Appendix J. Ecological Effects

Freshwater Fish: Acute Exposure (Mortality) Studies

Available data indicate that methidathion is very highly toxic on an acute basis to three surrogate freshwater fish species. As shown in **Table 1**, the bluegill sunfish 96-hour LC_{50} of 2.2 (0.9 – 5.1) µg/L will be used to calculate RQs for direct effects to the aquatic-phase CRLF. In this study, no mortality occurred at the lowest dosage level of 0.32 µg/L; 100% mortality occurred at the 8 highest levels from 5.6 to 1000 µg/L. The probit dose-response slope is 2.9 (1.9 – 4.0) for this study. According to the report, fish exposed to 1000 µg/L methidathion exhibited slight spastic motions and swam on their sides. The study was classified as supplemental primarily due to temperature, which was too low (15 - 18.3°C) and too variable.

Fish toxicity studies with the methidathion formulation 2E (25.2% a.i.) are also available for consideration in this risk assessment. These studies suggest that the tested formulation and technical grade methidathion exhibit similar toxicity on an acute basis.

Table 1. Freshwater Fish Acute Toxicity of Methidathion

Species	% ai	LC50 (µg/L ai)	Toxicity Category	MRID No. Author/Year	Study Classification
Rainbow trout *(Oncorhynchus mykiss)*	98.5	14	very highly toxic	40098001 F.L. Mayer/1986	core
	97.7	10	very highly toxic	00011841 /1965	supplemental
	2E (25.2%)	26.2 µg/L product (6.6 µg/L ai)	very highly toxic	42081703 /1991	core[1]
Bluegill sunfish *(Lepomis macrochirus)*	98.5	9	very highly toxic	40098001 F.L. Mayer/1986	core
	95	**2.2**[2]	very highly toxic	00011841 1965	supplemental
	2E (25.2%)	32.5 µg/L product (8.2 µg/L ai)	very highly toxic	42081702 /1991	core[1]
Goldfish (*Carassius auratus*)	97.7	6.8	very highly toxic	00011841 /1965	supplemental

[1] Formulation testing is required when a product is expected to reach surface water directly such as through direct application or drift.
[2] Bold indicates the endpoint is used to calculate RQs

Freshwater Fish: Chronic Exposure (Growth/Reproduction) Studies

The 35-day chronic toxicity of Supracide® (Methidathion) to the early life stage of Fathead Minnows (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs (140 eggs/level, 4 reps/level, 35 eggs/rep; <48 hrs old) of fathead minnow were exposed to 0 (negative and solvent controls), 0.93, 1.9, 3.8, 7.5 and 15 µg ai/L nominal concentrations. Time-weighted, mean-measured concentrations were <0.24-<0.42 (<LOQ; negative and solvent controls), 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L. The 35-day EC_{50} and NOAEC values, based on survival, total length and wet weight, were >12 and 6.3 µg ai/L, respectively. The sublethal effects included reduced juvenile survival and inhibitions of total length and dry weight. The most sensitive endpoints were percent survival, total length, and wet weight.

This toxicity study is scientifically sound and satisfies the requirements of §72-4a for an early life toxicity study with *Pimephales promelas*. It is classified as **Acceptable.**

Results Synopsis

Test Organism Size/Age(mean Weight or Length): Eggs, <48 Hrs
Test Type (Flow-through, Static, Static Renewal): Flow-Through

Percent Survival (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Total Length (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Wet Weight (Day 35):
EC_{50}: >12 µg ai/L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A
NOAEC: 6.3 µg ai/L
LOAEC: 12 µg ai/L

Endpoint(s) Affected: Percent Survival, Total Length and Wet Weight

Percent egg hatch [(no. of fry ÷ no. of eggs on day 0) x 100] was 78 and 94% in the negative and solvent controls, respectively, and 88, 91, 75, 89 and 75% in the measured 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L treatment groups, respectively. No significant differences were detected at any treatment levels relative to the controls.

Fry survival [(no. of fry on day 35 ÷ no. of fry introduced) x 100] was 91 and 98% in the negative and solvent controls, respectively, and 90, 90, 90, 90, 90 and 47% in the measured 0.68, 1.6, 3.2, 6.3 and 12 µg ai/L treatment groups, respectively. The percent survival at the highest treatment level, measured 12 µg ai/L, was significantly reduced relative to the pooled controls. The resulting NOAEC and LOAEC values were 6.3 and 12 µg ai/L.

Freshwater Invertebrates: Acute Exposure Studies

Available data indicate that methidathion is very highly toxic on an acute basis to a surrogate freshwater invertebrate species, *Daphnia magna*. As shown in **Table 3**, the daphnid 48-hour LC_{50} of 3.0 µg a.i./L will be used to calculate RQs for indirect effects to the aquatic-phase CRLF. Mean-measured concentrations measured 67% of nominal, on average. The slope of the dose response curve was 9.2. The NOAEC based on lethargy was 0.92 µg a.i./L.

Table 3. Freshwater Invertebrate Toxicity of Methidathion

Species	% ai	LC50/ EC50 (ppb ai)	Toxicity Category	MRID No. Author/Year	Study Classification
Waterflea	tech	7.2	very highly toxic	00011350	core

Table 3. Freshwater Invertebrate Toxicity of Methidathion					
(Daphnia magna)	2E (25.5%)	11.9 ppb product (**3.0 ppb ai**)	very highly toxic	Vilkas/1976 42081704 LeLievre/1991	core

Freshwater Invertebrates: Chronic Exposure Studies

Available chronic toxicity data indicate that methidathion adversely affects reproduction of a common freshwater zooplankton, *Daphnia magna*, at approximately one part per billion (**Table 4**). In this flow-through study, nominal test concentrations were 0 (control), 0.19, 0.38, 0.75, 1.5, and 3.0 µg a.i./L methidathion. Mean-measured concentrations were 0.11, 0.31, 0.66, 1.1, and 2.1 µg a.i./L (based on analytical verification on Days 7, 14, and 21). Survivorship was significantly reduced (97 – 100%) at 1.1 and 2.1 µg a.i./L; thus, the 21-day NOAEC (mortality) was 0.66 µg a.i./L. There were no significant reproductive or growth effects noted in the study (the two highest test concentrations were excluded from statistical analyses for reproduction and growth).

There is another chronic daphnid study available (Forbis, 1984); however, raw data were not provided for this study so it will not be used to quantitatively estimate risk. Results from this study are summarized below.

Table 4. Freshwater Aquatic Invertebrate Life-Cycle Toxicity of Methidathion

Species	% ai	NOAEC/ LOAEC (ppb)	Endpoints Affected	MRID No. Author/Year	Study Classification
Waterflea *(Daphnia magna)*	96.1	0.66/1.13	survival	42081707 Putt/1991	core
	97.2	0.51/1.0	survival; # young/female/repro. day	00157352 Forbis/1984	supplemental[1]

[1]Raw data were not provided.

Birds: Acute Exposure (Mortality) Studies

Based on acute oral toxicity studies for several bird species, methidathion is categorized as moderately to very highly toxic to birds (**Table 5**). The mallard duck was the most sensitive of the species tested, with an 8-day LD_{50} of 6.7 (5.4 – 8.4) mg/kg methidathion (MRID 00159201). This endpoint will be used for risk estimation in this assessment. Dosages used in this study were 0 (control), 1.0, 2.2, 4.6, 10, and 22 mg/kg methidathion. Mortality was 0% up to 4.6 mg/kg and 100% at 10 and 22 mg/kg. These data do not fit the probit dose-response model; thus, a default slope of 4.5 (2-9) will be used to calculate the probability of individual effects to the terrestrial-phase CRLF. Toxic symptoms included depression, reduced reaction to external stimuli, wing droop, convulsions, and salivation. This study was classified as supplemental primarily because the test organisms were only 14 days (instead of 15 weeks) old.

Table 5. Avian Acute Oral Toxicity of Methidathion

Species	% ai	LD50 (mg/kg)	Toxicity Category	MRID No. Author/Year	Study Classification
Mallard duck (*Anas platyrhynchos)*	93.8	28	highly toxic	00157347 Beavers/1979	core
Mallard duck *(Anas platyrhynchos)*	98.2	23.6	highly toxic	00060823 Tucker/1969	supplemental
Ring-necked Pheasant *(Phasianus colchicus)*	98.2	33.2	highly toxic	00060823 Tucker/1969	supplemental
Chukar *(Alectoris chukar)*	98.2	225	moderately toxic	00060823 Tucker/1969	supplemental
Canada goose *(Branta canadensis)*	98.2	8.4	very highly toxic	00160000 Hudson/1984	supplemental
Mallard duck *(Anas platyrhynchos)*	technical	**6.7**	very highly toxic	00159201 Fink/1976	supplemental

Avian subacute dietary studies are available for methidathion (TGAI) and a formulation (40% a.i.) (**Table 6**). Based on the available information, methidathion and the tested formulation appear to be similarly toxic to birds on a subacute dietary basis. The bobwhite quail *(Colinus virginianus)* was the most sensitive of the species tested, with an 8-day LC_{50} of 224 (177 – 281) ppm a.i. (MRID 42081701). This endpoint will be used for risk estimation in this assessment. Test concentrations were 0 (control), 100, 178, 316, 562, and 1000 ppm a.i. methidathion; percent mortality was 0, 0, 20, 90, 100, and 100%, respectively. The probit dose-response slope was 8.7 (3.5 – 13.8). Toxic symptoms including depression, reduced reaction to external stimuli, wing droop, loss of coordination, lower limb weakness, ruffled appearance, prostrate posture, and loss of righting reflex were observed at and above 178 ppm a.i. Body weight gain and food consumption were reduced at levels above 316 ppm a.i.

Table 6. Avian Subacute Dietary Toxicity of Methidathion

Species	% ai	LC50 (ppm)	Toxicity Category	MRID No. Author/Year	Study Classification
Mallard duck *(Anas platyrhynchos)*	93.8	543	moderately toxic	00159201 Beavers/1979	core
Bobwhite quail (*Colinus virginianus)*	93.8	224	highly toxic	42081701 Beavers/1979	core
Mallard duck *(Anas platyrhynchos)*	40	820 ppm product (328 ppm ai)	moderately toxic for formulation	0011841 Beliles/1965	supplemental
Northern bobwhite quail *(Colinus virginianus)*	40	600 ppm product (240 ppm ai)	moderately toxic for formulation	0011841 Beliles/1965	supplemental

Birds: Chronic Exposure (Growth, Reproduction) Studies

Several avian chronic toxicity studies are available for methidathion (**Table 7**). The mallard duck study (MRID 44381602) reported the most sensitive endpoint, an NOAEC of 1 ppm. In this study, the one-generation reproductive toxicity of methidathion technical to 6-month-old mallard ducks was assessed over 140 days. Methidathion technical was administered to the birds in the diet at 0, 1, 10, and 30 ppm diet. The NOAEC was determined to be 1 ppm diet based on eggs cracked and eggs not cracked/eggs laid at the 10 ppm diet level (LOAEC); however, this endpoint is not a relevant assessment endpoint for the CRLF. The number of normal hatchlings/live 3-week embryos was significantly reduced (5%) at the highest treatment, 30 ppm (LOAEC); the NOAEC for this effect is 10 ppm, which is the endpoint that will be used for risk estimation in this assessment. There were no apparent behavioral abnormalities or other treatment-related signs of toxicity on the parental generation.

Table 7. Avian Reproductive Toxicity of Methidathion

Species	% ai	NOAEC/ LOAEC (ppm)	Endpoints Affected	MRID No. Author/Year	Study Classification
Mallard duck *(Anas platyrhynchos)*	93.8	NOAEC: 10 LOAEC: 30	number of normal hatchlings/live 3-week embryos	44381602 Beavers/1980	core
Mallard duck *(Anas platyrhynchos)*	93.8	NOAEC: 35 LOAEC: >35	no effects at highest concentration tested	44381602 Beavers/1999	supplemental
Northern bobwhite quail *(Colinus virginianus)*	93.8	NOAEC: 35 LOAEC: >35	no effects at highest concentration tested	44381602 Beavers/1999	supplemental[a]
Northern bobwhite quail *(Colinus virginianus)*	93.8	NOAEC: 30 LOAEC: >30	no effects at highest concentration tested	44381601 Beavers and Fink/1980	supplemental[b]

a = 7 adults died in the controls in week 19; therefore, capability of test to detect effects in treatments was reduced.
b = Critical information in the study report (e.g., dietary analytical results) was omitted; also, test animals were exposed to methidathion technical for 8 weeks during egg laying rather than at least 10 weeks.

Mammals: Acute Exposure (Mortality) Studies

Available acute toxicity information suggests that methidathion is very highly toxic (Category I) to small mammals on an acute oral basis (Table 8). The most sensitive endpoint, the acute rat (weanling) LD_{50} of 12 mg/kg, will be used to estimate risk to the CRLF via indirect effects to mammals. The probit dose-response slope is assumed to be 4.5 (2–9) for this study.

Table 8. Mammalian Acute Toxicity of Methidathion

Species	% ai	Test Type	Toxicity Values/category	MRID No.
Laboratory mouse *(Mus musculus)*	tech	acute oral LD50	17 mg/kg very highly toxic	00012714

Table 8. Mammalian Acute Toxicity of Methidathion

Species	% ai	Test Type	Toxicity Values/category	MRID No.
Laboratory rat *(Rattus norvegicus)*	tech	acute oral LD50	28 mg/kg (adult male) 12 mg/kg (weanling) very highly toxic	00012714

Mammals: Chronic Exposure (Growth, Reproduction) Studies

Chronic mammalian reproduction toxicity studies are available for methidathion (**Table 9**). In a 2-generation reproduction study in rats (MRID 40079812, -13), rats were treated with 0 (control), 5, 25, or 50 ppm methidathion in the diet. The parental systemic NOAEC was 5 ppm and the LOAEC was 25 ppm, based on tremors and decreased food consumption during lactation, and decreased ovarian weight. In addition, there was also a slight decrease in body weight early in the F1 growth phase at 50 ppm. The reproductive NOAEC was 5 ppm and the LOAEC was 25 ppm based on a decreased mating index and a generalized indication of pup unthriftyness while nursing. In addition, there was an increase in stillbirths and decreased pup survival at birth and during lactation at the 50 ppm treatment level. An NOAEL of 5 ppm will be used for risk estimation in this assessment.

A 3-generation rat reproduction study (MRID 00011840) provided a slightly lower reproductive NOAEC of 4 ppm, based on offspring mortality at 32 ppm (LOAEC); however, it is unknown if this is formulation is relevant for consideration in this risk assessment.

Table 9. Mammalian Chronic Toxicity of Methidathion

Species	% ai	Test Type	Toxicity Values/category	MRID No.
Laboratory rat *(Rattus norvegicus)*	40 WP	3-generation reproduction	Repro NOAEL=4 ppm, LEL=32 ppm	00011840
Laboratory rat *(Rattus norvegicus)*	tech	2-generation reproduction	Repro and systemic NOAEL=5 ppm, LEL 25 ppm	400798-12 400798-13

Terrestrial Invertebrates: Acute Exposure (Mortality) Studies

Methidathion is classified as very highly toxic to bees, with an acute contact LD_{50} of 0.236 µg/bee or 1.84 ppm (**Table 10**). This endpoint will be used to quantitatively assess the risk to the CRLF via indirect effects to terrestrial invertebrates. The dose-response slope from this study was 9.06; confidence intervals on the slope are unknown since the raw data are unavailable.

In addition, a residual toxicity study indicates that the RT_{25} (i.e., the residual time to kill 25% of the tested population) is greater than 3 days when methidathion (Supracide 2E, 25.2% a.i.) is applied at a rate of 5 lbs a.i./A (**Table 11**). Mean mortalities in the control, 24-, 48-, and 72-hour treatment groups were 11, 84, 56, and 46%, respectively. These mortalities were dose responsive and appeared to be treatment related.

Table 10. Nontarget Insect Acute Contact Toxicity of Methidathion

Species	% ai	LD50 (µg/bee)	Toxicity Category	MRID No. Author/Year	Study Classification
Honey bee	technical	0.236	very highly toxic	0036935	core

Table 10. Nontarget Insect Acute Contact Toxicity of Methidathion

Species	% ai	LD50 (µg/bee)	Toxicity Category	MRID No. Author/Year	Study Classification
(Apis mellifera)				Atkins et al./1975	

Table 11. Nontarget Insect Toxicity of Methidathion Residues on Foliage

Species	Formulation	Toxicity (Lb /A)	MRID # Author/year	Guideline Classification
Honey bee *(Apis mellifera)*	2E (25.2%)	RT25 > 3 days at 5 lb ai/A	420817-08 Hoxter/1991	core