atropine sulfate (adult dose) may be given intramuscularly or even intravenously and repeated as necessary. Care should be taken to avoid overdosage of atropine, especially when treating children. In extreme cases, if the patient is unconscious or is in respiratory distress, oxygen may be required. Provide patient support as required, including; suction of secretions, maintenance of airways, intravenous fluids pro re nata and bladder catheterization. Morphine, aminophylline, phenothiazines, reserpine, furosemide and ethacrynic acid are contraindicated. Pralidoxime chloride is of doubtful value but if muscle weakness is severe a dilute solution may be given cautiously intravenously. If convulsions occur diazepam may be given, the patient must be monitored for respiratory depression and hypotensive reactions.

Prognosis - If the acute toxic effect is survived, the chances of complete recovery are very good. (FAO/UNEP (2004/2005)

The transport emergency card (http://www.inchem.org/documents/icsc/icsc/eics0122.htm) offers the following advice following exposure.

IN ALL CASES CONSULT A DOCTOR!
For inhalation - Fresh air, rest. Artificial respiration may be needed. Refer for medical attention. See Notes.
For spills on the skin - Remove contaminated clothes. Rinse and then wash skin with water and soap.
For splashing into the eye - First rinse with plenty of water for several minutes (remove contact lenses if easily possible), then refer for medical attention.
For ingestion - Give a slurry of activated charcoal in water to drink. Refer for medical attention. See Notes.
Notes - Specific treatment is necessary in case of poisoning with this substance; the appropriate means with instructions must be available.
Do NOT take working clothes home.
Carrier solvents used in commercial formulations may change physical and toxicological properties. If the substance is formulated with solvents also consult the ICSCs of these materials.

4.5 Waste management

Regulatory actions to ban a chemical should not result in creation of a stockpile requiring waste disposal. For guidance on how to avoid creating stockpiles of obsolete pesticides the FAO following guidelines are available: Guidelines on Prevention of Accumulation of Obsolete Pesticide Stocks (FAO, 1995), The Pesticide Storage and Stock Control Manual (FAO, 1996a) and Guidelines for the management of small quantities of unwanted and obsolete pesticides (FAO, 1999).

In all cases waste should be disposed in accordance with the provisions of the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal (1996), any guidelines thereunder, and any other relevant regional agreements.

It should be noted that the disposal/destruction methods recommended in the literature are often not available in, or suitable for, all countries; e.g., high temperature incinerators may not be available. Consideration should be given to the use of alternative destruction technologies. Further information on possible approaches may be found in the FAO Technical Guidelines for the Disposal of Bulk Quantities of Obsolete Pesticides in Developing Countries (FAO, 1996b).
Annexes

Annex 1  Further information on the substance
Annex 2  Details on Final regulatory action
Annex 3  Address of designated national authorities
Annex 4  References
Annex 1  Further information on the substance

Introduction
The information presented in this Annex reflects the conclusions of the notifying parties in three prior informed consent (PIC) regions: Europe (European Union), North America (Canada) and Africa (Cabo Verde, Chad, the Gambia, Mauritania, the Niger, Senegal and Togo). Summaries of the notifications were included in PIC Circular XXXV of June 2012, PIC Circular XL of December 2014 and PIC Circular XLI of June 2015, respectively.

Where possible, information on hazards provided by the notifying parties has been presented together, while the evaluation of the risks, specific to the conditions prevailing in the notifying Parties are presented separately. This information has been taken from the documents referenced in the notifications in support of their final regulatory actions to ban carbofuran from the European Union (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA Scientific Report 2006), Canada (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada 2009, 2010) and CILSS countries (UNEP/FAO/RC/CRC.11-INF-13.En, the Sahelian Pesticide Committee 2012).

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4 These seven parties share a common pesticide registration body, the Sahelian Pesticides Committee set up by the Permanent Interstate Committee for Drought Control in the Sahel (CILSS). As the CILSS member states take together decisions on the registration of pesticides at a regional level, the notifications submitted by the seven African parties refer to the same final regulatory action.
1. **Physico-Chemical properties (most of the information has been sourced from the EU notification UNEP/FAO/RC/CRC.11-INF-11.En and EFSA (2006), pp 51-53, except where indicated some additional is from the Canadian notification UNEP/FAO/RC/CRC.11-INF-12.En and Health Canada (2009) p10 – the former indicates these have been sourced from the Pesticides Manual, thirteenth edition, 2004)**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAS: 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (EFSA (2006), p50, Health Canada (2009), p9)</td>
</tr>
<tr>
<td><strong>1.2 Formula</strong></td>
<td>C_{12}H_{15}NO_{3} (UNEP/FAO/RC/CRC.11-INF-11.En EFSA (2006), p50;</td>
</tr>
<tr>
<td><strong>1.3 Colour and Texture</strong></td>
<td>Arysta: white crystalline solid, odourless (purified active substance)</td>
</tr>
<tr>
<td></td>
<td>FMC: off-white powder, aromatic acid-like odour (99.3%)</td>
</tr>
<tr>
<td><strong>1.4 Melting point</strong></td>
<td>Dianica: melting point 153.1°C (98.2%)</td>
</tr>
<tr>
<td></td>
<td>FMC: melting range 151.2 – 153.7°C (99.3%)</td>
</tr>
<tr>
<td><strong>1.5 Boiling Point</strong></td>
<td>Dianica: boiling with partial decomposition at 276°C (98.2%)</td>
</tr>
<tr>
<td></td>
<td>FMC: boiling at 254.1°C (no decomposition) (99.6%)</td>
</tr>
<tr>
<td><strong>1.6 Relative Density (g/cm³)</strong></td>
<td>Dianica: D_{20}^{20} = 1.228 (98.2%)</td>
</tr>
<tr>
<td></td>
<td>FMC: D_{22}^{22} = 1.290 (99.3%)</td>
</tr>
<tr>
<td></td>
<td>1.18 at 20°C</td>
</tr>
<tr>
<td><strong>1.7 Vapour Pressure</strong></td>
<td>Dianica: 2.25 X 10^-4 Pa at 20°C</td>
</tr>
<tr>
<td></td>
<td>FMC: 8 X 10^-8 Pa at 25°C</td>
</tr>
<tr>
<td></td>
<td>0.031 mPa at 20°C, 0.072 mPa at 25°C (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009) p10)</td>
</tr>
<tr>
<td><strong>1.8 Henry’s Law Constant</strong></td>
<td>Dianica: 1.58 X 10^2 Pa.m.mol^-1 at 20°C</td>
</tr>
<tr>
<td></td>
<td>FMC: 5 X 10^2 Pa.m.mol^-1 at 25°C</td>
</tr>
<tr>
<td></td>
<td>2.50 X10^-10 atm.m.mol^-1</td>
</tr>
<tr>
<td><strong>1.9 Solubility in Water</strong></td>
<td>Dianica: 315 mg/L at 19.5 ± 2.0°C, no effect of pH</td>
</tr>
<tr>
<td></td>
<td>FMC: 322 mg/L at 20.0 ± 0.5°C, no effect of pH</td>
</tr>
<tr>
<td></td>
<td>320 mg/L at 20°C, 351 mg/L at 25°C (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), p10)</td>
</tr>
<tr>
<td><strong>1.10 Solubility in Organic Solvents</strong></td>
<td>Dianica: solubility at 20°C (g/L) n-heptane 0.1, xylene 7.8, 1.2-dichloroethane 106.5, methanol 71.0, acetone 107.0, ethyl acetate 66.9.</td>
</tr>
<tr>
<td></td>
<td>FMC: solubility at 20°C (g/L) n-heptane 0.13, xylene 8.0, 1.2-dichloroethane 91.0, methanol 72.8, acetone 103.4, ethyl acetate 56.1.</td>
</tr>
<tr>
<td></td>
<td>In dichloromethane &gt;200, isopropanol 20-50, toluene 1-20 (all in g/L, 20°C) (Canadian notification form).</td>
</tr>
<tr>
<td><strong>1.11 Partition coefficient (log Kow)</strong></td>
<td>Dianica: 1.8 at 20°C, no effect of pH</td>
</tr>
<tr>
<td></td>
<td>FMC: 1.62 at 22°C, no effect of pH</td>
</tr>
<tr>
<td><strong>1.12 Dissociation Constant</strong></td>
<td>Dianica: no pKa in environmentally relevant pH range</td>
</tr>
<tr>
<td></td>
<td>FMC: no pKa in environmentally relevant pH range</td>
</tr>
<tr>
<td><strong>1.13 Surface tension</strong></td>
<td>Dianica: 48.9 mN/m at 20.3°C (90% saturated solution)</td>
</tr>
<tr>
<td></td>
<td>FMC: 54.7 mN/m at 20°C (90% saturated solution)</td>
</tr>
<tr>
<td><strong>1.14 Hydrolytic stability (DT50)</strong></td>
<td>Dianica: pH 4: hydrolytically stable; pH 7, 25°C: DT_{50} = 45.7 d; pH 9, 25°C: DT_{50} = 0.1 d</td>
</tr>
<tr>
<td></td>
<td>FMC: pH 7, 25°C: DT_{50} = 28 d; pH 7.5, 25°C: DT_{50} = 9.1 d; pH 8, 25°C: DT_{50} = 2.7 d</td>
</tr>
</tbody>
</table>
2 Toxicological properties

2.1 General

2.1.1 Mode of Action
Carbofuran is a broad spectrum, non-cumulative carbamate insecticide of very high toxicity. It is absorbed from the gastrointestinal tract and by inhalation, and only to a limited extent through the intact skin. Its mode of action is by reversible inhibition of acetyl cholinesterase. Erythrocyte cholinesterase is more inhibited than plasma cholinesterase. Symptoms of mild poisoning are short lasting and in case of occupational over-exposure occur without delay and at doses well below the fatal dose. Because of its rapid metabolism and excretion it does not accumulate in the tissues (FAO/UNEP (2004/2005), available at http://www.pic.int/Portals/5/DGDs/DGD_Dustable%20powder%20formulations_EN.pdf).

2.1.2 Symptoms of poisoning
Early symptoms of poisoning may include headache, weakness, giddiness and nausea. Later there may be perspiration, stomach pains, blurred vision, excessive salivation, slurred speech, and muscle twitching, tremor, diarrhoea and vomiting. Symptoms of poisoning also include excessive sweating, chest tightness, weakness, giddiness and nausea. Paraesthesia and mild skin reactions have also been reported. Diagnosis can be based on a recent history of activities and non-reactive pupils of the eyes (FAO/UNEP (2004/2005), available at http://www.pic.int/Portals/5/DGDs/DGD_Dustable%20powder%20formulations_EN.pdf).

2.1.3 Absorption, distribution, excretion and metabolism in mammals

**European Union**
Carbofuran is rapidly and completely absorbed and excreted in the rat (32 hours after dosing, 83% of the administered dose was excreted, and 96 hours after a dose, 92% and <4% were excreted in urine and faeces, respectively). In man, the two formulations have a dermal absorption value of 10%. Distribution is rapid, with the liver having the maximum concentration after 1 hour, and accumulation does not occur. Carbofuran is metabolized to form 3-hydroxy-carbofuran and then glucuronic acid, of which the latter is excreted in the bile. Enterohepatic recirculation may occur. Hydrolysis and hydroxylation of 3-hydroxy-carbofuran also yield 3-hydroxy-carbofuran-7-phenol and 3-ketocarbofuran, respectively, the latter is subsequently hydrolysed to 3-ketocarbofuran-7-phenol. These three metabolites are conjugated and excreted primarily in the urine. Oxidation of carbofuran to N-OH-methylcarbofuran also occurs, which is then hydroxylated to 3-OH-N-OH-methylcarbofuran and then carbon dioxide, which is excreted in expired air (UNEP/FAO/RC/CRC.11/6).

The EU notification adds that 92% of phenyl part is excreted within 48 h mainly via urine (89%) and faeces (2.5%); carbamate moiety excreted within 32 h in air as CO2. Carbofuran and metabolites with the carbamate moiety are the toxicologically significant compounds (animals, plants and environment) (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2006), p60).

**Canada**
Carbofuran was rapidly absorbed, metabolized and eliminated mainly in the urine after oral administration to mice and rats. The first step in the metabolic pathway is hydroxylation of carbofuran to 3-hydroxy-carbofuran then oxidation resulting in the formation of 3-ketocarbofuran. Breakage of the carbamate ester linkage results in liberation of the phenolic derivatives and their corresponding conjugates, principally glycosides. These degradation products are then excreted mainly as conjugates of glucuronic acid and sulfate. The most common carbamate metabolites are 3-hydroxy-carbofuran and 3-ketocarbofuran. There were no sex differences noted in the absorption, distribution, metabolism or excretion of carbofuran. Most metabolites
were found to be significantly less toxic than the parent compound in acute oral lethality tests. One metabolite 3-hydroxycarbofuran showed similar acute oral toxicity as carbofuran (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009, p11).

2.2 Toxicology studies

2.2.1 Acute toxicity

**European Union**
Carbofuran:
- is very toxic by ingestion (LD$_{50}$ 7 mg/kg bw);
- and by inhalation (LC$_{50}$ 0.05 mg/L);
- whereas toxicity during dermal exposure is moderate (LD$_{50}$ 1000-2000 mg/kg bw);
- Carbofuran is not a skin irritant, eye irritant, or skin sensitizer, but mortality was reported after exposure to eyes (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA, 2009, pp16-17).

**Canada**
In acute toxicity studies, carbofuran was highly toxic via the oral route of exposure in rats but showed low dermal toxicity. Acute inhalation studies were not available. Carbofuran was a minimal eye irritant and was not a dermal sensitizer. The acute effects observed in oral studies were typical for cholinesterase inhibition: ataxia, salivation, lacrimation, exophthalmos, hyperpnea, cyanosis and generalized tremors. As with other carbamate compounds, carbofuran’s cholinesterase-inhibiting effect is short-term and reversible (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009, p11).

**CILSS countries**
Carbofuran belongs to WHO class Ib (highly hazardous). Some formulations belong to class I (highly hazardous or extremely hazardous) or to class II (moderately hazardous). It is extremely toxic via oral route and by inhalation (LD$_{50}$ is 5 to 13 mg/kg in rats, 2 mg/kg in mice). Dermal toxicity is low. It is minimally irritating to the eyes and to the skin. It is not a skin sensitizer. Thermal degradation may release toxic vapours. Among all pesticides used in crops carbofuran presents the most acute toxicity to human health, apart from aldicarb and parathion. It is neurotoxic being a cholinesterase inhibitor. This is of short duration and reversible. A person exposed to doses higher than 0.25 mg/kg of body weight may present such symptoms as: salivation, abdominal pains, sleepiness, dizziness, anxiety, vomiting, loss of control, even coma and cardiac arrest. It is a strong endocrine disruptor which may affect the concentration of several human and animal hormones even at very low doses UNEP/FAO/RC/CRC.11-INF-13.En,SPC (2012).

2.2.2 Short term toxicity

**European Union**
The overall oral short term no-observed-adverse-effect-level (NOAEL) is 0.1 mg/kg bw/day from the 1-year dog studies with the NOAELs of 0.1 and 0.25 mg/kg bw/day, based on red blood cell (RBC) AChE inhibition and clinical signs of neurotoxicity and testicular degeneration (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA, 2009, p17).

**Canada**
In repeat-dose dietary studies in various species (mouse, rat and dog), the dog appeared to be the most sensitive species with respect to cholinergic symptoms. Cholinesterase inhibition was seen in all species with the mouse being the least sensitive. Inhibition of cholinesterase activity is also seen via the dermal route of entry in the rabbit. Repeat-dose inhalation studies were not available. No gender sensitivities were seen in repeat-dose dietary studies. Additional effects noted in the repeat-dose dietary studies include: a decrease in weight gain in mice and rats and testicular effects in dogs. The rodent studies highlight the differences between gavage and dietary dosing as animals tolerated
chronic dietary dose-levels that were equivalent to or even exceeded the LD₉₀ values in acute gavage studies. Repeat-dose dietary studies in the rat and dog did not indicate that an increase in the duration of dosing resulted in increased toxicity with respect to cholinesterase activity and/or effects (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009).

2.2.3 Genotoxicity (including mutagenicity)

**European Union**
Carbofuran is positive in *in vitro* studies, but negative in *in vivo* studies.

*In vitro* results were negative for the Ames test and V79 cell line assay using carbofuran from Arysta, but were positive for the Ames test and mouse lymphoma assays, with and without S9 metabolic activation, for carbofuran from FMC.

*In vivo* results were negative for the micronuclei assay using mouse bone marrow cells for carbofuran from Arysta and in chromosomal aberration for carbofuran from FMC (UNEP/FAO/RC/CRC.11/6).

**Canada**
Assessments of mutagenic potential in a variety of bacterial and mammalian *in vitro* and *in vivo* studies were performed for carbofuran.

Positive results in studies with bacteria have been recorded in *S. typhimurium* (TA 1535 and occasionally TA 98 & TA 1538), while negative results have been reported in other strains of *S. typhimurium*, *S. cerevisiae*, *E. coli* and *B. subtillis*.

In the mouse lymphoma mutagenesis assay, carbofuran displayed weak positive results. Positive evidence from other tests includes the in vivo chromosomal aberration assay and micronucleus assay; however, these positive results occurred at levels noted to induce lethality in the acute LD₉₀ studies. Negative results were achieved with the Drosophila sex-linked recessive lethal mutation, mitotic recombination in yeast, *in vitro* chromosome aberration, sister chromatid exchange and unscheduled DNA synthesis assays.


2.2.4 Long term toxicity and carcinogenicity

**European Union**
No carcinogenic potential was observed in four chronic studies (two in rat and two in mice). Tumours observed in the studies were considered to be spontaneous and unrelated to carbofuran treatment.

Rats (strain and sex unspecified, dietary, 2 years): NOAEL = 0.462 mg/kg bw/day (reduced bodyweight, reduced food efficiency and reduced red blood cell and brain AChE). Lowest relevant long-term NOAEL (UNEP/FAO/RC/CRC.11/6).

**Canada**
Studies for chronic toxicity/carcinogenicity were conducted on mice and rats. In all studies reviewed, there was no evidence of carcinogenicity (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009, p12).

**CILSS countries**
Carbofuran is not known to have carcinogenic effects. It has not been demonstrated that carbofuran is teratogenic or mutagenic, either (UNEP/FAO/RC/CRC.11-INF-13.En, SPC, 2012).
2.2.5 Effects on reproduction

**European Union**
Carbofuran induced decreased body weight in pups as well as pup survival at parental toxic doses. Results from the open literature demonstrated that in utero or lactational exposure to carbofuran during whole gestation or lactation period caused testicular effects and spermatotoxicity in pups at dose levels of 0.4 mg/kg bw not associated with inducing general toxic effects, these effects were reproduced in a more recent study with dietary administration, however, the effects were far less pronounced and occurred only at systemically toxic doses (18 mg/kg bw/day); they were not reproduced upon gavage administration.

Therefore, no classification regarding reproduction toxicity was proposed (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA, 2009, pp3-4).

**Canada**
The developmental toxicity studies in mice, rats and rabbits showed no evidence of teratogenicity and no additional sensitivity of the fetus following in utero exposure to carbofuran. Developmental effects in the fetuses included mortality, decreased weight and increased variations alongside maternal observations of mortality, clinical signs and reduced weight gain. At higher dose levels, carbofuran caused sperm and reproductive system damage when fed to either adult male rats or rats exposed in utero or during lactation. Degeneration was seen in the Sertoli cells along with atrophied seminiferous tubules. Disturbed spermatogenesis (decreased sperm count, abnormal sperm morphology and altered testicular enzymes) was noted in the rats. Effects on sperm quantity and quality were observed in carbofuran-treated rabbits. In the one-year dog study, testicular effects were manifested as decreased weight, degeneration of the seminiferous tubules and aspermatia. Despite these effects, no reproductive effects were noted in the multigeneration reproductive study. Parental effects were limited to reduced weight gain and food intake whereas offspring effects included reduced weight gain and viability. In view of the findings in the rat, rabbit and dog, carbofuran should be viewed as having some potential for reproductive toxicity (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009, pp12-13).

**CILSS countries**
Sub-chronic administration of carbofuran to rats may be toxic to sperm and testicles. Prolonged or repeated exposure to carbofuran may cause the same effects as an acute exposure. It has not been demonstrated that carbofuran can cause reproductive effects to humans and animals at expected exposure levels. However, chronic ingestion of high doses damages testicles in dogs. Doses of 5 mg/kg/day to rats and mice during two years showed loss of weight; carbofuran is known to affect reproduction and development. A daily diet of 100 ppm of carbofuran in pregnant rats considerably reduces newborn survival. However, in a three-generation reproductive toxicity study, Charles River rats were given carbofuran (95.6 % purity) at concentrations of 0, 20 or 100 mg/kg food, the NOAEL was 20 mg/kg food, equal to 1.2 mg/kg body weight per day, based on the reduction of body weight gain in parental generation and the reduction of growth and survival of offspring generation to 100 mg/kg food (UNEP/FAO/RC/CRC.11-INF-13.En, SPC, 2012).

2.2.6 Neurotoxicity/ delayed neurotoxicity, Special studies where available

**European Union**
At the occasion of the resubmission of carbofuran, new sets of acute neurotoxicity studies were assessed. No NOAEL could be established in pups at post-natal day 11 (PND11) based on a significant inhibition of the brain acetylcholinesterase, the low-observed-adverse-effect-level (LOAEL) was 0.03 mg/kg bw. In young adult rats, the NOAEL was 0.03 mg/kg bw; overall, clinical signs were observed from 0.3 mg/kg bw onwards (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA, 2009, p4).
Canada

Although no guideline acute neurotoxicity study was available, two published studies highlighted the short-acting effects typically associated with carbamate inhibitors of cholinesterase. Subchronic neurotoxicity studies (dietary) showed clinical signs, decreased motor activity and altered neurological functioning but lacked cholinesterase measurements. Results from the chronic rat study suggest that cholinesterase inhibition was occurring at the levels causing the neurological impairment. In a developmental neurotoxicity study (dietary), doses high enough to cause neonatal death, marked growth retardation and developmental delays did not cause persistent neurological effects. No evidence of neuropathology was noted in any of the available studies (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009, p12).

European Union

Carbofuran is rapidly and completely absorbed and excreted in the rat. It is very toxic by ingestion (LD₅₀ 7 mg/kg bw) and by inhalation (LC₅₀ 0.05 mg/L) whereas toxicity during dermal exposure is moderate (LD₅₀ 1000-2000 mg/kg bw). Carbofuran is not a skin irritant, eye irritant, or skin sensitizer, but mortality was reported after exposure to eyes. It is genotoxic in vitro but negative in in vivo studies. The relevant long term NOAEL is 0.462 mg/kg bw/day from the rat study. At the occasion of the resubmission of carbofuran, new sets of acute neurotoxicity studies were assessed. No NOAEL could be established in pups at post-natal day 11 (PND11) based on a significant inhibition of the brain acetylcholinesterase, the low-observed-adverse-effect-level (LOAEL) was 0.03 mg/kg bw. In young adult rats, the NOAEL was 0.03 mg/kg bw; overall, clinical signs were observed from 0.3 mg/kg bw onwards (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA, 2009).

Canada

A detailed review of the toxicological database for carbofuran was conducted. The toxicology database for carbofuran is primarily based on studies from the registrant. Carbofuran was rapidly absorbed, metabolized and eliminated mainly in the urine after oral administration to mice and rats. Most metabolites were found to be significantly less toxic than the parent compound in acute oral lethality tests. One metabolite, 3-hydroxycarbofuran, showed similar acute oral toxicity as carbofuran.

In acute toxicity studies, carbofuran was highly toxic via the oral route of exposure in rats but showed low dermal toxicity. Acute inhalation studies were not available. Carbofuran was a minimal eye irritant and was not a dermal sensitizer.

In repeat-dose dietary studies in various species (mouse, rat and dog), the dog appeared to be the most sensitive species with respect to cholinergic symptoms. Repeat-dose dietary studies in the rat and dog did not indicate that an increase in the duration of dosing resulted in increased toxicity with respect to cholinesterase activity and/or effects. Although no guideline acute neurotoxicity study was available, two published studies highlighted the short-acting effects typically associated with carbamate inhibitors of cholinesterase.

Subchronic neurotoxicity studies (dietary) showed clinical signs, decreased motor activity and altered neurological functioning but lacked cholinesterase measurements. Results from the chronic rat study suggest that cholinesterase inhibition was occurring at the levels causing the neurological impairment. In a developmental neurotoxicity study (dietary), doses high enough to cause neonatal death, marked growth retardation and developmental delays did not cause persistent neurological effects. No evidence of neuropathology was noted in any
of the available studies.

There is sufficient evidence to support weak mutagenic properties for carbofuran in bacteria and mammalian cells.

Studies for chronic toxicity/carcinogenicity were conducted on mice and rats. In all studies reviewed, there was no evidence of carcinogenicity.

In view of the findings in the rat, rabbit and dog, carbofuran should be viewed as having some potential for reproductive toxicity (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), pp11-13).

CILSS countries
Toxicological data
Acute toxicity - Carbofuran belongs to WHO class Ib (highly hazardous). Some formulations belong to class I (highly hazardous or extremely hazardous) or to class II (moderately hazardous). It is extremely toxic via oral route and by inhalation (LD50 is 5 to 13 mg/kg in rats, 2 mg/kg in mice). Dermal toxicity is low. It is minimally irritating to the eyes and to the skin. It is not a skin sensitizer. It is neurotoxic being a cholinesterase inhibitor. This is of short duration and reversible. It is a strong endocrine disruptor which may affect the concentration of several human and animal hormones even at very low doses. The exposure to carbofuran is presents a risk for the population, children and infants even if used normally. The antidote to carbofuran is atropine.

Chronic toxicity - Carcinogenic, teratogenic and mutagenic effects
- Carbofuran is not known to have carcinogenic effects. It has not been demonstrated that carbofuran is teratogenic or mutagenic, either.

Reproductive and development effects - Sub-chronic administration of carbofuran to rats may be toxic to sperm and testicles. Prolonged or repeated exposure to carbofuran may cause the same effects as an acute exposure. It has not been demonstrated that carbofuran can cause reproductive effects to humans and animals at expected exposure levels.

The Decision Guidance Document for dustable powder formulations containing a combination of benomyl at or above 7%, carbofuran at or above 10% and thiram at or above 15%, FAO/UNEP (2004/2005) contains the FAO/WHO Data Sheets on Pesticides No 56 Carbofuran as an Annex which also contains a more extensive summary on human and mammalian toxicology (UNEP/FAO/RC/CRC.11-INF-13.En, SPC (2012).

Residues
From the available data it can be concluded that the degradation and metabolism of carbofuran in plants following a soil application proceeds primarily via hydroxylation on the furan ring to yield the major metabolite 3-hydroxycarbofuran, which forms due to successive oxidation and hydrolysis steps 3- ketocarbofuran, 2-hydroxymethyl-3-ketocarbofuran and the phenol metabolites 3-OH-7-phenol and 3-keto-7-phenol. The first two metabolites were considered as toxicologically relevant but the others are of lower toxicity than carbofuran and 3-hydroxycarbofuran. It is proposed to define the residue for risk assessment purposes as the sum of carbofuran and 3-hydroxycarbofuran, expressed as carbofuran (soil applied uses). Residue trial data with carbofuran under field conditions from both European regions were submitted by both applicants on sugar beet and maize, and on sunflowers. The data indicate residues being below the respective LOQ for both analytes in maize grain. In maize silage positive residues (0.03 mg/kg) were found in Northern and Southern European trials. Taking all the available results on sugar beets from both applicants together (complete data set), it was considered a

3 Human exposure/Risk evaluation

3.1 Food European Union

Due to the data gaps identified the consumer risk assessment could not be finalized. Though the RMS had provided a comprehensive dietary exposure and risk assessment for consumers using both the EFSA PRIMo and the UK model. The sum of intakes of carbofuran and 3-hydroxycarbofuran from the primary crop, rotational crops and food of animal origin was considered and compared to the toxicological reference values for carbofuran (ADI and ARfD, both 0.00015 mg/kg bw/day). This approach was deemed to be appropriate as the metabolite 3-hydroxycarbofuran is assumed to be of comparable toxicity as carbofuran based on acute toxicity studies.

An exceedance of the ADI was noted for UK toddlers in both models (EFSA PRIMo 173% ADI; UK model 101% ADI). The risk assessment could be further refined when residues in sugar are not considered at the level of the LOQ of the analytical method for sugar beet, but at a level of 0 mg/kg.

However, the acute consumer risk assessment indicates the ARfD is significantly exceeded for a number of crops consumed by children and by adults/the general population. A great exceedence of the ARfD was observed for leafy (up to 1800% ARfD) and root/tuber crops (up to 615% ARfD). These results highlight the importance of residue data on succeeding crops to enable further refinement of the dietary risk assessment for consumers (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2009), pp37-38).

Canada

Acute dietary risk from food-only exposure to carbofuran is of concern for all subpopulations. Chronic dietary risk from food-only exposure to carbofuran is not of concern for all subpopulations (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009).

Acute dietary exposure to carbofuran as a percentage of the acute reference dose ranges from 141% for adults aged 50+ years old to 733% for children aged 1 to 2 years old, and is 339% for the general population. The acute dietary exposure to carbofuran is higher than the acute reference dose for all population subgroups; therefore, it is of concern. Chronic dietary exposure to carbofuran as a percentage of the acceptable daily intake ranges from 19% for adults aged 50+ years old to 76% for children aged 1 to 2 years old, and is 30% for the general population. The chronic dietary exposure to carbofuran is less than the acceptable daily intake for all population subgroups; therefore, it is not of concern (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2010).

3.2 Air

The general population is not expected to be exposed to carbofuran via air as carbofuran is not very stable in air.

3.3 Water European Union

In the consumer risk assessment performed by the rapporteur Member State the possible intake of carbofuran through drinking water derived from groundwater had not been considered. EFSA noted that significant contribution to the acute and chronic exposure might be expected if any restrictions that might be considered were not effective. To assess this situation EFSA estimated consumer exposure (not peer reviewed) with regard to carbofuran residues in ground water used as drinking water on the basis of the predicted PEC groundwater levels (annual average, based on the model FOCUS PEARL) in order to reflect the worst case. The estimates were based on the default assumptions laid down in the WHO Guidelines for drinking water quality for the consumer groups of adults (weighing 60 kg), toddlers
(10 kg) and bottle-fed infants (5 kg) with a daily per capita consumption of 2 L, 1 L and 0.75 L, respectively.

It is further noted that the toxicological reference values of carbofuran are also applicable to the metabolites 3-hydroxycarbofuran and 3-ketocarbofuran. Therefore the sum of all 3 compounds leaching into groundwater was expressed as carbofuran equivalents and considered in the consumer risk assessment.

The predicted concentrations of carbofuran toxicological equivalents in the most vulnerable scenarios may lead to the exceedance of the toxicological reference values ADI and ARfD for toddlers and infants. In terms of the acute assessment it is noted that the daily consumption figures used might rather reflect a mean consumption than a high consumption that is normally considered for acute intake estimates, and thus the actual acute consumer exposure (single day event) might be even higher than estimated (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2009), pp38-39).

**Canada**

The following was concluded in relation to dietary risk from drinking water. Since acute dietary exposure exceeds the ARfD for food alone, there is concern about any additional exposure through drinking water. Health Canada (2010), p4 notes that an aggregate risk assessment combining exposure from food and drinking water was conducted using either estimated environmental concentrations (EECs) from the modelling assessment or EECs from monitoring data. The dietary risks from food and drinking water are of concern whether EECs from modelling or monitoring data are used (UNEP/FAO/RC/CRC.11-INF-11.En, Health Canada (2009), p37).

### 3.4 Occupational exposure

**European Union**

The acceptable operator exposure level (AOEL) is 0.0003 mg/kg bw/day, based on the NOAEL of 0.03 mg/kg bw in young adults from the acute neurotoxicity studies and a safety factor of 100 applied. For granular formulations the estimated operator exposure according to the US Pesticide Handler’s Exposure Database (PHED) is below the AOEL i.e. 95 % if personal protective equipment (PPE) as gloves, normal work wear and respiratory protective equipment (RPE) are worn during loading and spreading of the product and assuming an application rate of 0.6 kg carbofuran/ha and a maximum work rate of 10 ha/day. Worker exposure is unlikely to occur, as the formulation is incorporated by mechanical means into the soil when sowing (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2009), p4).

**Canada**

The following was concluded in relation to Occupational Risk. Risk estimates associated with applying, mixing and loading activities for certain proposed agricultural label uses are of concern even when engineering controls or personal protective equipment are used. Post-application risks for workers were of concern for certain scenarios; mitigation measures that would diminish the risk were considered, however, the mitigation measures calculated to reduce post-application risk may be agronomically unfeasible (UNEP/FAO/RC/CRC.11-INF-11.En, Health Canada (2009), p37).

Risk estimates associated with certain mixing, loading and applying activities are of concern to the PMRA. Based on the precautions and directions for use on the existing carbofuran product labels, post-application risks to workers performing activities, such as thinning, pruning and harvesting of most crops, did not meet current standards and are also of concern (UNEP/FAO/RC/CRC.11-INF-11.En, Health Canada (2009), p5).
3.5 Medical data contributing to regulatory decision

**European Union**
A low number of carbofuran intoxications have been reported. The majority of the incidents resulted from maintenance or equipment cleaning work. Under normal work conditions, employees wear rubber gloves, long sleeve shirts, eye protection and head covering (UNEPA/FAO/RC/CRC.11-INF-11.En, EFSA (2009), p4).

**Canada**
Starting April 26, 2007, registrants are required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. There was one incident report related to human health that was submitted to the PMRA for carbofuran. The report indicates that the protective clothing required by carbofuran labels for the use was not worn during spraying. The individual was treated and released from hospital. No other incidents involving human health have been reported to the PMRA as of 29 September 2008 (UNEPA/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), p20).

However, in the United States, in 2007 the USEPA published that more than 700 possible carbofuran poisoning incidents were reported. In most cases, symptoms for carbofuran incidents were specific to cholinergic poisoning and most resulted from dermal and inhalation exposure, rather than oral exposure, and the majority of illnesses were of a systemic type. Eye problems were also widely reported, accounting for approximately one quarter of all recorded incidents. Causes of these incidents included: failure to wear appropriate personal protective equipment, exposure during cleaning or repair of spray equipment, spray drift or early entry into treated fields. The majority of incidents occurred among handlers who mix, load, and apply carbofuran in agricultural fields. The USEPA concluded that the number and rate of poisoning cases due to carbofuran exposure is sufficient to warrant priority attention to risk reduction measures for this pesticide (UNEPA/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), p20-21).

**CILSS countries**
2342 cases of carbofuran poisoning have been reported in farmers in Thailand in 2003. Carbofuran caused farmers’ skin and eye burns strongly affecting their health. The long term effects may cause permanent damage to the nervous system (UNEPA/FAO/RC/CRC.11/6 under Acute toxicity).

3.6 Public exposure

**European Union**
The granular formulation is applied by ground-directed equipment that is nearly dust free; therefore, the level of bystander exposure to vapour or airborne particles at the time of application is likely to be negligible (UNEPA/FAO/RC/CRC.11-INF-11.En, EFSA (2009).

**Canada**
The following was concluded in relation to Non-Occupational Risk. Given that there are no residential uses of carbofuran, a risk assessment for this scenario was not conducted. UNEPA/FAO/RC/CRC.11-INF-11.En, Health Canada (2009), p37

3.7 Summary—overall risk evaluation

**European Union**
It was concluded that carbofuran was not demonstrated to fulfil the safety requirements laid down in Article 5 (1) (a) and (b) of Directive 91/414/EEC (replaced by Regulation (EC) 1107/2009). The consumer risk assessment, which raised a concern about the acute exposure of vulnerable groups of consumers, in particular children, could not be finalised due to the lack of information as regards certain relevant residues.
Canada
Health Canada concluded that an evaluation of available scientific information found that, under the then-current conditions of use, carbofuran products posed an unacceptable risk to human health and the environment and therefore did not meet Health Canada’s standards for human health and environmental protection. As a result, all uses of carbofuran were proposed for phase-out. This included registered uses on canola, mustard, sunflower, corn (sweet, field and silage), sugar beet, green pepper, potato, raspberry and strawberry as well as temporary emergency uses on turnip and rutabaga. The proposal affected all end-use products registered in Canada that contained carbofuran (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada 2009 and 2010).

CILSS countries
Carbofuran presents risks to human health and especially to non-target organisms in the environment, making it very difficult to handle it without risks for users in Sahel countries. These risks have justified its ban in many countries of the world among which all the European Union countries. The Sahelian Pesticides Committee has stopped the registration of carbofuran based pesticides in CILSS countries since 2006 taking into account:

- The fragile ecology of CILSS countries already characterized by an imbalance of ecosystems and the disappearance of organisms useful to the environment;
- Non-compliance with recommended measures for a safe use of carbofuran by users in the context of CILSS countries;
- Non-compliance with the pre-harvest intervals (PHI) in particular, entailing the presence of pesticide residues in harvested foodstuff;
- The low utilization rate of protective equipment by growers:
- The existence of alternatives to the use of carbofuran.

The Coordinating Ministry of CILSS Countries issued this ban to make public the decision to ban carbofuran based pesticides, and this in a transparent way, in order to improve human health and to preserve the environment in these countries (UNEP/FAO/RC/CRC.11-INF-13.En, Sahelian notification SPC 2012).

4 Environmental fate and effects
4.1 Fate
4.1.1 Soil

European Union
Variable results have been obtained from different laboratory degradation experiments, which indicate that carbofuran may be of low to high persistency in soil (lab DT$_{50}$ = 5.7 - 387 days, field DT$_{50}$ = 1.3 - 27 days).

Field studies have indicated that 3-hydroxycarbofuran, 3-keto-carbofuran and carbofuran-7-phenol are formed, with some levels being reported as 3% of the total residue (TR), 20% TR and <LOD, respectively. EU field trials have indicated that the half-life of carbofuran (as a metabolite of carbosulfan) is 1.3 - 27 days. However, US field studies (at a similar climate compared to the EU) indicate that the half-life for carbofuran as the parent compound is 5-121 days. Only the EU studies were considered applicable.

A 56 day laboratory study under dark aerobic conditions at 20°C and 10°C examined the metabolism of carbofuran in four soils. No metabolites over 10% AR were detected in the study performed at 20°C, however, at 10°C 3-ketocarbofuran reached a 7.7% AR. Minor
uncharacterised metabolites were detected at <2.5% AR, unextractable residue was up to 57.7% and mineralisation was 66% AR after 120 days. A second study under dark aerobic conditions at 25°C used a sandy loam soil. 3-ketocarbofuran peaked at 12.41% AR after 181 days, with minor metabolites being 3-hydroxycarbofuran (maximum 1.32% after 122 days), 3-keto-7-phenol and carbofuran-7-phenol. Another aerobic metabolism study reported that 3-hydroxycarbofuran and carbofuran-7-phenol reached maximums of 0.9% AR and 9% AR, respectively, after 184 days.

The same metabolites were also detected in an aerobic/anaerobic study; after the aerobic phase, 3-ketocarbofuran reached a maximum of 6.2% AR. An anaerobic soil study under dark conditions at 20°C found that after 28 days, carbofuran-7-phenol was the major metabolite at a maximum of 62.9% AR and other minor unspecified metabolites were reported. After 120 days, mineralisation was low (CO2 6.2% AR) and bound residues reached a maximum of 62.7% AR.

Although conflicting results regarding photolysis have been reported, it is concluded that photolysis in soil does not occur (as study limitations are reported for the results of the conflicting study).

Based on a Koc of 17-28 mL/g, carbofuran is classified as being of very high mobility in soil. Additionally, an aged column leaching study reports that carbofuran, 3-ketocarbofuran and carbofuran-7-phenol are mobile and may leach (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA, 2006, pp3-4 and pp26-28).

**Canada**

Carbofuran is classified as relatively non-volatile under field conditions. Phototransformation is not an important route of transformation for carbofuran in soil.

Transformation of carbofuran in aerobic soil appears to have resulted from a combination of hydrolysis and biotransformation. In an acidic soil (pH 5.7), carbofuran degraded with a half-life of 321 days, but in soil of pH 7.7, the half-life dropped to 149 days. The major identified transformation product was 3-ketocarbofuran. The persistence of carbofuran may decrease in soils that have been previously treated with carbofuran because of microbial adaptations.

No information was available addressing the soil biotransformation of carbofuran under anaerobic conditions.

Soil adsorption studies indicate that carbofuran has a high to very high mobility in soils. Koc values ranged from 10 to 63 in a variety of soils. Carbofuran was shown to be mobile in soil column leaching studies with 33 to 78% of the radioactivity in the aged soils collected in the leachate. Carbofuran was the major extractable residue in both the aged soils and the leachate.

Carbofuran would be considered non-persistent to moderately persistent from field soil dissipation studies conducted in the U.S. according to the classification of Goring et al. (1975) (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009, p. 21-22).

Table 1 of Appendix IX, in Health Canada (2009, p. 71-72) contains a detailed table of environmental fate and toxicity data.

**CILSS countries**

The GUS (groundwater ubiquity score) of carbofuran is 3.02, which represents a high risk of ground water pollution through leaching.

Carbofuran is soluble in water and has a high to very high mobility in sandy and loamy soil and a moderate mobility in clay soil.

Photolysis half-life in soil is 78 days. It is very persistent in soil in aerobic conditions. Its half-life varies according to soil pH.
(half-life=149 d at pH 7.7, and half-life = 321 d at pH 5.7).

Carbofuran degrades fairly slowly in non-sterile, neutral or acid aerobic soils, with half-lives ranging from 1 to 8 weeks. It is more stable in sterile soils and instable in alkaline conditions. Under anaerobic conditions, carbofuran may take twice as long to degrade (UNEP/FAO/RC/CRC.11-INF-13.En, SPC 2012).

4.1.2 Water

European Union

In water, hydrolysis of carbofuran is extremely dependent on pH; half-lives of none, 28-45.7 days and 0.1 days were observed under acidic (pH 4), neutral (pH 7) and alkaline (pH 9) conditions, respectively, at 25°C. In all cases, the major metabolite was carbofuran-7-phenol.

Photolysis does not significantly occur and no indication of ready biodegradation is apparent.

A 102 day water sediment dissipation study showed that under acidic conditions, degradation of carbofuran occurred with a half-life of 70 days, 32.8% AR occurred as bound residues and mineralisation was low. Half-lives of 6.9 - 8.5 days in the water phase were reported from dark aerobic systems under neutral or alkaline conditions, with half-lives of 9.0 - 11.6 days being reported for degradation in the whole system. Carbofuran-7-phenol (maximum 12% AR after 4 days) was the only major metabolite in the water phase and in the sediment, only carbofuran exceeded levels of 10% AR. Minor unspecified metabolites were identified (max. 5.9% AR). The maximum amount of bound residues at the end of the study (after 120 days) was 74-78% AR (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2006), p. 4-5 and 28-29).

Canada

The reported solubility of carbofuran in water (700 mg/L at 25°C), would classify it as very soluble.

Carbofuran is stable to hydrolysis at pHs < 6, but becomes increasingly susceptible to hydrolysis as the pH increases, hydrolyzing rapidly at alkaline pHs (half-lives of less than a day).

Phototransformation is an important route of transformation for carbofuran in shallow clear water. Biotransformation was an important route of transformation in aquatic habitats under aerobic conditions. The major transformation product formed in aquatic systems was carbofuran phenol. Biotransformation was also a route of transformation in aquatic systems under anaerobic conditions, however degradation may not have been due strictly to anaerobic metabolic processes, hydrolysis may have also contributed. The major transformation product was carbofuran phenol and was predominantly associated with the sediment fraction.

In alkaline environments, carbofuran appears to have a low potential to accumulate in fish (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada, 2009, p. 22).

Table 1 of Appendix IX, in Health Canada (2009, p. 71-72) contains a detailed table of environmental fate and toxicity data.

CILSS countries

Carbofuran is also very persistent in water in anaerobic conditions where its half-life is 189 days. Because of its high mobility, carbofuran presents a risk of surface water pollution in sandy areas. This pesticide has been detected in surface waters in a few rivers in Quebec at maximum concentrations ranging from 0.14 to 2.7 ppb. Following its percolation into the soil, carbofuran leaches into soil and has been detected in ground waters after it had been used in agriculture (UNEP/FAO/RC/CRC.11-INF-13.En, SPC 2012).
4.1.3 Air **European Union**
In air, long range transport of carbofuran is not expected. At environmental temperatures (20-25°C), carbofuran has a vapour pressure of 1 x 10^{-6} - 2.25 x 10^{-4}Pa, a Henry's Law constant of 5 x 10^{-5} - 1.58 x 10^{-4} Pa.m^3/mol and a photochemical degradation half-life of <5 hours (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA, 2006, p30).

**CILSS countries**
Carbofuran exists in the air both in the form of vapour and absorbed to suspended particulates (UNEP/FAO/RC/CRC.11-INF-13.En, SPC 2012).

4.1.4 Bioconcentration **European Union**
Bioaccumulation: Maximum BCFs for carbofuran have been reported to be 3.8 (fillet), 22 (viscera) and 12 (whole fish), which indicate it is unlikely to bioaccumulate. This is supported by the rapid clearance time CT_{50} (1.4 days). Indeed, the level of residues in organisms after the 14 day depuration phase is <5% (whole fish) (UNEP/FAO/RC/CRC.11/6).

4.1.5 Persistence
Based on the above summaries carbofuran may range from low to high persistence in soil and in water with the latter depending on the pH, with much slower degradation at acidic pH.

4.2 Effects on non-target organisms

4.2.1 Terrestrial vertebrates

**Birds** **European Union**
Acute toxicity: LD_{50} Mallard (*Anas platyrhynchos*, male) = 0.71 mg a.i./kg b.w.
Dietary toxicity: LC_{50} Mallard (*Anas platyrhynchos*) = 1.6 mg a.i./kg b.w./day
Reproductive toxicity: No agreed endpoint
(UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2006), Appendix 1.6, p82)

**Canada**
Acute oral toxicity (Carbofuran technical):
Fulvous Whistling-Duck (*Dendrocygna bicolor*) LD_{50} = 0.24 mg a.i./kg bw
Mallard (*Anas platyrhynchos*) LD_{50} = 0.37 - 0.63 mg a.i./kg bw
Red-winged Blackbird (*Agelaius phoeniceus*) LD_{50} = 0.42 mg a.i./kg bw
Red-billed Quelea (*Quelea quelea*) LD_{50} = 0.422-0.562 mg a.i./kg bw
American Kestrel (*Falco sparverius*) LD_{50} = 0.6 mg a.i./kg bw
House Finch (*Carpodacus mexicanus*) LD_{50} = 0.75 mg a.i./kg bw
House Sparrow (*Passer domesticus*) LD_{50} = 1.33 mg a.i./kg bw
Rock Dove (*Columba livia*) LD_{50} = 1.33 mg a.i./kg bw
Brown-headed Cowbird (*Molothrus ater*) LD_{50} = 1.33 mg a.i./kg bw
Common Grackle (*Quiscalus quiscula*) LD_{50} = 1.33 - 3.16 mg a.i./kg bw
Japanese Quail (*Coturnix coturnix*) LD_{50} = 1.7 - 1.9 mg a.i./kg bw
Eastern Screech-Owl (*Otus asio*) LD_{50} = 1.9 mg a.i./kg bw
Ring-necked Pheasant (*Phasianus colchicus*) LD_{50} = 4.2 mg a.i./kg bw
Northern Bobwhite (*Colinus virginianus*) LD_{50} = 5.0 - 12 mg a.i./kg bw
European Starling (*Sturnus vulgaris*) LD_{50} = 5.6 mg a.i./kg bw
Dietary: Mallard duck (*Anas platyrhynchos*) LD_{50} = 79 mg a.i./kg diet
Chronic: Mallard duck (*Anas platyrhynchos*) LOAEC < 2.0 mg a.i./kg diet
(UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), Appendix IX, Table 2, pp73-76).
CILSS countries
Several sources agree that carbofuran is highly toxic to birds. One single grain may kill a bird (LD$_{50}$ oral of 0.4 mg/kg body weight) (UNEP/FAO/RC/CRC.11-INF-13.En, SPC 2012).

4.2.2 Aquatic species

European Union
The data below are for the most sensitive species from each group:

Fish
Bluegill sunfish (*Lepomis macrochirus*) 96 hours semi-static LC$_{50}$ = 0.18 mg/L. Sheepshead minnow (*Cyprinodon variegatus*) 35 day fish early life stage NOEL = 0.006 mg/L.

Invertebrates
Water flea (*Daphnia magna*) 48 hours static EC$_{50}$ (mortality) = 0.0094 mg/L. Water flea (*Daphnia magna*) 21 days semi-static NOEC (reproduction) = 0.008 mg/L. Water flea (*Ceriodaphnia dubia*) 7 days semi-static NOEC (reproduction) = 0.00016 mg/L.

Scud (*Gammarus fasciatus*) 96 hours static LC$_{50}$ = 0.0028 mg/L.

Algae
Green algae (*Pseudokirchneriella subcapitata*). Green algae (*Pseudokirchneriella subcapitata*) 72 hours static EbC$_{50}$ (biomass) = 6.5 mg/L. Green algae (*Pseudokirchneriella subcapitata*) 72 hours static ErC$_{50}$ (growth) = 19 mg/L. (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2006), Appendix 1.6, p83).

Canada
Fish (freshwater, carbofuran technical)
Acute: Bluegill sunfish (*Lepomis macrochirus*) 96 h LC$_{50}$ = 88 μg a.i./L. Yellow perch (*Perca flavescens*) 96 h LC$_{50}$ = 120 μg a.i./L. Lake trout (*Salvelinus namaycush*) 96 h LC$_{50}$ = 164 μg a.i./L. Channel catfish (*Ictalurus punctatus*) 96 h LC$_{50}$ = 248 μg a.i./L. Brown trout (*Salmo trutta*) 96 h LC$_{50}$ = 280 μg a.i./L. Rainbow trout (*Oncorhynchus mykiss*) 96 h LC$_{50}$ = 362 μg a.i./L. Brown trout (*Oncorhynchus kisutch*) 96 h LC$_{50}$ = 530 μg a.i./L. Fathead minnow (*Pimephales promelas*) 96-h LC$_{50}$ = 872 μg a.i./L. Chronic (Early Life Stage): Rainbow trout (*Oncorhynchus mykiss*) 101-d NOEC = 24.8 μg a.i./L.

Fish (salt water, carbofuran technical)
Acute: Atlantic silverside (*Menidia menidia*) juvenile ) 96 h LC$_{50}$ > 1000 μg a.i./L. Pink shrimp (*Penaeus duorarum*) 96 h LC$_{50}$ = 7.3 μg a.i./L. Opossum shrimp (*Neosiphas mercedis*) 96 h LC$_{50}$ = 2.7 μg a.i./L. Copepod (*Tigriopus brevicornis*) 96 h LC$_{50}$ = 17.7 μg a.i./L. Chronic: Mysid shrimp (*Mysidopsis bahia*) 28-d NOEC = 0.4 μg a.i./L.

Amphibians (Acute formulation)
Bog Frog (*Rana limnocharis*) 48 h LC$_{50}$ = 11,226 μg a.i./L.

Aquatic invertebrates (freshwater, carbofuran technical)
Acute: Water flea (*Daphnia magna*) 48 h LC$_{50}$ = 29 μg a.i./L. Water flea (*Ceriodaphnia dubia*) 48 h LC$_{50}$ = 2.6 μg a.i./L. Crayfish (*Procambarus clarkii*) 48 h LC$_{50}$ = 2700 μg a.i./L. Chronic water flea (*Daphnia magna*) 21 d NOEC 9.8 μg a.i./L.

Aquatic Invertebrates (saltwater , carbofuran technical)
Acute: Eastern oyster (*Crassostrea virginica*) 96 h LC$_{50}$ > 1000 μg a.i./L. Pink shrimp (*Penaeus duorarum*) 96 h LC$_{50}$ = 7.3 μg a.i./L. Opossum shrimp (*Neosiphas mercedis*) 96 h LC$_{50}$ = 2.7 μg a.i./L. Copepod (*Tigriopus brevicornis*) 96 h LC$_{50}$ = 17.7 μg a.i./L. Chronic: Mysid shrimp (*Mysidopsis bahia*) 28-d NOEC = 0.4 μg a.i./L. Algae (Chronic)
Green algae (Chlorella pyrenoidosa) 75% a.i. 8-10 week NOEC = 750 μg a.i./L  
**Vascular Plants** (Acute, 40.6% a.i.)  
Duckweed (Lemna minor) 48 h NOEC > 10,000 μg a.i./L  
Sago pondweed (Potamogeton pectinatus) 48 h NOEC > 10,000 μg a.i./L  
(UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), Appendix IX, Table 2, pp73-76).  
**CILSS countries**  
Carbofuran is moderately to high toxic to freshwater fish (LC₅₀ 96 h = 88 at 1 990 ppb). It is extremely toxic to Daphnia magna, LC₅₀ is 0.015 mg/L, on algae LC₅₀ is 19.9 mg/L  

### 4.2.3 Honeybees and other arthropods

#### European Union

**Honeybees**

Acute oral toxicity: No data.  
Honeybees, acute contact toxicity: LD₅₀ (48 h) = 0.0357 μg a.i./bee  

**Arthropod species**

- Ground beetle (Poecilus cupreus), adults Diafuran 5G 12 kg/ha = 20% mortality  
- Beetle (Aleochara bilineata), adult females Diafuran 5G 12 kg/ha = 100% mortality  
- Beetle (Aleochara bilineata), adults Diafuran 5G 12 kg/ha = 4.5% mortality & 60.4% reduction in parasitism rate  
- Beetle (Aleochara bilineata), adults Furadan 5G 1-10 kg/ha (extended test) LD₅₀ = 3.58 g/ha  
- Thin legged wolf spiders (Pardosa sp.), adults and sub-adults Diafuran 5G 12 kg/ha = 100% mortality.  
- Thin legged wolf spiders (Pardosa sp.), adults and sub-adults Diafuran 5G 12 kg/ha = 13.3% mortality & 5.2% increase in food consumption  
- Thin legged wolf spiders (Pardosa sp.), adults and sub-adults Furadan 5G 3.2-32 kg/ha (extended test) LD₅₀ = 2.7 kg/ha  
- Predatory mite (Typhlodromus pyri), protonymphs carbofuran 1.8-18 g/ha (extended test) LD₅₀ = 3.65 g/ha  
- Cereal aphid parasite (Aphidius rhopalosiphi), adults carbofuran 1-32 g/ha (extended test) LD₅₀ = 2.68 g/ha.  

**Canada**

Acute contact Honey bee (Apis mellifera) Carbofuran Technical 48 h LD₅₀ = 0.16 μg a.i./bee  
(UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), Appendix IX, Table 2, pp73-76).  

**CILSS countries**  
Carbofuran is extremely toxic to bees, with LD₅₀ acute by contact of 0.16 μg/bee.  

### 4.2.4 Earthworms

#### European Union

**Earthworm:**

Acute toxicity LC₉₀ = 4487 mg Diafuran 5G/kg dry soil  
LC₉₀ > 1000 mg Furadan 5G/kg dry soil  
Reproductive toxicity NOEC <16.8 mg Diafuran 5G/kg dry soil  

**Canada**

Earthworm (Allolobophora caliginosa ) 14 d LC₅₀ = 0.28 mg a.i./kg soil  
Earthworm (Eisenia fetida) 14 d LC₅₀ = 3.09 - 28.3 mg a.i./kg soil  
Earthworm (Lumbricus terrestris) 14 d LC₅₀ = 4.7 mg a.i./kg soil
4.2.5 Soil microorganisms

**European Union**

**Nitrogen mineralisation:**
No adverse effects of Furadan 5G at 0.8 and 4 mg carbofuran/kg soil after 28 days

**Carbon mineralisation:**
No adverse effects of Furadan 5G at 0.8 and 4 mg carbofuran/kg soil after 28 days (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2006), Appendix 1.6, p86).

4.2.6 Terrestrial plants

No non-target plant toxicity data was provided in the EU, Canadian and Sahelian notifications.

5 Environmental Exposure/Risk Evaluation

5.1 Terrestrial vertebrates

**European Union**

A risk assessment for birds and mammals was conducted based on a granule size of 0.4-0.85 mm and an average weight of 0.87 mg. The loading of one granule was assumed to be 0.0437 mg a.i./granule. The number of granules to reach the acute and dietary LD50 was calculated to be 0.2 and 0.5 granules for a 15 g bird, indicating a potential high risk to birds.

A high risk to birds was identified in the first-tier risk assessment for the uptake of contaminated food items (sugar beet seedlings, earthworms and arthropods). Reduced fraction of food type in diet and fraction of diet obtained in treated area value were suggested in the refined risk assessment together with measured residues in food items. However, a higher tier risk assessment could not be completed due to data deficiencies in the residue trials conducted on these food items.

No long-term reproductive NOEL could be derived from the reproduction study because parental mortality was observed even at the lowest tested dose. It was not clear if the effects of carbofuran are only acute effects

The number of granules to reach the acute LD50 and the long-term NOAEL was calculated to be 1.82 for a small mammal of 15 g indicating a potential high acute risk to mammals. A risk assessment for unintentional uptake of granules conducted according to the European and Mediterranean Plant Protection Organization (EPPO) scheme resulted in an acceptable risk to mammals. The refined risk assessment was based on measured residues in sugar beet seedlings, earthworms and arthropods, but the residue values were not accepted to be used in the risk assessment (see discussion above for birds). Further refinements were also judged to be not acceptable.

A risk assessment for birds and mammals for the uptake of contaminated drinking water was also available. The resulting acute TER for small granivorous mammals was 20 but the acute TER for birds was significantly below the trigger of 10, suggesting a potential risk only for the latter. However, it was noted that a high risk could prevail for situations where puddles are formed at locations where high numbers of granules are left on the soil surface (e.g. end of row) (UNEP/FAO/RC/CRC.11-INF-11.En, edited version of the summary of the environmental risk assessment contained in EFSA (2009), Section 5.1 Effects on Terrestrial Vertebrates, pp 50-53).

**Canada**

A risk assessment of carbofuran to terrestrial organisms was based upon an evaluation of toxicity data for fifteen bird and one mammal species representing vertebrates (acute, dietary, reproduction exposure). For the assessment of risk, toxicity endpoints chosen from the most sensitive species were used as surrogates for the wide range of species that can be potentially exposed following treatment with
carbofuran. The risk assessment for birds did not include a screening level risk assessment but instead used the conclusions of a special review conducted in Canada and the results of a refined probabilistic risk assessment conducted by the USEPA, since the label rates used for the USEPA risk assessment were similar to Canadian label rates.

The conclusions of the USEPA risk assessment and Canadian special review for flowable carbofuran were that evidence from field studies and incident reports support the modelled estimations, showing that approved or registered agricultural use of liquid carbofuran sprays results in mortality to birds. In addition to direct avian mortality, these field studies and bird kill incident reports indicate that flowable carbofuran has the potential to cause secondary avian mortality in cases where raptors ingest prey species, such as small birds and mammals that have previously succumbed to carbofuran intoxication.

The acute oral risk to small wild mammals feeding on the site of carbofuran applications from standard exposure scenarios on vegetation and other food sources showed the level of concern from acute exposure is exceeded for most generic body weights and feeding guilds of small wild mammals feeding on the site of carbofuran applications. Small wild mammals feeding on the site of carbofuran applications are therefore at risk from acute exposure to contaminated vegetation.

The chronic risk to small wild mammals feeding on the site of carbofuran applications showed the level of concern from chronic exposure is exceeded for all the generic weights and feeding guilds following one or two applications at 528 g a.i./ha and single applications at 1132 g a.i./ha and 2500 g a.i./ha. The chronic level of concern is exceeded for all 15 and 35 g insectivores and 35 g herbivores for all of the application rates, and for 1000 g herbivores at all the application rates. Small wild mammals feeding on the site of carbofuran applications are therefore at risk from chronic exposure to contaminated vegetation.

Some small wild mammals were also estimated to be at risk from acute and chronic exposure from the consumption of food items contaminated from spray drift off the site of application following both ground boom and aerial applications of carbofuran (UNEP/FAO/RC/CRC.11-INF-12.En, edited version of the summary of the environmental risk assessment contained in Health Canada (2009), Section 4.2.1 Effects on Terrestrial Organisms, pp 23-27).

5.2 Aquatic species

European Union

Aquatic invertebrates were the most sensitive group of aquatic organisms tested. The acute and long term TERs did not indicate a high risk for fish, algae and sediment dwellers with the model FOCUS step3 PECsw. The TERs indicated a high risk for crustaceans (Daphnia magna, Ceriodaphnia dubia) in the FOCUS model scenarios which are based on drainage (D3, D4). The exposure via run-off was negligible in the FOCUS model run-off scenarios R1 and R3.

No further refinement of the aquatic risk assessment was provided and a high risk to the aquatic environment cannot be excluded for the representative use of carbofuran at an application rate of 600 g a.i./ha for environmental conditions represented by the FOCUS model drainage scenarios.

The risk from the metabolites 3-ketocarbofuran, 3-hydroxycarbofuran and carbofuran-phenol was assessed as low (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2009), p54).

Canada

A risk assessment of carbofuran to freshwater aquatic organisms was based upon an evaluation of toxicity data for the following four
freshwater invertebrate species (acute and chronic exposure); eight freshwater fish species (acute and chronic exposure); one freshwater algae; two freshwater vascular plant species; one amphibian species; five estuarine/marine invertebrate species (acute and chronic exposure) and three estuarine/marine fish species (acute and chronic exposure).

The initial conservative screening level EEC calculations for aquatic systems were based on a direct application to water depths of 15 and 80 cm. The 15 cm depth was chosen to represent a temporary body of water that could be inhabited by amphibians. The 80 cm depth was chosen to represent a typical permanent water body for applications of pest control products in agriculture. The screening level risk assessment indicated that carbofuran poses both an acute and chronic risk to freshwater and estuarine/marine aquatic invertebrates and fish for most of the application rates. The level of concern was not exceeded for freshwater algae and vascular plants. The level of concern was only exceeded for amphibians at the highest application rate of 2500 g a.i./ha.

A refined risk assessment to aquatic organisms from carbofuran spray drift and runoff was conducted for those taxa that exceeded the level of concern in the screening level risk assessment. This showed the acute and chronic levels of concern for freshwater aquatic invertebrates were exceeded for all use-patterns following ground boom applications with the exception of one application at 72 g a.i./ha. The acute and chronic levels of concern for freshwater aquatic invertebrates were also exceeded for all use-patterns following aerial applications. The risk assessment also concluded the level of concern for benthic invertebrates, and the acute and chronic levels of concern for freshwater fish as well as estuarine/marine fish and invertebrates were also exceeded following ground boom and aerial applications, but generally at higher rates.

The refined risk assessment to aquatic organisms from carbofuran runoff showed that the acute and chronic level of concern for freshwater aquatic invertebrates, estuarine/marine invertebrates and for estuarine/marine fish is exceeded for all of the use-pattern scenarios, and for benthic aquatic invertebrates and for freshwater fish for all of the use-pattern scenarios with the exception of the New Brunswick potato scenario (UNEP/FAO/RC/CRC.11-INF-12.En, edited version of the summary of the environmental risk assessment contained in Health Canada (2009), Section 4.2.2 Effects on Aquatic Organisms, pp 27-30).

5.3 Honey bees and above ground arthropods

**European Union**

Carbofuran is very toxic to bees with acute oral and contact LC_{50} values ranging from 0.0357 μg a.i./bee to 0.05 μg a.i./bee. No exposure of bees is expected from the use in sugar beet since sugar beets are wind pollinated and the production crop is harvested before flowering. Therefore the risk to bees from the representative use in sugar beets is considered to be low.

Effects of >50% were observed in extended laboratory studies and semi-field tests with the ground dwelling beetles *Aleochara bilineata* and *Poecilus cupreus* and the formulation Curaterr GR5. A field study was conducted at an application rate of 375 g a.i./ha where recovery was observed within 2 months of all invertebrate taxa investigated. The application rate in the field study does not cover the supported use of 600 g a.i./ha in sugar beet. Therefore a data gap remains to address the risk to non-target arthropods for an application rate of 600 g a.i./ha (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA (2009), p54).

**Canada**

The screening level risk assessment indicated that the level of concern for bees was exceeded at application rates of 528 g a.i./ha and higher. However, a higher level risk assessment could not be located in the
5.4 Earthworms and other soil macro-organisms

**European Union**

The acute risk to earthworms was assessed as low but the long-term TER values were below the trigger of 5 indicating a high long-term risk to earthworms. However, it was concluded that the information provided by the applicants is not sufficient to address the potential high long-term risk to earthworms.

In laboratory studies with the formulation Furadan 5G and *Folsomia candida* and *Hypoaspis aculeifer* the NOECs (reproduction) were 0.21 mg a.i./kg dry soil and 10.4 mg a.i./kg dry soil. The resulting TERs based on the initial PECsoil of 0.8 mg a.i./kg dry soil were 0.26 and 13, indicating a potential high risk to collembola. Collembola were also investigated in the field study with non-target arthropods (see above). Recovery was observed in this study but the application rate of 375 g a.i./ha did not cover the supported use of 600 g a.i./ha in sugar beets. Therefore the risk to other soil non-target macro organisms needs to be addressed further (data gap) (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA 2009, p55).

**Canada**

The screening level risk assessment indicated that the level of concern for earthworms was exceeded at application rates of 528 g a.i./ha and higher. However, a higher level risk assessment could not be located in the reference and appears not to have been carried out (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada (2009), Section 4.2.1 Effects on Terrestrial Organisms, pp 23-26).

5.5 Soil microorganisms

**European Union**

No effects on soil respiration and nitrification were observed after 28 days of exposure to a concentration of 0.8 and 4 mg carbofuran/kg soil equivalent to an application rate of 12 kg Furadan 5G/ha and 60 kg Furadan 5G/ha. A strong impact on nitrogen turnover was observed at days 7 and 14. However, the risk to soil micro-organisms is considered to be low for the representative uses since the nitrogen level in the treated samples was similar to controls after 28 days (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA 2009, p56).

5.6 Terrestrial plants

**European Union**

While no data on the risk to non-target organisms (flora and fauna) was provided, due to the mode of application (in furrow) exposure of non-target plants was assumed to be negligible suggesting a low risk to non-target plants. (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA 2009, p56).

5.7 Summary – overall risk evaluation

**European Union**

Overall it was concluded that a high risk to birds and mammals was indicated for the representative use evaluated. The EPCO experts for ecotoxicology expressed their doubts that a safe use could be demonstrated even with further refinement of the risk assessment.

Overall it can be concluded that a high risk to aquatic organisms cannot be excluded for the application rate of 600 g a.i./ha and environmental conditions represented by the FOCUS model drainage scenarios (D3, D4). Further refinement of the risk assessment is needed. The risk was considered to be low for environmental conditions represented by the run-off scenarios (R1 and R3).

The risk to bees from the representative use in sugar beets is considered to be low, but data gaps remain to address the risk to non-target arthropods and other soil non-target macro organisms for an application rate of 600 g a.i./ha, as well as the potential high long-term risk to earthworms (UNEP/FAO/RC/CRC.11-INF-11.En, EFSA 2009,
Canada
The risk assessment of carbofuran indicates adverse effects on non-target terrestrial invertebrates and vertebrates and aquatic organisms some of which cannot be mitigated. There is potential that carbofuran may appear in surface water through runoff and in groundwater through leaching (UNEP/FAO/RC/CRC.11-INF-12.En, Health Canada 2009, Section 7.2 Environmental Risk, p 38).

The CILSS countries
The Sahelian Pesticide Committee has stopped the registration of carbofuran-based pesticides in CILSS countries in 2006 taking into account the fragile ecology of CILSS countries already characterized by an imbalance of ecosystems and the disappearance of organisms useful to the environment.

Further to the pollution of Sahel ground water which constitutes the main drinking water resource with open wells, several sources agree that Carbofuran is highly toxic to birds. One single grain may kill a bird (oral LD$_{50}$ of 0.4 mg/kg body weight.

Carbofuran is highly toxic to fresh water invertebrates and extremely toxic to birds.

Carbofuran is moderately to highly toxic to fresh water fish (UNEP/FAO/RC/CRC.11/6).
### Annex 2 – Details on final regulatory actions reported

<table>
<thead>
<tr>
<th>1</th>
<th>Effective date(s) of entry into force of actions</th>
<th>The complete entry into force of all provisions of Commission Decision 2007/416/EC of 13 June 2007 was 13 December 2008 since all uses of plant protection products containing carbofuran were prohibited as from that date at the latest.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Succinct details of the final regulatory action(s)</td>
<td>It is prohibited to place on the market or use plant protection products containing carbofuran. Carbofuran is not included in the list of approved active ingredients under Regulation (EC) No 1107/2009, which replaces Directive 91/414/EEC.</td>
</tr>
<tr>
<td>3</td>
<td>Reasons for action</td>
<td>Human health: it has not been demonstrated that risks are acceptable for consumers, in particular children. Environment: it has not been demonstrated that risks are acceptable for ground water contamination and for birds and mammals, aquatic organisms, bees, non-target arthropods, earthworms, and soil non-target organisms.</td>
</tr>
<tr>
<td>4</td>
<td>Basis for inclusion into Annex III</td>
<td>The final regulatory action to ban carbofuran was based on a risk evaluation taking into consideration local conditions in the EU Member States.</td>
</tr>
</tbody>
</table>
| 4.1 | Risk evaluation | **Human Health**  
A risk assessment was carried out on the basis of Directive 91/414/EEC (replaced by Regulation (EC) 1107/2009), which provides for the European Commission to issue a work programme for the examination of existing active substances used in plant protection products with a view to their possible inclusion in Annex I to the Directive, and in accordance with the provisions of Article 8(7) of Regulation (EC) No 451/2000.  
A Member State (Belgium) was designated to undertake the risk assessment based on the information submitted by the notifiers and to establish a draft assessment report, which was subject to peer review organised by the European Food Safety Authority (EFSA). The conclusions provided by EFSA were reviewed by the Member States and the Commission and submitted to the Standing Committee on the Food Chain and Animal Health (SCFCAH).  
The evaluation was based on a review of scientific data, taking into account the conditions prevailing in the European Union (intended uses, recommended application rates, good agricultural practices). Only data that had been generated according to scientifically-recognised methods were validated and used for the evaluation. Moreover, data reviews were performed and documented according to generally recognised scientific principles and procedures.  
The risk assessment resulted in several documents, including: Review Report for the active substance carbofuran finalised in the Standing Committee on the Food Chain and Animal Health at its meeting on 24 November 2006 (SANC0/10054/2006 final) [http://ec.europa.eu/food/plant/pesticides/eu-pesticides-](http://ec.europa.eu/food/plant/pesticides/eu-pesticides-) |
The risk assessment concluded that carbofuran was not demonstrated to fulfil the safety requirements laid down in Article 5 (1) (a) and (b) of Directive 91/414/EEC (replaced by Regulation (EC) 1107/2009). The consumer risk assessment, which raised a concern about the acute exposure of vulnerable groups of consumers, in particular children, could not be finalised due to the lack of information as regards certain relevant residues (UNEP/FAO/RC/CRC.11/6, section 2.4.2.1, p. 8).

Environment

It was concluded that carbofuran was not demonstrated to fulfil the safety requirements laid down in Article 5 (1) (a) and (b) of Directive 91/414/EEC (replaced by Regulation (EC) 1107/2009). The environmental risk assessment identified a number of concerns with regard to ecotoxicology. The risk for ground water contamination was assessed to be high, but could not be concluded, in particular because the data did not provide sufficient information about a number of metabolites which have a hazardous profile. Furthermore, concerns remain as regards the risk assessment for birds and mammals, aquatic organisms, bees, non-target arthropods, earthworms, and soil non-target organisms.

4.2 Criteria used

Human Health and the Environment

Relevance to other States and Region

Similar health and environmental problems are likely to be encountered in other countries where the substance is used particularly those with similar climatic conditions as well as in developing countries.

5 Alternatives

None reported

6 Waste management

None reported

7 Other

None reported
<table>
<thead>
<tr>
<th>Country Name: Canada</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 Effective date(s) of entry into force of actions</strong></td>
</tr>
<tr>
<td><strong>2 Succinct details of the final regulatory action(s)</strong></td>
</tr>
<tr>
<td><strong>3 Reasons for action</strong></td>
</tr>
<tr>
<td><strong>4 Basis for inclusion into Annex III</strong></td>
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<tr>
<td><strong>Human Health</strong></td>
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<tr>
<td><strong>Environment</strong></td>
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<tr>
<td><strong>4.2 Criteria used</strong></td>
</tr>
<tr>
<td><strong>Relevance to other States and Region</strong></td>
</tr>
<tr>
<td><strong>5 Alternatives</strong></td>
</tr>
<tr>
<td><strong>6 Waste management</strong></td>
</tr>
<tr>
<td><strong>7 Other</strong></td>
</tr>
<tr>
<td>Country Name: CILSS countries (Cabo Verde, Chad, the Gambia, Mauritania, the Niger, Senegal and Togo)</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
</tr>
</tbody>
</table>

| 1 Effective date(s) of entry into force of actions | Carbofuran was banned by the decision of CILSS Coordinating Minister N 008/MAE-MC/2015 of 08 April 2015. |
| Reference to the regulatory document | Carbofuran was banned by the decision of CILSS Coordinating Minister N 008/MAE-MC/2015 of 08 April 2015. The decision was based on the reasons stated in Sahelian Pesticide Committee: Annex to the decision to ban Carbofuran; June 2012/reviewed in November 2014. |

| 2 Succinct details of the final regulatory action(s) | Carbofuran was banned in these CILSS countries as of 08 April 2015. |

| 3 Reasons for action | Human health: unacceptable risk to users and to consumers due to exposure from food and drinking water. Environment: high risk to birds and fresh water invertebrates. |

| 4 Basis for inclusion into Annex III | The final regulatory action to ban carbofuran was based on a risk evaluation taking into consideration local conditions in the Sahel. |

| 4.1 Risk evaluation | Carbofuran presents risks to human health and especially to non-target organisms in the environment, making it very difficult to handle it without risks for users in Sahel countries. These risks have justified its ban in many countries of the world among which (are) all the European Union member states. A consultation mission conducted on behalf of the Sahelian Pesticide Committee (SPC) concluded that the SPC should stop the registration of the pesticides of toxicity class Ib since they are used by poorly trained small farmers who don’t respect the safety measures (CILSS countries supporting documentation p. 32 paragraph 4.2.4). The Sahelian Pesticide Committee stopped the registration of carbofuran based pesticides in CILSS countries in 2006 taking into account: |

- The fragile ecology of CILSS countries already characterized by an imbalance of ecosystems and the disappearance of organisms useful to the environment; |
- Non-compliance with recommended measures for a safe use of carbofuran by users in the context of CILSS countries; |
- The presence of pesticide residues in harvested crops and the behaviour of local people make the risk unacceptable |

Further to the pollution of Sahel ground water which constitutes the main drinking water resource with open wells, several sources agree that carbofuran is highly toxic to birds. One single grain may kill a bird (oral LD$_{50}$ of 0.4 mg/kg body weight). Carbofuran is highly toxic to fresh water invertebrates and extremely toxic to birds. Carbofuran is moderately to highly toxic to fresh water fish. |

| 4.2 Criteria used | Human Health and the Environment |
| Relevance to other States and Region | Similar health and environmental problems are likely to be encountered in other countries where the substance is used particularly those with similar climatic conditions. |

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5 These seven parties share a common pesticide registration body, the Sahelian Pesticides Committee set up by the Permanent Interstate Committee for Drought Control in the Sahel (CILSS). As the CILSS member states take together decisions on the registration of pesticides at a regional level, the notifications submitted by the seven African parties referred to the same final regulatory action.
5 Alternatives

**Chemical alternatives:** Several alternative molecules to carbofuran exist. The India Committee of pesticide experts recommended the following pesticides on paddy rice and other crops: chlorantraniliprole, flubendiamide and quinalphos.

According to Jon Tollefson and Erin Hodgson, from the Department of Entomology of IOWA State University in the USA, the alternative for the protection of corn against root worms is to add seeds treated with a neonicotinoid pesticide like Poncho™ in the applicator. In case of post-emergence liquid treatment Lorsban™ 4E, an ethylchloropyriphos-based formulation is an option. Currently five formulations authorized by the Sahelian Pesticide Committee under the name of Dursban are ethylchloropyriphos based.

Capture™ 2EC of the new generation of pyrethroids is an alternative to carbofuran thanks to its effectiveness.

**Integrated Pest and production management (IPPM):** The experience in IPPM launched by FAO in collaboration with the Ministries of Agriculture in several countries of the Sahel yielded important results in agricultural production and pest management. This initiative of Good Agricultural Practices (GAP) will improve the agricultural productivity and train several growers who are potential facilitators. IPPM is based on the following principles:

- A sound and judicious use of pesticides;
- The acquisition of knowledge and practical skills critical to pest control;
- The reinforcement of decision-making capacity of growers at a field level;
- The development of a better low-cost productivity which protects the environment.

6 Waste management

None reported

7 Other

None reported

**Previous notifications**  A severely hazardous pesticide formulation, i.e. dustable powder formulations containing a combination of benomyl at or above 7 percent, carbofuran at or above 10 per cent and thiram at or above 15 percent is already listed in Annex III of the Convention.
## Annex 3 – Addresses of designated national authorities

### European Union

<table>
<thead>
<tr>
<th>Address</th>
<th>Phone</th>
<th>Fax</th>
<th>E-mail</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+32 2 296 7617</td>
<td><a href="mailto:Juergen.Helbig@ec.europa.eu">Juergen.Helbig@ec.europa.eu</a></td>
</tr>
</tbody>
</table>

### Canada

<table>
<thead>
<tr>
<th>Address</th>
<th>Phone</th>
<th>Fax</th>
<th>E-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pest Management Regulatory Agency 2720 Riverside Drive Ottawa ON K1A 0K9 Canada Trish MacQuarrie Director General of the Policy, Communications and Regulatory Affairs Directorate</td>
<td>1-613-736-3660</td>
<td>1-613-736-3659</td>
<td><a href="mailto:Trish.Macquarrie@hc-sc.gc.ca">Trish.Macquarrie@hc-sc.gc.ca</a></td>
</tr>
</tbody>
</table>

### CILSS countries

<table>
<thead>
<tr>
<th>Address</th>
<th>Phone</th>
<th>Fax</th>
<th>E-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ministère de l’Agriculture et de l’Environnement BP 1551 Ndjamena Tchad Moussa Abderrarman Abdoulaye Directeur de la Protection des Végétaux et du Conditionnement Chad</td>
<td>00235 516 00 89</td>
<td>-</td>
<td><a href="mailto:charafara2009@gmail.com">charafara2009@gmail.com</a></td>
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<tr>
<td>National Environment Agency Jimpex Road, Kanifing PMB 48, Banjul, The Gambia Omar S Bah Designated National Authority, Rotterdam Convention The Gambia</td>
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<td>220 4399430</td>
<td><a href="mailto:Omar16bah@yahoo.ca">Omar16bah@yahoo.ca</a></td>
</tr>
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<td>-</td>
<td><a href="mailto:ouldmaouloudm@yahoo.fr">ouldmaouloudm@yahoo.fr</a></td>
</tr>
<tr>
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<td>0022720 74 19 83</td>
<td><a href="mailto:dpv@intnet.ne">dpv@intnet.ne</a>, <a href="mailto:douki_a@yahoo.fr">douki_a@yahoo.fr</a></td>
</tr>
</tbody>
</table>
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C Industrial chemicals
CP Pesticides and industrial chemicals
P Pesticides
Regulatory actions

European Union:


Canada:


CILSS countries:


Supporting documentation provided by the European Union:


Supporting documentation provided by Canada:


Supporting documentation provided by CILSS countries:


Other Documents

FAO/UNEP (2004/2005) Decision Guidance Document dustable powder formulations containing a combination of benomyl at or above 7%, carbofuran at or above 10% and thiram at or above 15%. Available at: http://www.pic.int/Portals/5/DGDs/DGD_Dustable%20powder%20formulations_EN.pdf


Relevant guidelines and reference documents


