

## APPENDIX F. Summary of Ecotoxicity Data for PCNB and its Degradates

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### 1. Toxicity to Terrestrial Organisms

#### 1.1 Acute and Subacute Toxicity to Birds

The acute oral toxicity of PCNB to 26 week-old bobwhite quail (*Colinus virginianus*) was assessed over 14 days (MRID 40618001). The 14 day-acute oral LD<sub>50</sub> exceeded the highest dose tested (2,250 mg a.i./kg-bw) (**Table 1**). There was no mortality during the study. No physiological or behavioral abnormalities were observed and body weights and food consumption remained unaltered. According to the US EPA classification, PCNB is practically non-toxic to bobwhite quail on an acute exposure basis. The study is classified as scientifically sound and is consistent with guideline testing requirements for avian oral toxicity studies using bobwhite quail.

**Table 1. Summary of avian acute toxicity data for PCNB.**

Species (Compound)	Study Type	% a.i.	LD <sub>50</sub> (mg a.i./kg-bw)	MRID	Toxicity Category	Study Classification
Bobwhite quail <i>Colinus virginianus</i> (PCNB)	Acute Oral	98.0	>2,250 mg/kg-bw	40618001	Practically non-toxic	Acceptable

Two subacute dietary studies using the technical grade active ingredient (TGAI) are recommended to establish the toxicity of PCNB to birds. The preferred test species are the bobwhite quail (*Colinus virginianus*) and the mallard duck (*Anas platyrhynchos*). Four studies were submitted for review. In a 9-day quail study with PCNB (MRID 40652602), marked anorexia and lethargy were observed at the two highest test concentrations (**Table 2**). A total of five birds died during the study (one at the 27,000 mg a.i./kg-diet dose and four at the 54,000 mg a.i./kg-diet dose). Gross pathological examination of the five birds revealed no abnormalities. In

a 5-day bobwhite quail study with PCNB (MRID 40618003), the LC<sub>50</sub> exceeded the highest concentration tested (5,620 mg a.i./kg-diet). Anorexia and significant low weight gain were observed in the 5,620 mg a.i./kg-diet treatment group. In a 9-day mallard duck study with PCNB (MRID 40652603), marked anorexia and lethargy were observed at the two highest test concentrations (27,000 and 54,000 mg a.i./kg-diet). Gross pathological examination of the seven birds that died during testing revealed abnormalities in four of the birds; three had gas-filled intestines and the fourth had what was characterized as “yolk” in the abdominal cavity. The dietary LC<sub>50</sub> value was reported as greater than the highest concentration tested (54,000 mg a.i./kg-diet). In a 5-day mallard duck study with PCNB (MRID 40618002), the LC<sub>50</sub> again exceeded the highest concentration (5620 mg a.i./kg-diet) tested. No mortalities were observed in this experiment; however, anorexia and significantly reduced weight gain were reported in the highest treatment group. Based on the results of these studies, PCNB is classified as practically non-toxic to birds on a subacute dietary exposure basis. All of the dietary studies are classified as acceptable and are consistent with guideline subacute avian dietary testing requirements.

**Table 2. Summary of avian subacute dietary toxicity data for PCNB.**

Species (Compound)	Study Type	% a.i.	LC <sub>50</sub> (mg a.i./kg-diet)	MRID	Toxicity Category	Study Classification
Bobwhite quail <i>Colinus virginianus</i> (PCNB)	9-day acute dietary	99.0	>54,000	40652602	Practically non-toxic	Acceptable
	5-day acute dietary	98.0	>5,620	40618003	Practically non-toxic	Acceptable
Mallard duck <i>Anas platyrhynchos</i> (PCNB)	5-day acute dietary	98.0	>5,620	40618002	Practically non-toxic	Acceptable
	9-day acute dietary	99.0	>54,000	40652603	Practically non-toxic	Acceptable

## 1.2 Chronic Toxicity to Birds

Six avian reproduction dietary studies with PCNB were submitted for review (**Table 3**). In a study with bobwhite quail (MRID 43980301), the NOAEC was determined to be 1,000 mg a.i./kg-diet based upon a reduction in 14-day survivor weight and percentage of 14-day hatchling survivors; the LOAEC was 2,500 mg a.i./kg-diet. This avian reproduction study is scientifically sound and is classified as acceptable. In a study with mallard ducks (MRID 43903301), there were no overt signs of toxicity or reductions in body weights or feed consumption at any of the test concentrations when compared to controls. For all concentrations tested, there were no statistically significant effects on any reproductive parameters. The NOAEC was determined to be 5,500 mg a.i./kg-diet (the highest concentration tested) and no LOAEC was determined. This study is scientifically sound and is classified as acceptable.

Two additional studies with bobwhite quail (MRID 41321101) and mallard ducks (MRID 41321201) with PCNB were classified as supplemental (**Table 3**). These studies are scientifically sound and document adverse effects on reproductive endpoints. The quail study detected significant reductions in the number of 14-day survivors, the number of eggs set, the percentage of hatchlings of eggs set and the body weight of offspring at all treatment levels. There was a significant reduction in the number of eggs laid, the number of hatchlings, viable

embryos and number of eggs set at the 11,000 and 22,000 mg a.i./kg-diet treatment concentrations. Additionally, the number of viable embryos and live 15- to 20-day embryos were decreased at a dietary concentration of 22,000 mg a.i./kg-diet. This study was classified as supplemental due to an unusually high percentage of defective eggs in the control group (40.8%) and the lack of 14-day-old survivors/pen data. The unusually high percentage of cracked eggs in the control group confounds an analysis of the effects of PCNB on egg quality. The mallard duck study detected decreases in egg shell thickness and female body weight at all treatment levels. The number of eggs laid, viable embryos, live 15- to 20-day embryos and hatchlings were reduced at the 11,000 and 22,000 mg a.i./kg-diet treatment levels. The number of defective (cracked) eggs was significantly increased and the number of eggs set was decreased at the 22,000 mg a.i./kg-diet treatment concentration. The NOAECs in these studies were therefore less than the lowest concentration (<5,500 mg a.i./kg-diet) tested. This study was classified as supplemental due to the lack of 14-day-old survivors/pen data. These two studies, while providing useful information on the effects of PCNB on avian reproduction, do not comply with guideline testing requirements to establish a chronic NOAEC.

Two other avian reproduction studies with bobwhite quail (MRID 41332801) and mallard ducks (MRID 41332802) with PCNB were submitted for review (**Table 3**). PCNB exposure did not result in treatment-related effects on mortality, behavior, food consumption or adult body weight of bobwhite quail. Based on a reduction of 14-day survivors as a percentage of hatchlings, the only reproductive endpoint significantly affected, the NOAEC was determined to be 600 mg a.i./kg-diet, while the LOAEC was 1200 mg a.i./kg-diet. For the mallard ducks, PCNB exposure did not result in treatment-related effects on reproduction, survival, behavior, or food consumption. Due to a decrease in adult body weight, the NOAEC was determined to be 600 mg a.i./kg-diet, while the LOAEC was 1200 mg a.i./kg-diet. Both studies are scientifically sound and are consistent with guideline testing requirements for an avian reproduction studies and are classified as acceptable.

**Table 3. Summary of avian reproduction data for PCNB.**

Species (Compound)	% a.i.	NOAEC/LOAEC (mg a.i./kg-diet)	Most sensitive endpoint	MRID	Study Classifications
Bobwhite quail <i>Colinus virginianus</i> (PCNB)	97.4	1,000/2,500	14-day survivor weight, 14-day survivors	43980301	Acceptable
Mallard duck <i>Anas platyrhynchos</i> (PCNB)	97.4	5,500/ND	No significant effects noted at any concentration	43903301	Acceptable
Bobwhite quail <i>Colinus virginianus</i> (PCNB)	98.5	<5,500/5,500	14-day survivors, number of eggs set, body weight offspring	41321101	Supplemental
Mallard duck <i>Anas platyrhynchos</i> (PCNB)	98.5	<5,500/5,500	Egg shell thickness, female body weight	41321201	Supplemental
Bobwhite quail <i>Colinus virginianus</i> (PCNB)	99.4	600/1,200	14-day survivors	41332801	Acceptable
Mallard duck <i>Anas platyrhynchos</i> (PCNB)	99.4	600/1,200	Adult body weight	41332802	Acceptable

ND = Not Determined

### 1.3 Acute and Chronic Toxicity to Mammals

Wild mammal testing is required on a case-by-case basis, depending on the results of lower-tier laboratory mammalian studies, intended use pattern and pertinent environmental fate characteristics.

In an acute oral toxicity study (MRID 41443101), rats exposed to PCNB developed toxic symptoms that included slight piloerection, diarrhea and slight to moderate constriction of pupils at the higher treatment concentrations; all animals were asymptomatic after three days (**Table 4**). The acute oral LD<sub>50</sub> exceeded the maximum dose (>5,050 mg/kg-bw) tested.

In a 2-generation reproduction study with rats exposed to PCNB (MRIDs 43469301, 43469302, 43469303) reviewed by the Health Effects Division, the data evaluation record reports that there were no compound-related effects in parental body weight, food consumption or reproductive performance. In addition, there were no clinical signs of toxicity in parental animals. In the P<sub>1</sub> generation, hepatocellular hypertrophy was observed at 1000 mg/kg in both sexes and thyroid follicular cell hypertrophy/hyperplasia was observed at 100 and 1000 mg/kg in males and at 1000 mg/kg in females. There were no changes observed in the reproductive organs. In the P<sub>2</sub> generation, the effects were the same except that the liver effects were only observed in high

dose males. No treatment-related effects were observed in litter size, pup viability, pup body weight or pup macroscopic examinations. No microscopic examinations were conducted on pups. The LOEL is 100 mg/kg/day in males and 1000 mg/kg/day in females based on increases in hepatocellular hypertrophy and thyroid follicular cell hypertrophy/hyperplasia. The NOEL is 10 mg/kg/day in males and 100 mg/kg/day in females. The reproductive study in the rat is classified as acceptable and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800) in the rat.

In another 2-generation study with rats (MRID 41918701), decreased body weight was observed in parents and in offspring exposed to PCNB levels as low as 169 mg/kg-bw/day (LOAEL). Decreased body weight gain was also observed at the LOAEL in parents. The resulting NOAEL for this study was 1.2 mg/kg-bw/day (equivalent to a NOAEC of 24 mg/kg-diet/day using standard FDA lab rat conversion); because of a lack of data to the contrary, PCNB degradates are presumed to have similar toxicity to that of the parent compound.

**Table 4. Summary of acute and chronic rat toxicity data for PCNB.**

Species (Compound)	Endpoint	MRID	Study Classification
Rat (PCNB)	LD <sub>50</sub> >5,050 mg/kg-bw	41443101	Acceptable
	LOAEL = 2,000 mg/kg-diet NOAEL = 200 mg/kg-diet	43469301 43469302 43469303	Acceptable
	LOAEL = 3,000 mg/kg-diet NOAEL = 24 mg/kg-diet	41918701	Acceptable

#### 1.4 Toxicity to Terrestrial Invertebrates

A honey bee acute contact study was conducted with PCNB (MRID 40506102), and the resulting contact LD<sub>50</sub> was greater than the highest dose tested (>100 µg/bee; **Table 5**). No sublethal effects were noted in any of the control or test animals throughout the duration of the study. The study is scientifically sound and is consistent with guideline testing requirements.

**Table 5. Summary of honey bee acute contact and oral toxicity data for PCNB.**

Species (Compound)	% a.i.	LD <sub>50</sub> µg/bee	MRID	Toxicity Category	Study Classification
Honey Bee <i>Apis mellifera</i> (PCNB)	99.5	>100 (contact)	40506102	Practically nontoxic	Acceptable

Two studies were identified in the open-literature evaluating toxicity to earthworms.

In the first study (Roark and Dale, 1979; ECOTOX No. E53634), earthworms were exposed to PCNB mixed into soil at a concentration of 108 µg/cm<sup>3</sup> and observed at 10, 29, 52, 64, and 84 days of exposure. By day 29, there was 100 percent mortality in the PCNB treatment group but only 8 percent mortality in control worms. Earthworms treated with PCNB showed little feeding

activity and did not reproduce during the test. This study was classified as qualitative because EFED currently does calculate soil-specific exposure concentrations in order to evaluate risk to worms.

Another study investigated the toxicity of pentachlorobenzene (PCB) and other compounds to two earthworm species, *Lumbricus rubellus* and *Eisenia andrei*, exposed through soil (Van Gestel et al. 1991; ECOTOX No. E40464). The overall goal of the study was to determine if development of QSARs for earthworms is feasible. As a part of this study, 2-week LC<sub>50</sub> toxicity values were derived for each earthworm species. The two-week LC<sub>50</sub> values for *L. rubellus* were 115 (95% CI: 109-122) and 201 (95% CI: 150-270) mg/kg in natural and artificial soils, respectively. The two-week LC<sub>50</sub> values for *E. andrei* were 134 (95% CI: 100-180) and 238 (95% CI: 180-320) mg/kg in natural and artificial soils, respectively. This study was classified as qualitative because EFED currently does not have a method to evaluate risk to earthworms. Therefore, this data can only be used for characterization purposes.

### 1.5 Toxicity to Terrestrial Plants

There are no available toxicity data for terrestrial plants.

## 2. Toxicity to Aquatic Organisms

### 2.1 Acute Toxicity to Freshwater Fish

Results of toxicity tests with PCNB for freshwater fish are listed in **Table 6**. Since the LC<sub>50</sub> values for the species tested are in the 100 to 550 µg a.i./L range, PCNB is classified as highly toxic to freshwater fish on an acute exposure basis. In acute toxicity testing with bluegill sunfish (MRID 40849001 and 41060801), sublethal effects including loss of equilibrium, fish at the bottom of the test chamber, and quiescence were noted at exposure concentrations ranging from 43 to 350 µg a.i./L. Similar sublethal effects were also noted in rainbow trout at PCNB exposure concentrations ranging from 130 to 450 µg a.i./L (MRID 40992701). All of the freshwater fish studies are consistent with guideline testing requirements and are classified as acceptable.

**Table 6. Summary of freshwater fish acute toxicity data for technical PCNB.**

Species (Compound)	% a.i.	96-hour LC <sub>50</sub> (µg a.i./L)	Toxicity Category	MRID	Study Classification
Rainbow Trout	99	320	Highly Toxic	40992701	Acceptable
<i>Oncorhynchus mykiss</i> (PCNB)	98.2	550	Highly Toxic	40618005	Acceptable
Bluegill Sunfish	99	210	Highly Toxic	40849001	Acceptable
<i>Lepomis macrochirus</i> (PCNB)	98.2	100	Highly Toxic	40618004	Acceptable

Acute toxicity was determined for formulated end-use products containing PCNB as well; 96-hr LC<sub>50</sub> values ranged from 240 to 1,660 µg a.i./L, indicating that formulated PCNB can be classified as moderately to highly toxic to freshwater fish (**Table 7**). In acute toxicity testing of formulated product with bluegill sunfish (MRID 41060801), sublethal effects including

hemorrhaging, loss of equilibrium, fish at the bottom of the test chamber, and quiescence were noted at exposure concentrations ranging from 73 to 580 µg a.i./L. Sublethal effects in rainbow trout were similar to those observed in bluegill sunfish and occurred at exposure concentrations ranging from 99 to 760 µg a.i./L (MRID 41060802). All of the formulated product tests were consistent with guideline testing requirements.

**Table 7. Summary of freshwater fish acute toxicity data for formulated products of PCNB.**

Species (Compound)	% a.i.	96-hour LC <sub>50</sub> (µg a.i./L)	Toxicity Category	MRID	Study Classification
Rainbow Trout <i>Oncorhynchus mykiss</i> (PCNB)	26.6	1,660	Moderately toxic	40650301	Acceptable
	24	310	Highly Toxic	41060802	Acceptable
Bluegill Sunfish <i>Lepomis macrochirus</i> (PCNB)	26.6	750	Highly Toxic	40650302	Acceptable
	24	240	Highly Toxic	41060801	Acceptable

## 2.2 Chronic Toxicity to Freshwater Fish

Results of a freshwater fish early life-stage (ELS) study with PCNB is summarized in **Table 8**. Exposure of rainbow trout to PCNB at 32 µg a.i./L significantly reduced ( $p < 0.05$ ) larval growth in terms of both length and weight (MRID 41663401). Significant reductions in percentage (27%) of eggs hatched and fry survival were observed at 52 µg a.i./L. This study is scientifically sound and is consistent with the guideline requirements for a freshwater fish early life stage toxicity test.

An open-literature study (Metcalf et al. 2008; ECOTOX No. E110757) with PCNB was identified for Japanese medaka fish (*Oryzias latipes*), which resembles the first portion of a fish ELS test design (OPPTS 850.1400 guideline). Typically, guideline ELS studies are performed with newly fertilized fish embryos and exposure to the test substance occurs up to approximately 30 days post-hatch (depending on test species). In this study, Japanese medaka fish (*Oryzias latipes*) were exposed to multiple PCNB concentrations until only 17 days from the fertilized egg stage. Since hatching occurs within the first 17 days of eggs fertilization in this species, the percent hatch endpoint was adequately targeted in this test design; growth data and post-hatch mortality were not appropriately captured. The nominal PCNB concentrations tested in this study were 1, 9, 46, 92, 229, 686, 915, 2288, 4575, and 6863 µg active ingredient (a.i.)/L based (note: nominal concentrations were corrected for percent a.i. based on analytical measurements of test material). Based on data reported in the manuscript, the proportion of fish that hatched while exposed to PCNB was statistically lower than controls at concentrations  $\geq 9$  µg a.i./L, resulting in a study NOAEL of 1 µg a.i./L. Deformities were also observed in developing embryos. Anisophthalmia (defined in manuscript as cases where eyes become fused or where one eye is smaller than the other or completely absent) occurred at statistically significant levels relative to control fish at concentrations of  $\geq 686$  µg a.i./L. Other abnormalities including lack of development of brain, notochord, and heart occurred at concentrations of  $\geq 46$  µg a.i./L. This study was classified as qualitative for the following reasons (1) environmental parameters, including dissolved oxygen levels in test water, were not reported; (2) measured concentrations of test material were not reported; therefore exposure levels are uncertain; (3) Only 10-20 fish

embryos were used per treatment level, which may have affected the statistical power of the test. ELS guidance states that 60 embryos divided into two replicates should be used; (4) control organism performance data was not provided and therefore cannot be assessed.

**Table 8. Summary of freshwater fish early life stage toxicity data for PCNB.**

Species (Compound)	% a.i.	NOAEC/LOAEC (µg a.i./L)	Endpoints Affected	MRID	Study Classification
Rainbow Trout <i>Oncorhynchus mykiss</i> (PCNB)	99	13/32	Growth (length and weight)	41663401	Acceptable

### 2.3 Acute Toxicity to Freshwater Invertebrates

A freshwater invertebrate acute toxicity test (Acc. No. 114167) was submitted using the preferred test species *Daphnia magna* and is summarized in **Table 10**. The 48-hour LC<sub>50</sub> was 770 µg a.i./L and the NOAEC was 130 µg a.i./L. All daphnids exhibited lethargy in the two highest concentrations tested. Additionally, several daphnids were caught at the surface of test solution in the next two lower concentrations. PCNB is categorized as highly toxic to aquatic invertebrates on an acute exposure basis. The study is consistent with guideline testing requirements and is classified as acceptable.

**Table 9. Summary of freshwater invertebrate acute toxicity data for PCNB.**

Species (Compound)	% a.i.	48-hour LC <sub>50</sub> (µg a.i./L)	Toxicity Category	MRID	Study Classification
<i>Daphnia magna</i> (PCNB)	99.08	770	Highly toxic	114167 (accession no.)	Acceptable

Several open-literature studies on aquatic invertebrates are also available for PCB.

*Chironomus riparius* (Roghair et al. 1994; ECOTOX No. E4072) larvae were exposed to PCB at nominal concentrations of 0, 0.10, 0.18, 0.32, 0.56, 1.0, 1.8, and 3.2 mg/L for 48 hours, and mortality, appearance, and behavior were observed. When PCB was delivered through use of dimethyl sulfoxide (DMSO) as a solvent, the resulting 48-hour LC<sub>50</sub> value was 0.23 mg/L. When PCB was delivered using a generator column, the resulting 48-hour LC<sub>50</sub> value was >0.32 mg/L. Temperature, pH, and test substance concentration showed minimal variability during the test. This study was classified as qualitative because DMSO was used as a solvent to dissolve PCB. This solvent has been known to interfere with toxicity test results.

*Gammarus pseudolimnaeus* (amphipods) were exposed to PCB for 96 hours under flow-through conditions, and mortality and behavioral effects were recorded (Brooke, 1987; ECOTOX No. E14339). The 96-hour LC<sub>50</sub> value was 51.1 µg/L (95% CI: 39.2 to 66.6 µg/L). During the test, amphipods were placed in the same container as fathead minnows during flow-through testing, although they were separated by wire mesh; however, it cannot be precluded that physical,



chemical, or behavioral cues between amphipods and fathead minnows did not affected the outcome of the test. Therefore, this study was classified as qualitative.

*Hyalella azteca* were exposed to PCB in beakers at time-weighted average concentrations ranging from 0.029 to 4.57  $\mu\text{mol/L}$  and mortality was observed (Landrum et al. 2004; E75146). This study derived  $\text{LC}_{50}$  values for time periods ranging from 26.5 to 600 hours. The resulting  $\text{LC}_{50}$  values ranged from 0.5 to 2.3  $\mu\text{mol/L}$  and occurred at the longest (600 hours) and shortest (26.5 hours) time intervals, respectively. The most relevant endpoints for risk assessment are those  $\text{LC}_{50}$  values occurring around 48 to 96 hours, as these data reflect the time-frame in which acute toxicity is typically measured in OCSPP guideline studies. The lowest toxicity value from this general timeframe was the 97-hour  $\text{LC}_{50}$  of 0.84  $\mu\text{mol/L}$  (210.3  $\mu\text{g/L}$ ). The duration and method of exposure in this assessment is in line with general practices for conducting acute toxicity studies as outline in OCSPP 850 guidelines for toxicity testing on aquatic organisms (e.g., 850.1000). However, this study was classified as qualitative because multiple experiments were used to derive  $\text{LC}_{50}$  data and it is not possible to determine the results or the number of sampling points from each experiment. Consequently, it is not possible to verify  $\text{LC}_{50}$  values or calculate them for individual experiments.

## 2.4 Chronic Toxicity to Freshwater Invertebrates

Two freshwater aquatic invertebrate life-cycle tests using the TGAI were submitted for PCNB (MRID 40823204 and 41321301) using the preferred test species, *Daphnia magna*, and are summarized in **Table 11**. The first study (MRID 40832304) observed a NOAEC of 19  $\mu\text{g a.i./L}$  and LOAEC of 34  $\mu\text{g a.i./L}$ . Reproduction was significantly reduced at higher treatment levels. Because the authors of this study did not submit the raw data for evaluation, this study is classified as supplemental since the statistical analysis could not be verified. A second 21-day toxicity study performed with the water flea estimated a NOAEC and a LOAEC of 18  $\mu\text{g a.i./L}$  and 30  $\mu\text{g a.i./L}$ , respectively, based on affected reproduction. The number of young per adult per day decreased significantly with increasing concentration of PCNB. A one hundred percent mortality rate was observed at the highest treatment level of 23  $\mu\text{g a.i./L}$ .

**Table 10. Summary of freshwater aquatic invertebrate life-cycle toxicity data for PCNB.**

Species (Compound)	% ai	21-day NOAEC/ LOAEC ( $\mu\text{g a.i./L}$ )	Endpoints Affected	MRID	Study Classification
<i>Daphnia magna</i> (PCNB)	98.2	19/34	Reproduction	40832304 41918803	Supplemental
	99.0	18/30	Reproduction	41321301	Acceptable

## 2.5 Acute Toxicity to Estuarine/Marine Fish

Three estuarine/marine fish acute toxicity tests were submitted for PCNB using the preferred test species, sheepshead minnow (*Cyprinodon variegatus*) (**Table 12**). Two of the studies (MRIDs 42336901 and 40832302), were classified as supplemental. For study MRID 42336901, an  $\text{LC}_{50}$  of 1,700  $\mu\text{g a.i./L}$  was calculated; however, there is uncertainty in this endpoint because undissolved test material was observed at all concentrations except the lowest (260  $\mu\text{g a.i./L}$ ) and the analytical samples were not filtered/centrifuged to remove precipitate. For study MRID

40832302, the calculated LC<sub>50</sub> value was 1,500 µg a.i./L (95% CI of 1,200 to 1,800 µg a.i./L). One hundred percent mortality occurred after 96 hours at the highest analytically-determined level of 7,500 mg a.i./L of PCNB technical. At the next lower treatment level (4,300 µg a.i./l), all surviving fish (40%) showed complete loss of equilibrium. The lowest test concentration of 1,200 µg a.i./L had mortality of 25%. In this study, precipitate was observed at all concentrations tested, resulting in uncertainty in the level of exposure to the test substance. Study MRID 40882903 determined the 96-hour LC<sub>50</sub> to be 7,900 µg a.i./L; therefore. Complete loss of equilibrium, erratic swimming behavior, lethargy, and swimming at the surface were all observed in this test at concentrations ranging from 1,400 to 15,000 µg a.i./L. Throughout the test period, and at all test concentrations the test solutions showed cloudiness and a white precipitate which could be seen on the bottom of the test vessels. This study is scientifically sound and is consistent with guideline testing requirements.

Since all three estuarine/marine fish acute toxicity studies reported precipitate in the majority of treatment groups, but no effort was made to remove the precipitate from the test solution, the endpoint values derived from these studies are considered uncertain.

**Table 11. Summary of estuarine/marine fish acute toxicity data for PCNB.**

Species (Compound)	% a.i.	96-hour LC <sub>50</sub> (µg a.i./L)	Toxicity Category	MRID	Study Classification
Sheepshead Minnow <i>Cyprinodon variegatus</i> (PCNB)	98.98	1,700	Moderately Toxic	42336901	Supplemental
	98.2	1,500	Moderately Toxic	40832302	Supplemental
	97.7	7,900	Moderately Toxic	40882903	Acceptable

## 2.6 Chronic Toxicity to Estuarine and Marine Fish

No data were available to assess the chronic toxicity of PCNB to estuarine/marine fish.

## 2.7 Acute Toxicity to Estuarine and Marine Invertebrates

As shown in **Table 13**, the 96-hour mysid shrimp LC<sub>50</sub> for technical PCNB is 12 µg a.i./L (MRID 40832301). Thus, this chemical is categorized as very highly toxic to estuarine/marine crustaceans on an acute exposure basis. This chemical is also very highly toxic to mollusks (LC<sub>50</sub> range: 23 to 24 µg a.i./L; MRIDs 40832303 and 40882902). The studies are scientifically sound and are consistent with guideline testing requirements. For study MRID 40832303, samples were not filtered prior to chemical analysis, so the dissolved levels of PCNB to which the oysters were exposed in the test chambers are unknown and; hence, PCNB is likely to be more toxic than reported. For study MRID 40882902, the registrant failed to provide the raw data for shell thickness measurements making it impossible to verify the statistical results.

**Table 12. Summary of estuarine/marine invertebrate acute toxicity data for PCNB.**

<b>Species (Compound)</b>	<b>% a.i.</b>	<b>96-hour LC<sub>50</sub> (µg a.i./L)</b>	<b>Toxicity Category</b>	<b>MRID</b>	<b>Study Classification</b>
Mysid ( <i>Americamysis bahia</i> ) (PCNB)	98.2	12	Very Highly Toxic	40832301	Acceptable
	97.9	23	Very Highly Toxic	40882901	Acceptable
Eastern Oyster ( <i>Crassostrea virginica</i> ) (PCNB)	98.2	24	Very Highly Toxic	40832303	Supplemental
	97.7	23	Very Highly Toxic	40882902	Supplemental

## **2.8 Chronic Toxicity to Estuarine and Marine Invertebrates**

There are no available chronic toxicity data for estuarine/marine invertebrates.

## **2.9 Toxicity to Aquatic Plants**

There are no available toxicity data for vascular or nonvascular aquatic plants.