# RTC Attachment 1. Review of toxicity endpoints used in draft BEs (atrazine, simazine and glyphosate)

EPA received public comments regarding the toxicity data used in the biological evaluations and endpoints selected for the analysis. In this appendix, the endpoint tables from Chapter 2 are provided along with an additional column noting if an endpoint was changed from the endpoint used in the draft assessment. Additionally, endpoints that were revised or those that were re-evaluated but not revised, are discussed further in sections below the tables. Regarding the overall toxicity data set for the three herbicides, it is EPA’s intention to communicate with the Services during the consultation period regarding the variability of the data and considerations for the use of other endpoints during consultation. For atrazine, focused reanalysis was conducted with the MAGtool using updated toxicity endpoints, as well as other input parameters, which may be useful for consideration when assessing species for impacts to an individual vs. impacts that would jeopardize the population or result in adverse modification of critical habitat and evaluating mitigation options. Further information on the MAGtool reanalysis is provided in Attachment 2.

## Atrazine Toxicity data

Below is a copy of Tables 2-1 through 2-6 from Chapter 2 of the Biological Evaluation, with a column added signifying if an endpoint was changed. Endpoints that represent new endpoints are highlighted in bold. Further discussion of endpoints evaluated is provided below the tables.

Table 2‑1. Terrestrial mortality endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Threshold** | **Taxon** | **Test Species** | **Type of endpoint** | **Value** | **Units** | **Slope** | **Weight of test animal (g)** | **Comments** | **Reference** | **Change in Endpoint?** |
| DOSE BASED MORTALITY | Mammals | Norway Rat | LD50 | 1,869 | mg ai/kg-bw | 4.5 | 350 | Norway rat; no slope available, default slope | MRID 00024706 | No |
| Birds | Bobwhite quail (*Colinus virginianus*) | LD50 | 783 | mg ai/kg-bw | 3.8 | 35 | Northern Bobwhite quail (Colinus virginianus); MRID 00024721 | MRID 00024721 | No |
| Reptiles | Bobwhite quail (*Colinus virginianus*) | LD50 | 783 | mg ai/kg-bw | 3.8 | 35 | bird used as surrogate; Northern Bobwhite quail | MRID 00024721 | No |
| Terrestrial Invertebrates | Honeybee | LD50 | >756 | mg ai/kg-bw | 4.5 | NA | Non definitive endpoint; honeybee, contact exposure | MRID 00036935 | No |
| DIETARY BASED MORTALITY | Mammals | No Data | | | | | | | |  |
| Birds | Bobwhite quail (*Colinus virginianus*) | LC50 | 5760 | mg ai/kg-diet | 4.5 | NA | No slope reported in study, default value used; Bobwhite quail | MRID 00059214 | No |
| Reptiles | Bobwhite quail (*Colinus virginianus*) | LC50 | 5760 | mg ai/kg-diet | 4.5 | NA | bird used as a surrogate; Bobwhite quail | MRID 00059214 | No |
| MORTALITY | Terrestrial Invertebrates | Honeybee | LC50 | >756 | mg ai/kg-bw | 4.5 | NA |  | MRID 00036935 | No |
| Terrestrial Invertebrates | Earthworm | LC50 | 273 | mg ai/kg-soil | 4.5 | NA | earthworm, default slope | E40493; Haque  and Ebing 1983 | No |
| Terrestrial Invertebrates | Honeybee | LD50 | >97 | µg ai/bee | 4.5 | NA | Non definitive endpoint; honeybee, contact exposure | MRID 00036935 | No |

Table 2‑2. Terrestrial sublethal endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Threshold** | **Taxon** | **Test Species** | **NOAEC (or LOAEC if no NOAEC)** | **MATC or LOAEC** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| DOSE BASED SUBLETHAL ENDPOINTS | Mammals | Norway Rat | 3.7 | 12 | mg ai/kg-bw | 12%-15% reduction in body weight; Norway rat; MATC used, LOAEC = 39 | MRID 40431306 | No |
| Birds | Mallard Duck  (*Anas platyrhynchos)* | 7 | 7 | mg ai/kg-bw | 5.3% reduction in hatchling weight; Mallard; LOAEC only; dose endpoint derived from dietary study | MRID 42547101 | No1 |
| Reptiles | Mallard Duck  (*Anas platyrhynchos)* | 7 | 7 | mg ai/kg-bw | 5.3% reduction in hatchling weight; Bird surrogate; Mallard; LOAEC only; dose endpoint derived from dietary study | MRID 42547101 | No1 |
| DIETARY BASED SUBLETHAL ENDPOINTS | Mammals | Norway Rat | 50 | 158 | mg ai/kg-diet | 12%- 15% reduction in body weight; rat; MATC used; LOAEC= 500 | MRID 40431306 | No |
| Birds | Mallard Duck  (*Anas platyrhynchos)* | 75 | 75 | mg ai/kg-diet | 5.3% reduction in hatchling weight; Mallard; LOAEC only | MRID 42547101 | No1 |
| Reptiles | Mallard Duck  (*Anas platyrhynchos)* | 75 | 75 | mg ai/kg-diet | 5.3% reduction in hatchling weight; Bird surrogate; Mallard; LOAEC only | MRID 42547101 | No1 |
| SUBLETHAL/  Mortality | Terrestrial Invertebrates | Honeybee | 756 | 756 | mg ai/kg-bw | 5% mortality; honeybee, contact exposure | MRID 00036935 | No |
| Terrestrial Invertebrates | NA | NA | NA | mg ai/kg-diet | NA | NA | NA |
| Terrestrial Invertebrates | Springtails | 2.5 | 2.5 | mg ai/kg-soil | 2.5 mg/kg-soil, 18% mortality; Exposure to springtail (*O. apuanicus*) at 2.5 mg/kg-soil, 18% mortality | E71417; Mola et a. 1987 | No |
| Terrestrial Invertebrates | Honeybee | 97 | 97 | µg ai/bee | 5% mortality; honeybee, contact exposure | MRID 00036935 | No |

Table 2‑3. Aquatic mortality endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Taxon** | **Test Species** | **Type of endpoint** | **Value**  **(ug ai/L)** | **Slope** | **Duration of study (days)** | **Reference** | **Change in Endpoint?** |
| FW FISH | Rainbow Trout  (*Oncorhynchus mykiss*) | LC50 | 5,300 | 4.5 | 4 | MRID 00024716 | No |
| E/M FISH | Sheepshead Minnow (*Cyprinodon variegates*) | LC50 | 2,000 | 4.5 | 4 | MRID 45208303; MRID 45227711 | No |
| **AQ AMPHIBIANS** | **American Toad (*Bufo americanus*)** | **LC50** | **10,700** | **4.5** | **4** | **E18805; Howe et al.** | Yes1 |
|  | *Previous endpoint: American Bullfrog (Lithobates catesbeianus)* | *LC50* | *410* | *4.5* | *4* | *E6187; Birge et al.* |  |
| FW INVERTEBRATES | Midge (*Chironomus tentans*) | LC50 | 720 | 4.5 | 4 | MRID 00024377 | No |
| E/M INVERTEBRATES | Opposum shrimp  (*Neomysis integer*) | LC50 | 48 | 4.5 | 4 | E103334; Noppe et al. 2007 | No1 |
| MOLLUSKS | Eastern oyster  (*Crassostrea virginica*) | LC50 | >1,700 | 4.5 | 4 | MRID 46648201 | No |

Table 2‑4. Aquatic sublethal endpoints used to evaluate impacts to species and impacts to PPHD.

| **Taxon** | **Test Species** | **NOAEC** | **MATC or LOAEC** | **Units** | **Duration of study (days)** | **Comments** | **Reference** | **Change in Endpoint?** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| FW FISH | Atlantic Salmon (*Salmo salar*) | 8.5 | 26.7 | ug ai/L | 212 | decreased weight (5.6%), decreased length (~18%), decreased growth rate based on weight (60% reduction), mortality (9%) and decreased food consumption (12-15%); MATC used as input; LOAEC = 84.3 | Nieves-Puigdoller et al. 2007 | No1 |
| E/M FISH | Atlantic Salmon (*Salmo salar*) | 8.5 | 26.7 | ug ai/L | 212 | decreased weight (5.6%), decreased length (~18%), decreased growth rate based on weight (60% reduction), mortality (9%) and decreased food consumption (12-15%); MATC used as input; LOAEC = 84.3 | Nieves-Puigdoller et al. 2007 | No1 |
| AQ AMPHIBIANS | African clawed frog (*Xenopus laevis*) | 9.7 | 30.8 | ug ai/L | 212 | ~27% decrease in gonad weight and GSI; MATC used as input; LOAEC = 97.7 | Sai et al. 2016 | No1 |
| FW INVERTEBRATES | Scud2  (*Gammarus fasciatus*) | 60 | 92 | ug ai/L | 21 | Based on reduced development (% effect) of F1 to seventh instar; MATC used as input; LOAEC = 140 µg a.i./L | MRID 00024377 | No |
| E/M INVERTEBRATES | Copepod (*Amphiascus tenuiremis*) | 3.5 | 3.5 | ug ai/L | 21 | Based on 32% reduction in offspring in F1 females; LOAEC used; no NOAEC available | E73333; Bejarano et al. | No1 |
| MOLLUSKS | Copepod (*Amphiascus tenuiremis*) | 3.5 | 3.5 | ug ai/L | 21 | Based on 32% reduction in offspring in F1 females; LOAEC used; no NOAEC available | E73333; Bejarano et al. | No1 |

Table 2‑5. Aquatic plant endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **EPA Category** | **Species** | **NOAEC** | **MATC or LOAEC** | **IC50** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| NON-VASCULAR | Cyanobacteria (*Pseudanabaena galeata*) | 3 | 3.87 | 14.4 | ug ai/L | 4% reduction in average growth rate at LOAEC; NOAEC and MATC for Pseudanabaena galeata; MATC used for threshold; IC50 based on HC05 species from all plant SSD | E6712; Carrasco and Sabater 1997 | No |
| VASCULAR | Canadian waterweed  (*Elodea canadensis*) | 4.6 | 4.6 | 14.4 | ug ai/L | 50% decrease in biomass for Elodea canadensis; Threshold based on lowest EC50; IC50 based on HC05 species from all plant SSD | MRID 48261124 | No |

Table 2‑6. Terrestrial plant endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **EPA Category** | **Species** | **NOAEC** | **MATC or LOAEC** | **IC25** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| MONOCOT | Onion (*Allium cepa*) | 0.0011 | 0.0011 | 0.0037 | lb ai/A | Dry weight; Step 1 and 2 thresholds based on IC15 for soybean; IC25 based on HC05 species from SSD for seedling emergence | MRID 49639102 | No |
| DICOT | Soybean (*Glycine max)* | 0.018 | 0.018 | 0.0037 | lb ai/A | Dry weight; Step 1 and 2 thresholds based on IC15 for onion; IC25 based on HC05 species from SSD for seedling emergence | MRID 49639102 | No |

1 Public comment considerations and further discussion for this taxa provided below.

2 Correction from draft table.

## Birds, Reptiles and Terrestrial-Phase Amphibians – Chronic endpoints

The chronic endpoint for birds was based on decreased hatchling weight in a study in the mallard duck. In this study, decreased hatchling weight was significant at all concentrations tested, with decreases ranging from 5.3 to 12.3% at 75 to 675 mg a.i./kg-diet, respectively. At a concentration of ≥225 mg a.i./kg-diet, there were effects on egg production and mean food consumption while live embryos and hatchlings per eggs set and male weight gain were affected at 675 mg a.i./kg-diet (MRID 42527101).

Commenters noted there was no science-based evidence or rationale to shift the NOAEC from 225 to 75 mg/kg-diet for decreased hatchling weight, as was previously used prior to the 2016 PRA. This comment has been previously addressed in the response to comment in the RTC for the 2016 PRA and was also discussed in Chapter 2 of the BE. From the BE, “Several public comments were received on the above study when published with the 2016 DRA. Comments included concern about the statistical analysis method utilized in the analysis, the relevance of the endpoint of hatchling weight to risk analysis and the inclusion of a deceased hen in the statistical analysis. These comments were considered in the re-evaluation of the endpoint. Although removal of the deceased hen from the statistical analysis changes the endpoint to a NOAEC = 75 mg a.i./kg-diet and LOAEC = 225 mg a.i./kg-diet, the hen was found dead at week 20, well after the egg laying/setting period and at the end of the exposure. Removal of the hen from the analysis is not appropriate given the effect endpoint of hatchling weight. Additionally, the relevance of the endpoint and the statistical analysis chosen were reevaluated and determined to be suitable; there is variability across the control and treatment groups, but the effects are both dose dependent and biologically and statistically significant.”

In addition, commenters discussed how reproductive endpoints should be applied when using the MCNest model. These comments were confusing as it pertains to the BE, as the MCNest model was not used for the BE. The MCNest model was used in the 2016 PRA. As stated in the RTC to the 2016 PRA, the EPA appreciated the additional data provided on dermal absorption and triazine specific parameters that could be applied in TIM-MCNest modeling. If the model is seen as useful in further evaluating jeopardy to listed birds or adverse modification of their critical habitat, EPA will work with the Services to ensure the additional data is utilized and model inputs are properly parameterized.

The comments on the BE also state there was no amended DER published on this chronic bird reproduction study. This is incorrect. The revised DER was posted to the docket on July 17, 2016 at the time the PRA was published (<https://www.regulations.gov/document/EPA-HQ-OPP-2013-0266-0350>).

Although the endpoint was not changed for the BE, the use of other endpoints in the analysis for impacts to birds is further explored in the MAGtool analysis provided in Attachment 2.

## Aquatic-phase Amphibians – Acute endpoint (LC50)

The acute endpoint used for aquatic-phase amphibians was reevaluated. In review, it was noted that when using data presented in the LC50 summary table provided in the study, it is not possible to replicate the results for the LC50 based on the data presented. It appears the LC50 value reported for this species could possibly be a NOAEC value and does not appear logical based on the % mortality reported at each treatment level in the study (for example, at the reported LC50 concentration of 410 ug/L in the table, the survival is still noted at 93%; survival does not drop below 50% until a concentration of 26,400 ug/L). No other information was provided to further investigate this discrepancy. Therefore, this endpoint was deemed to not be suitable as the most sensitive LC50 for quantitative analysis.

In review of the ECOTOX spreadsheet report, the next most sensitive endpoints were an LC50 of 1,690 in the Pacific Chorus Frog and 5,520 in the Foothill Yellow-Legged Frog (ECOTOX # 118706; Kerby JL. 2006. Pesticide Effects on Amphibians: A Community Ecology Perspective Ph.D. Thesis, University of California, Davis, CA : 146 p.). The study author referred to these values as “rough estimates of LC50 values with each of the pesticides.” Well water was used in the study (without chemical analysis of the water), survival at each test concentration was not included and there was no discussion of control group performance. More significantly, it appeared based on the description of methods, that the amount of solvent (99.9% methanol) varied between treatment groups. For the reported results, the standard error for the LC50 was very high compared to the reported value (± 1561.43) and the higher LC50 was extrapolated outside of the tested dose range. For these reasons, this endpoint was not used as a quantitative endpoint in the analysis.

The next lowest endpoint in the ECOTOX spreadsheet was an LC50 of 10,700 ug/L in the American Toad late stage larvae (*Bufo americanus*) (Ecotox #18805; Howe GE;Gillis R;Mowbray RC. 1998. Effect of Chemical Synergy and Larval Stage on the Toxicity of Atrazine and Alachlor to Amphibian Larvae Environ. Toxicol. Chem. 17(3): 519-525). This study was suggested by some commenters as the best available endpoint for use as an LC50 in aquatic-phase amphibians. This study used a 40% formulation product of atrazine, control mortality was not reported, and individual treatment level survival was not reported. It was unclear from the study what test concentrations were used in the definitive test as the author stated, “Exposure concentrations were determined from range-finding tests that consisted of 96-h static exposures of test organisms to a control and five concentrations of herbicide (0.1, 1, 10, 100, and 1,000 mg/L). In definitive tests, each test series consisted of nine selected exposure concentrations and a control.” Although this study consisted of some similar uncertainties as the Kerby et al. study, there appeared to be better confidence bounds around the reported results and no potential solvent effects and was therefore used as the quantitative endpoint for aquatic-phase amphibians.

## Fish and Aquatic-phase Amphibians – sublethal endpoints

Commenters cited what were viewed as deficiencies in the studies used of establishing fish and aquatic-phase amphibian endpoints, including for amphibians sourcing the eggs from only one female, lack of a water-only control, use of DMSO as a solvent, infrequent measures of exposure concentrations, and lack of information on water-quality and husbandry. In Appendix 2-7 of the BE, EPA provided additional information on other studies with or without effects to fish and aquatic-phase amphibians. In addition, EPA referenced the multiple other previous reviews of fish and aquatic-phase amphibian data provided in previous EPA documents, including multiple SAPs, the California Reg-legged Frog ESA assessment and the 2016 PRA. For the assessment of effects to aquatic vertebrates for the BE and assessing potential impacts to an individual, EPA feels the endpoints utilized in the BEs are protective of effects based on the body of the available data. For the purposes of assessing jeopardy to a species or adverse modification of critical habitat and potential mitigation, a wider analysis of the endpoints should be considered, including other studies where other types of effects (e.g., reproductive effects) may or may not have been observed and potential species differences. Some of these types of other analyses are further explored in the MAGtool analysis provided in Attachment 2.

## Estuarine/Marine Invertebrates – Acute endpoint (LC50) and sublethal endpoints

Commenters were critical of the study used to establish the estuarine/marine acute toxicity endpoint (LC50= 48 ug/L) citing the study lacked raw data, corresponding figures of trends, measured test concentrations, or report of control performance. EPA agrees that the lack of the concentration of the solvent in this study does raise uncertainty, even if it was stated concentrations were “similar” to treatment groups, and the lack of control performance data raises uncertainty for understanding potential solvent effects. However, these deficits were similar to the study discussed above that EPA is now using for the acute aquatic-phase amphibian endpoint; it was the same commenters that recommended the study above by Howe as an acceptable endpoint for acute toxicity to amphibians despite having the same deficits. Commenters suggested an endpoint of 5,400 ug/L as the best available data for this endpoint; however there are 14 additional studies reporting more sensitive LC50 values between the endpoint of 48 and 5,400 ug/L (and it is notable that most of these endpoints are associated with copepods). In order to apply consistent screening criteria for acute studies and to preserve the conservative nature of the BE assessment, the endpoint of 48 ug/L is retained or the BE analysis.

For the purposes of assessing jeopardy to a species or adverse modification of critical habitat and potential mitigation, a wider analysis of the endpoints should be considered. As discussed above, for both acute and chronic endpoints, many of the more sensitive endpoints are associated with studies involving copepods. Consideration may be given to the subclassifications of listed aquatic invertebrate species, many of which are mollusks, and how relevant endpoints based on copepods are compared to endpoints available from other invertebrate groups, such as those in the same order as listed species. Consideration should also be given to appropriate endpoints to use when considering aquatic invertebrates as a dietary source when impacts are predicted, rather than a direct effect to a listed invertebrate.

## Aquatic plants

For nonvascular plants, the LOAEC used to calculate the MATC is based on a low effect, with a 4% difference in the growth rate at 4 ug/L. The EC50 from the same study is 12 ug/L, so the LOAEC therefore appears to be within a range where biologically significant results could be expected.

The endpoint for vascular plants is based on a root EC50 for *Elodia canadensis* in a cosm study. Particular care must be taken in root growth measurements to ensure accuracy and there is wide variability in the study. Recognizing these limitations, EPA does not rely on the endpoint to represent precisely 50% effects, and instead uses this endpoint as a LOAEC to retain the conservativeness of the initial screen.

## Simazine Toxicity data

Below is a copy of Tables 2-1 through 2-6 from Chapter 2 of the Biological Evaluation, with a column added signifying if an endpoint was changed. Endpoints that represent new endpoints are highlighted in bold. Further discussion of endpoints evaluated is provided below the tables. The only comments received during the public comment period on the endpoints used in the Simazine BE referred back to the comments on the 2016 PRA.

Table 2‑1. Terrestrial mortality endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Threshold** | **Taxon** | **Test Species** | **Type of endpoint** | **Value** | **Units** | **Slope** | **Weight of test animal (g)** | **Comments** | **Reference** | **Change in Endpoint?** |
| DOSE BASED MORTALITY | Mammals | Gray-Tailed Vole | LD50 | 2014 | mg ai/kg-bw | 4.5 | 27.7 | Species was Gray-Tailed vole; 95% CI: 1401-2896; assumed slope | ECOTOX # 70756; Cholakis et al. 1978; USEPA ORD) | No |
| Birds | Mallard Duck | LD50 | 4640 | mg ai/kg-bw | 4.5 | 1580 | Non-definitive (>) value, Mallard duck; assumed slope | MRID 00072798 | No |
| Reptiles | Mallard Duck | LD50 | 4640 | mg ai/kg-bw | 4.5 | 1580 | Non-definitive (>) value, Mallard duck; assumed slope | MRID 00072798 | No |
| Terrestrial Invertebrates | Honeybee | LD50 | 756 | mg ai/kg-bw | 4.5 | NA | Honeybee; non-definitive; assumed slope; calculated with Reverse BeeRex | MRID 00036935 | No |
| DIETARY BASED MORTALITY | Mammals | No Data | | | | | | | |  |
| Birds | Mallard Duck | LC50 | 5000 | mg ai/kg-diet | 4.5 |  | Mallard duck; Non-definitive (>) value; slope assumed | MRID 00022923 | No |
| Reptiles | Mallard Duck | LC50 | 5000 | mg ai/kg-diet | 4.5 |  | bird used as a surrogate; Mallard duck; Non-definitive (>) value; slope assumed | MRID 00022923 | No |
| MORTALITY | Terrestrial Invertebrates | Earthworm | LC50 | 100 | mg ai/kg-soil | NA |  |  | ECOTOX #58170 Martin, 1982 | No |
| Terrestrial Invertebrates | Honeybee | LD50 | 96.7 | µg ai/bee | 4.5 |  | honeybee, non-definitive; assumed slope; contact exposure | MRID 00036935 | No |

Table 2‑2. Terrestrial sublethal endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Threshold** | **Taxon** | **Test Species** | **NOAEC (or LOAEC if no NOAEC)** | **MATC or LOAEC** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| DOSE BASED SUBLETHAL ENDPOINTS | Mammals | Lab Rat | 0.54 | 1.77 | mg ai/kg-bw | rat; MATC used, LOAEC = 5.41; 6% decrease in body weight, 31% decrease in maternal body weight gain | MRID 41803601; MRID 40614405 | No1 |
| Birds | Bobwhite Quail | 13 | 28 | mg ai/kg-bw | LOAEC = 61; Average BW female controls | MRID 43576901 | No |
| Reptiles | Bobwhite Quail | 13 | 28 | mg ai/kg-bw | LOAEC = 61; Average BW female controls | MRID 43576901 | No |
| DIETARY BASED SUBLETHAL ENDPOINTS | Mammals | Lab Rat | 10 | 32 | mg ai/kg-diet | rat; MATC used, LOAEC = 100; 6% decrease in body weight, 31% decrease in maternal body weight gain | MRID 41803601; MRID 40614405 | No1 |
| Birds | Bobwhite Quail | 100 | 223 | mg ai/kg-diet | MATC used, LOAEC = 500; 32-33% reduction in 3-week old embryos, hatchling survival and 14-day old chick survival | MRID 43576901 | No |
| Reptiles | Bobwhite Quail | 100 | 223 | mg ai/kg-diet | Bird used as a surrogate; MATC used, LOAEC = 500; 32-33% reduction in 3-week old embryos, hatchling survival and 14-day old chick survival | MRID 43576901 | No |
| SUBLETHAL/Mortality | Terrestrial Invertebrates | Honeybee | 756 | 756 | mg ai/kg-bw | honeybee; Mortality (6.9%) | MRID 00036935 | No |
| Terrestrial Invertebrates | Earthworm | 100 | 100 | mg ai/kg-soil | Earthworm, NOAEC values; No effects seen on growth over 7 days | MRID 00036935 | No |
| Terrestrial Invertebrates | Honeybee | 96.7 | 96.7 | µg ai/bee | honeybee; Mortality (6.9%) | MRID 00036935 | No |

Table 2‑3. Aquatic mortality endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Taxon** | **Test Species** | **Type of endpoint** | **Value**  **(ug ai/L)** | **Slope** | **Duration of study (days)** | **Reference** | **Change in Endpoint?** |
| FW FISH | Fathead Minnow | LC50 | 6400 | 4.5 | 4 | MRID 00033309 | No |
| E/M FISH | Sheepshead Minnow (*Cyprinodon variegatus*) | LC50 | 4300 | 4.5 | 4 | MRID 42503702 | No |
| AQ AMPHIBIANS | Western Clawed Frog | LC50 | 7550 | 4.5 | 4 | ECOTOX # 178499; Saka et al. 2018 | No |
| FW INVERTEBRATES | Stonefly (*Pteronarcys californica*) | LC50 | 1900 | 4.5 | 4 | MRID 40098001; ECOTOX # 6797 | No |
| E/M INVERTEBRATES | Stonefly (*Pteronarcys californica*) | LC50 | 1900 | 4.5 | 4 | MRID 40098001; ECOTOX # 6797 | No |
| MOLLUSKS | Eastern Oyster (*Crassostrea virginica*) | LC50 | 3700 | 4.5 | 4 | MRID 42503703 | No |

Table 2‑4. Aquatic sublethal endpoints used to evaluate impacts to species and impacts to PPHD.

| **Taxon** | **Test Species** | **NOAEC** | **MATC or LOAEC** | **Units** | **Duration of study (days)** | **Comments** | **Reference** | **Change in Endpoint?** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| FW FISH | African clawed frog | 1.2 | 3.6 | ug ai/L | 100 | MATC used as input; LOAEC = 11.1; 19% increase in morality; decrease in gonad weight and time reach metamorphosis; amphibian used as surrogate | ECOTOX # 178653; Sai et al. 2016 | No1 |
| E/M FISH | African clawed frog | 1.2 | 3.6 | ug ai/L | 100 | MATC used as input; LOAEC = 11.1; 19% increase in morality; decrease in gonad weight and time reach metamorphosis; amphibian used as surrogate | ECOTOX # 178653; Sai et al. 2016 | No1 |
| AQ AMPHIBIANS | African clawed frog | 1.2 | 3.6 | ug ai/L | 100 | MATC used as input; LOAEC = 11.1; 19% increase in morality; decrease in gonad weight and time reach metamorphosis | ECOTOX # 178653; Sai et al. 2016 | No1 |
| FW INVERTEBRATES | Saltwater mysid (*Americamysis bahia*) | 63 | 98 | ug ai/L | 21 | MATC used as input; LOAEC = 151; EM invertebrate used as surrogate; 15% reduction in F0 survival | MRID  48680006 | No1 |
| E/M INVERTEBRATES | Saltwater mysid (*Americamysis bahia*) | 63 | 98 | ug ai/L | 21 | MATC used as input; LOAEC = 151; 15% reduction in F0 survival | MRID 48680006 | No1 |
| MOLLUSKS | Saltwater mysid (*Americamysis bahia*) | 63 | 98 | ug ai/L | 21 | MATC used as input; LOAEC = 151; EM invertebrate used as surrogate; 15% reduction in F0 survival | MRID 48680006 | No1 |

Table 2‑5. Aquatic plant endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **EPA Category** | **Species** | **NOAEC** | **MATC or LOAEC** | **IC50** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| NON-VASCULAR | Green Algae (*Anabaena flos aqua*) | 5.4 | 5.4 | 12.19 | ug ai/L | reduced growth rate; IC05 used for both NOAEC and LOAEC; 96 -hour exposure; HC05 species from all aquatic plant SSD used for IC50 | MRID 42662401 | No |
| VASCULAR | *Lemna gibba* | 50 | 74 | 12.19 | ug ai/L | 39% reduction in number of fronds; MATC used for Step 2 threshold; 7-d exposure; endpoints from same study; HC05 from all aquatic plant SSD used for IC50 | MRID 42503704 | No |

Table 2‑6. Terrestrial plant endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **EPA Category** | **Species** | **NOAEC** | **MATC or LOAEC** | **IC25** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| MONOCOT | Oat | 0.016 | 0.028 | 0.0129 | lb ai/A | 20% reduction in dry weight at the LOAEC; NOAEC/MATC based on reductions in weight for oat; IC50 based on HC05 species of the SSD for seedling emergence | MRID 42634603 | No |
| DICOT | Lettuce | 0.0018 | 0.0031 | 0.0129 | lb ai/A | 25% reduction in dry weight at the LOAEC; NOAEC/MATC based on reductions in weight for lettuce; IC50 based on HC05 species of the SSD for seedling emergence | MRID 42634603 | No |

1 Public comment considerations and further discussion for this taxon provided below.

## Aquatic and terrestrial animal sublethal endpoints

In public comments on both the simazine BE and the 2016 simazine PRA, commenters expressed concern regarding the endpoints used for sublethal (or chronic) effects to mammals, fish, aquatic-phase amphibians and aquatic invertebrates. These comments pertained to potential study deficiencies that may raise uncertainty around the chosen endpoint, comparison of endpoints to other studies in the same taxa and the ecological relevance of the effect noted in the studies. For the purposes of the BE and determining potential impacts to one individual and maintaining the conservatism of this step of the analysis, EPA did not make modifications to the reported endpoints. However, for the purposes of assessing jeopardy to a species or adverse modification of critical habitat and potential mitigation, a wider analysis of the endpoints should be considered. Through the consultation process, EPA will provide the Services with the additional information from the public comment periods and assistance in interpreting this material. Additional endpoints should be considered in the analysis when assessing these impacts in a similar manner as demonstrated in **Attachment 2** for atrazine (additional MAGtool runs).

## Aquatic and terrestrial plant endpoints

Commenters state the EPA did not sufficiently address the concerns raised in comments on the simazine PRA. EPA will continue to work with the Services during consultation to interpret the available data, including information provided in public comments on the simazine draft BE and the PRA.

## Glyphosate Toxicity Data

Table 2-1. Terrestrial mortality endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Threshold** | **Taxon** | **Test Species** | **Type of endpoint** | **Value** | **Units** | **Slope** | **Weight of test animal (g)** | **Comments** | **Reference** | **Change in Endpoint?** |
| DOSE BASED MORTALITY | Mammals | Norway Rat | LD50 | 357 | mg ai/kg-bw | 4.5 | 350 | TEP (11.4% formulation) | MRID 00024706 | No |
| Birds | Bobwhite quail (*Colinus virginianus*) | LD50 | 1,131 | mg ai/kg-bw | 4.5 | 120 | TEP (68.5% formulation) | MRID 00024721 | No |
| Reptiles | Bobwhite quail (*Colinus virginianus*) | LD50 | 1,131 | mg ai/kg-bw | 4.5 | 120 | TEP (68.5% formulation); bird used as surrogate | MRID 00024721 | No |
| Terrestrial Invertebrates | Honeybee | LD50 | >757 | mg ai/kg-bw | 4.5 | NA | TEP (65.6% formulation) | MRID 00036935 | No |
| DIETARY BASED MORTALITY | Mammals | No Data | | | | | | | | |
| Birds | Bobwhite quail (*Colinus virginianus*) | LC50 | >4971 | mg ai/kg-diet | 4.5 | NA | TGAI | MRID 00059214 | No |
| Reptiles | Bobwhite quail (*Colinus virginianus*) | LC50 | >4971 | mg ai/kg-diet | 4.5 | NA | TGAI; bird used as a surrogate | MRID 00059214 | No |
| MORTALITY | Terrestrial Invertebrates | Honeybee | LC50 | >1128 | mg ai/kg-diet | 4.5 | NA |  | MRID 00026489 | No1 |
| Terrestrial Invertebrates | Earthworm | LC50 | >6560 | mg ai/kg-soil | 4.5 | NA | TEP (64.9% formulation) | MRID 45767109 | No |
| Terrestrial Invertebrates | Honeybee | LD50 | >97 | ug ai/bee | 4.5 | NA | TEP (65.6% formulation) | MRID 00036935 | No1 |

Table 2-2. Terrestrial sublethal endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Type of Threshold** | **Taxon** | **Test Species** | **NOAEC (or LOAEC if no NOAEC)** | **MATC or LOAEC** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| DOSE BASED SUBLETHAL ENDPOINTS | Mammals | Norway Rat | 408 | 709.56 | mg ai/kg-bw | Delayed age and increased weight at male sexual development; TGAI; rat; MATC used as input, LOAEC = 1234 mg a.e./kg-bw | MRID 48865101; MRID 48865105 | No |
| Birds | Mallard Duck (Anas platyrhynchos*)* | 64.6 | 64.6 | mg ai/kg-bw | NA; Calculated based on average BW and food consumption from MRID 48876602 | MRID 48876602 | No |
| Reptiles | Mallard Duck (Anas platyrhynchos*)* | 64.6 | 64.6 | mg ai/kg-bw | NA; Calculated based on average BW and food consumption from MRID 48876602 | MRID 48876602 | No |
| DIETARY BASED SUBLETHAL ENDPOINTS | Mammals | Norway Rat | 5000 | 8660 | mg ai/kg-diet | Delayed age and increased weight at male sexual development; TGAI; rat; MATC used as input; LOAEC= 15,000 | MRID 48865101; MRID 48865105 | No |
| Birds | Mallard Duck (Anas platyrhynchos*)* | 501 | 501 | mg ai/kg-diet | 10% decrease in 14-d survivor body weight; TGAI; mallard duck; LOAEC used as input | MRID 48876602 | No1 |
| Reptiles | Mallard Duck (Anas platyrhynchos*)* | 501 | 501 | mg ai/kg-diet | 10% decrease in 14-d survivor body weight; TGAI; bird used as surrogate | MRID 48876602 | No1 |
| SUBLETHAL/  Mortality | Terrestrial Invertebrates | Honeybee | 39.8 | 54.4 | mg ai/kg-bw | Reduced food consumption; TEP (46.1% formulation); NOAEC and MATC; honey bee; daily dose divided by default bee weight (0.128g) | MRID 50603803 | No1 |
| Terrestrial Invertebrates | Honeybee | 2.5 | 2.5 | mg ai/kg-diet | Reduced gustatory response and olfactory learning; TGAI | E179312; E179310 | No 1 |
| Terrestrial Invertebrates | Earthworm (*Octolasion tyrtaeum*) | 5000 | 5000 | mg ai/kg-soil | Reduced Biomass and fecundity, no mortality; TGAI | E170666 | No |
| Terrestrial Invertebrates | Honeybee | 5.1 | 6.96 | ug ai/bee | Reduced food consumption; TEP (46.1% formulation); NOAEC and MATC | MRID 50603803 | No1 |
| Terrestrial Invertebrates | Predatory mite (*Typhlodromus pyri*) | 0.193 | 0.193 | lb ai/A | 85% reduction in number of eggs laid at LOAEL; TEP (64.9% formulation) | MRID 45767105 | No |

Table 2-3. Aquatic mortality endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Taxon** | **Test Species** | **Type of endpoint** | **Value**  **(ug ai/L)** | **Slope** | **Duration of study (days)** | **Comments** | **Reference** | **Change in Endpoint?** |
| FW FISH | Rainbow Trout (*Oncorhynchus mykiss*) | LC50 | 1,000 | 4.5 | 4 | TEP (30% formulation) | MRID 40098001 | No |
| E/M FISH | Sheepshead Minnow (*Cyprinodon variegates*) | LC50 | 2,700 | 4.5 | 4 | TEP (30.75% formulation) | MRID 48934205 | No |
| AQ AMPHIBIANS | Wood frog (*Lithobates sylvaticus*) | LC50 | 580 | 4.5 | 4 | TEP (5.18 g a.e/L formulation) | E173391 | No |
| **FW INVERTEBRATES** | **Horsehair worm (*Chordodes nobilii*)** | **LC50** | **67** | **4.5** | **2** | **TEP** | **E178535** | **Yes1** |
| FW INVERTEBRATES | Previous endpoint: San Diego Fairy Shrimp  (*Branchinecta sandiegonensis*) | LC50 | 8.7 | 4.5 | 1 | TEP | E178782 |  |
| E/M INVERTEBRATES | Mysid (*Americamysis bahia*) | LC50 | 765 | 4.5 | 4 | TEP | MRID 48934292 | No |
| Mollusks | Freshwater mussel (*Utterbackia imbecillis*) | LC50 | 13,542 | 4.5 | 1 | TEP (18% formulation) | E74236 | No |

Table 2-4. Aquatic sublethal endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Taxon** | **Test Species** | **NOAEC** | **MATC or LOAEC** | **Units** | **Duration of study (days)** | **Comments** | **Reference** | **Change in Endpoint?** |
| FW FISH | Characin (*Leporinus obtusidens*) | 704 | 704 | ug ai/L | 90 | LOAEC only based on reduced body length and weight gain; TEP (48% formulation); LOAEC used as input | E1618033 | No |
| E/M FISH | Characin (Leporinus obtusidens) | 704 | 704 | ug ai/L | 90 | LOAEC only based on reduced body length and weight gain; TEP (48% formulation); LOAEC used as input; FW fish used as surrogate | E1618033 | No |
| AQ AMPHIBIANS | Wood frog (*Lithobates sylvaticus*) | 580 | 580 | ug ai/L | 4 | LC50; TEP (5.18 g/L formulation); LC50 used as input | E173391 | No |
| FW INVERTEBRATES | *Daphnia magna* | 333 | 576.8 | ug ai/L | 55 | MATC; TGAI | E161204 | No |
| E/M INVERTEBRATES | Blue crab (*Callinectes sapidus*) | 4070 | 4070 | ug ai/L | 1 | LOAEC based on reduced time to metamorphosis and 20% mortality; TEP (50.2% formulation); LOAEC used as input | E161498 | No |
| Mollusks | Eastern oyster (*Crassostrea virginica*) | 930 | 930 | ug ai/L | 2 | EC50; EC50 used as input; TEP (30.75% formulation) | MRID 48934204 | No |

Table 2-5. Aquatic plant endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **EPA Category** | **Species** | **NOAEC** | **MATC or LOAEC** | **IC50** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| Non-vascular | *Skeletonema costatum* | 17.3 | 25.2 | 100 | ug ai/L | Cell density; TEP; 96-hr IC50 and NOAEC/LOAEC; uncertain whether it contains POEA, MRID 45666703 | MRID 45666703 | No |
| Vascular | *Lemna gibba* | 290 | 580 | 1500 | ug ai/L | frond number; TEP; 14d IC50; formulation assumed to contain POEA, MRID 44125714. | MRID 44125714 | No |

Table 2-6. Terrestrial plant endpoints used to evaluate impacts to species and impacts to PPHD.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **EPA Category** | **Species** | **NOAEC** | **MATC or LOAEC** | **IC25** | **Units** | **Comments** | **Reference** | **Change in Endpoint?** |
| Monocot | Wheat (*Triticum aestivum*) | 0.049 | 0.086 | 0.021 | lb a.e/A | aboveground dry weight; LOAEC used to derive MATC: MRID 44125715, for formulation; IC25: the HC05 of an SSD, all terrestrial plant vegetative vigor data | MRID 44320636 | No1 |
| Dicot | Radish (*Raphanus sativus*) | 0.07 | 0.07 | 0.021 | lb a.e/A | aboveground dry weight; Formulation LOAEC: MRID 44125715, non-definitive (<) for formulation; IC25: the HC05 of an SSD, all terrestrial plant vegetative vigor data | MRID 44125715 | No1 |

1 Public comment considerations and further discussion for this taxon provided below.

Commenters expressed concern regarding the endpoints used for acute effects to mammals and birds, sublethal (or chronic) effects to birds, effects to terrestrial and aquatic invertebrates, aquatic vertebrates, and plants. These comments pertained to potential study deficiencies that may raise uncertainty around the chosen endpoint, comparison of endpoints to other studies in the same taxa and the ecological relevance of the effect noted in the studies. For the purposes of the BE and determining potential impacts to one individual and maintaining the conservatism of this step of the analysis, EPA made only limited modifications to the reported endpoints. However, for the purposes of assessing jeopardy to a species or adverse modification of critical habitat and potential mitigation, a wider analysis of the endpoints should be considered. Through the consultation process, EPA will provide the Services with the additional information from the public comment periods and assistance in interpreting this material. Additional endpoints should be considered in the analysis when assessing these impacts in a similar manner as demonstrated in Attachment 2 for atrazine (additional MAGtool runs).

## Birds, Reptiles, and Terrestrial Phase Amphibians

Public comments disputed the use of a non-definitive (<) sublethal avian endpoint derived from a chronic toxicity test in Mallard duck (*Anas platyrhynchos*) (MRID 48876602) on the grounds that the effect is not monotonic and male mallard food consumption may be affected by feeding behavior in the study. Commenters contend that the reported effects are therefore not due to the active ingredient. As noted in the response to comments on the glyphosate Preliminary Risk Assessment (PRA), EPA continues to support the use of the non-definitive endpoint as the most sensitive chronic avian endpoint. Like other acceptable dietary studies, the control data are considered adequate to account for feeding behavior in the study. EPA also notes that strict monotonicity is not required for effects to be attributed to the active ingredient.

## Terrestrial Invertebrates

As stated in Chapter 2 for the BE, both the formulated product and technical grade glyphosate showed little or no effect on mortality in most tests on honeybees. Commenters recommended the use of Thompson *et al*. 2014 as the source for the chronic larval toxicity endpoint for terrestrial invertebrates. EPA has evaluated Thompson *et al*. 2014 and finds that the study provides useful information on the potential for effects to honey bee larvae, with some limitations. The study is of limited value in quantitatively assessing individual toxicity since exposure to individual larvae cannot be reliably quantified. Because individual toxicity could not be determined, there is additional uncertainty in using these data as surrogates for endangered terrestrial invertebrates. Commenters state that a chronic larval toxicity study has been conducted according to OECD guidelines, but has not yet been submitted to the EPA. That study will be evaluated for use after it has been submitted to the EPA.

Commenters stated thatproboscis extension reflex(PER) studies are not suitable for deriving sublethal endpoints due to variability in the test results, lack of reproducibility, and highly artificial test conditions. EPA concurs that the PER tests are conducted under highly artificial conditions and notes that guidelines for testing sublethal effects to foraging behavior have not been established. Additional studies in the open literature point to the potential for sublethal effects on foraging behavior, as provided in the ECOTOX spreadsheet EPA considers feeding behavior a relevant line of evidence in evaluating sublethal effects on honey bees, and notes that effects on foraging and/or homing in less social bees could be more likely to result in nest failure. To preserve conservatism at the screening level, EPA is continuing to use the endpoint identified in the draft BE. However, EPA will work with the Services during consultation on the interpretation of the range of sublethal toxicity data available for glyphosate, and the relevance of gustatory response and foraging behavior as endpoints for listed bees and other taxa.

Commenters note that food consumption is not a validated endpoint for honey bee toxicity studies using the OECD guidelines. EPA recognizes that the OECD guideline tests are designed to test mortality, rather than sublethal effects. Where sublethal effects are reported, however, that information is provided. The food consumption endpoints presented in the draft BE are provided for context but were not used quantitatively in the analysis, as risk at the screening step is driven by the endpoint provided in mg/kg-diet.

## Terrestrial Plants

Commenters noted inconsistencies between reported endpoint values in different sections of the BE. Table 2-6 above provides the correct endpoints for terrestrial vertebrates.

Commenters noted that the IC25 values were not consistent with IC25 values reported in the studies used to derive the NOAEC, LOAEC, or MATC. This is correct, as the IC25 endpoints used to evaluate potential impacts to monocots and dicots is derived from a species sensitivity distribution, rather than a single study.

Commenters noted that the glyphosate PRA used different endpoints than the BE. In the BE, the most sensitive geometric mean (MATC) of the NOAEC and LOAEC were used as the endpoints, rather than the lowest NOAECs used in the PRA. Although the lowest NOAEC was 0.049 lb a.e./A in the PRA (MRID 44320636), the spacing of the treatment levels in that study was such that the MATC was higher than the lowest LOAEC in MRID 44125715. Therefore, the lowest LOAEC was selected as the endpoint.

Typographical errors to the monocot entry in Table 2-5 of the draft BE did not impact the effects determinations and have been corrected.

## Aquatic Invertebrates

Commenters objected to the use of formulation endpoints for aquatic taxa. EPA notes that consideration of the toxicity of technical grade active ingredient is also presented through the alternative analysis.

Commenters provided additional information regarding the composition of the test substance reported in Ripley *et al*. 2002. Commenters state that the reported test substance is not sold at a 1% a.e. concentration, as reported in the study, but is instead sold at a 99% concentration. In conjunction with the lack of analytical test provided in the study, commenters state that this throws the reported test concentrations into considerable doubt. In addition, commenters note the lack of control mortality data and assert that the EPA has misinterpreted the available description of control mortality. Commenters also note that the resulting endpoints are significantly lower than other available endpoints for freshwater invertebrates.

Regarding the comments above on Ripley *et al.* 2002, EPA records of registration number 71995-18 (used in the study) indicate that the product was conditionally registered in 1996 with a concentration of 41% glyphosate isopropylamine (GLY-IPA) as the active ingredient. This differs from the concentration reported in Ripley *et al*. 2002 (1% GLY-IPA). This disparity results in substantial uncertainty in the concentrations tested, since it is not known whether the authors incorrectly reported the product name, concentration, and/or the registration number. Therefore, the EC50 is not suitable for quantitative use. The relative sensitivity of the tested aquatic invertebrates can still be informative, which shows that the endangered San Diego Fairy Shrimp (*Branchinecta sandiegonensis*) is slightly more sensitive than *Cerio daphnia* to glyphosate and more than five times more sensitive than *Thamnocephalus platyurus*.

The lowest quantitative endpoint for freshwater invertebrates using a formulation registered in the U.S. is 1.6 mg a.e./L, from an acute toxicity study using the water flea, *Daphnia magna* (MRID 00070893). Sixteen freshwater crustacean endpoints lower than 1.6 mg a.e./L are presented in the data array in Figure 2-15 of the BE. Of these lower endpoints, three are from Ripley *et al*. 2002 and are therefore unsuitable for quantitative use. The remaining lower endpoints, which range from 67 – 1,170 ug a.e./L, were derived using formulations from outside the U.S., including formulations purchased in Argentina, Canada, China, South Africa, Sri Lanka, and Ukraine. In addition, experiments conducted *in situ* using a Canadian formulation as part of a Ph.D. thesis reported an overall 50% reduction in crustacean abundance at 710 ug a.e./L (Wojtaszek, 2004). Overall, these data indicate that the 1.6 mg a.e./L endpoint may not be protective of freshwater aquatic invertebrate toxicity. Either there is inadequate testing to generate a protective endpoint for formulations registered in the U.S., or the formulations registered in the U.S. are less toxic to aquatic invertebrates than those formulations sold abroad. To retain protectiveness, the Step 2 endpoint was adjusted to 67 ug a.e./L, as reported in Achiorno *et al*. 2018, with further characterization that will be provided to the Services during consultation.

## Aquatic plants

Commenters request the use of endpoints from studies on the technical grade active ingredient and note that glyphosate trimesium salt is not included in any registered formulations. EPA notes that, where available, endpoints derived from the use of technical glyphosate are incorporated in the alternative analysis. The typographical errors noted in the table have been fixed, the endpoint values have not changed. EPA will continue to consider the toxicity of registered glyphosate formulations as well and provide characterization of the available endpoints to the Services during consultation.