

CONCLUSIONS

Degradation - Photodegradation on Soil

1. This study and addendum provide *supplemental* information, but do not fully satisfy the data requirement. The study may be upgradable if the registrant supplies additional supporting data and/or explanations or corrections. The registrant, without solicitation from the Agency, had apparently realized or been informed by Canadian regulators of some shortcomings in the original submission. The addendum was a revision and reanalysis of that submission intended to remedy the deficiencies. The addendum rectifies certain, but not all, of the shortcomings. Principal remaining deficiencies are: 1) the viability status of the soils was not confirmed; 2) the dark control soil results (provided only for the phenyl label study) were questionable based on data provided in other submitted studies which clearly showed relatively rapid soil metabolism. Additionally, the registrant-calculated half-lives of the parent were questionable due to anomalous variability in the data (possibly as the result of other undetermined processes occurring); inappropriate unequal weighting of data points at different time intervals; and, although apparently minor due to unexplained reasons, the result from the dark control did not appear to have been subtracted from the dosed treatments.

However, based on results of other fate studies and the physical and chemical properties of mesotrione, the absence of upgraded or new soil photolysis data is not critical to the fate assessment and does not play a prominent role in standard simulation modeling to estimate environmental concentrations. Therefore, because there appears to be little value added to the overall fate assessment if the registrant supplies additional data/explanations/corrections to this photolysis study or submits a new study, the Agency is not requesting any additional soil photolysis information at this time.

2. Some additional reasons why this study does not meet Subdivision N Guidelines for the fulfillment of EPA data requirements on photodegradation on soil are the following:
 - (i) data on the pattern of formation and decline of the degradates was not adequately presented for review;
 - (ii) the soil moisture content was not maintained at 75% of 0.33 bar during the incubation;
 - (iii) the incubation temperature was not held constant ($\pm 1^\circ\text{C}$); and
 - (iv) total light intensity was not reported. For future submissions, the use of actinometers and the determination of quantum yield is recommended, and will become standard when internationally harmonized of guidelines are adopted.

(v) although apparently minor,

The Comments Section of this review cites additional deficiencies in the original study submission and provides some detail on those given above, *but has not been modified to incorporate possible changes that may have issued from the addendum*. There will be minor additional discussion (not presented here) on the results of these data in the forthcoming environmental fate assessment which will accompany the registration eligibility decision. The portion of the study conducted under EEC guidelines is also not discussed here; however, because of the essential similarity (within apparent experimental error), these results will be equally weighted and incorporated in the risk assessment.

3. Uniformly phenyl ring-labeled [¹⁴C]mesotrione, at a nominal application rate of 300 g/ha, degraded with a registrant-calculated half-life of 21.3 sunlight-equivalent days ($r^2 = 0.68$) on silt loam soil incubated at 20-24°C for up to 15 days while irradiated continuously with a xenon arc lamp. However, the half-life was *incorrectly* calculated from the reported pseudo first-order rate constant, and the observed first "half-life" occurred between 11.7 and 19.4 sunlight-equivalent days (partially corrected in the addendum). Degradation was also possibly biphasic, with slower degradation occurring from 19.4 to 36.4 sunlight-equivalent days. However, data variability between replicates and between sampling intervals may be the result of undetermined real processes or experimental artifacts. In contrast, the parent was stable in the dark control soil.

All data below, designated as percentages of the applied, represent percentages of the nominal application and are reviewer-calculated means of multiple replicates unless otherwise noted. In the irradiated soil, the parent was initially present at 92.4% of the applied radioactivity, decreased to 44.3% by 19.4 sunlight-equivalent days posttreatment, and was 30.4-47.6% (range of six replicates) at 36.4 days. Two degradates tentatively identified from the phenyl- radiolabel were MNBA (up to 10-12% dose) and AMBA (up 8 to 9% of dose); tabular data were not reported in the original submission, but were in the addendum. Other minor degradates were separated as well. Carbon dioxide was up to 12-15% of dose. Nonextractable [¹⁴C]residues (reviewer-calculated) were <7% of the applied radioactivity throughout the incubation. Evolved ¹⁴CO₂ accounted for 10.9% of the applied radioactivity by 31.1 sunlight-equivalent days posttreatment and 12.1% of the applied by 36.4 days; [¹⁴C]organic volatiles were negligible. In the dark control soil, the parent was present at 85.7-91.5% of the applied radioactivity from 0 to 7 days posttreatment and was 84.5% at 15 days.

Cyclohexane ring-labeled [2-¹⁴C]mesotrione, at a nominal application rate of 300 g/ha, degraded with a registrant-calculated half-life of 19.3 sunlight-equivalent days ($r^2 = 0.41$) on silt loam soil incubated at 20-24°C for up to 14 days while irradiated continuously with a xenon arc lamp. However, the half-life was incorrectly calculated from the reported pseudo first-order rate constant and the half-life is questionable due to data variability over time. In the irradiated soil, the parent was initially present at 95.5% of

the applied radioactivity, decreased to 68.7% of the applied by 4.5 sunlight-equivalent days posttreatment, and was 40.1-54.9% (range of individual replicates) from 13.2 to 30.9 days with the exception of 25.8% (single replicate) at 21.9 days. Nonextractable [^{14}C]residues (reviewer-calculated) were <5% of the applied radioactivity throughout the incubation. Evolved, $^{14}\text{CO}_2$ accounted for 14.9% of the applied radioactivity at 4.5 sunlight-equivalent days posttreatment, increased to 25.4% by 13.2 days, and was 31.8% at 30.9 days; [^{14}C]organic volatiles were negligible. Dark controls were not prepared for cyclohexane ring-labeled [2- ^{14}C]mesotrione.

METHODOLOGY

A sample (5-5.8 g) of Radford silt loam soil (from Walworth County, WI; 17.1% sand, 57.7% silt, 25.2% clay, 2.7% organic matter, pH 6.2, CEC 12.0 meq/100 g; p. 15) was sieved (2 mm) and mixed with water (3 mL) to form a slurry (p. 18). Subsamples of the slurry were transferred to glass slides using a spatula and circular template to form duplicate soil disks with a surface area of 1.3 cm² and a mean weight of 0.059 g each. The soil was air dried overnight, then treated by microcapillary pipet with either uniformly phenyl ring-labeled [^{14}C]mesotrione {2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; radiochemical purity 96.3-98.2%, specific activity 30.4 mCi/mmol; p. 13} OR cyclohexane ring-labeled [2- ^{14}C]mesotrione (radiochemical purity 98.0%, specific activity 36.6 mCi/mmol), dissolved in acetonitrile:water (1:2, v:v), at a nominal application rate of 300 g/ha (p. 18); the phenyl ring-labeled parent was enriched with ^{13}C at the carbonyl position to aid in compound identification. The samples were placed in a stainless steel photolysis reactor equipped with a quartz glass lid, air inlet and outlets, and a recirculating water bath (p. 15; Figure 1A, p. 44). Soil slurries used for dark controls were prepared and treated with uniformly phenyl ring-labeled [^{14}C]mesotrione as previously described except the photoreactor was wrapped with foil; dark controls were not prepared for cyclohexane ring-labeled [2- ^{14}C]mesotrione. Temperatures were continuously monitored with a thermocouple placed in a soil disk; temperatures were maintained at 20-24°C (p. 15). Humidified air was passed through the chamber and drawn into a series of traps, including an XAD-2 (styrene/ divinylbenzene polyaromatic resin) column for [^{14}C]organic volatiles and two ethanolamine traps for $^{14}\text{CO}_2$ (Figure 1B, p. 45). Samples were continuously irradiated for up to 15 days (phenyl label study) or 14 days (cyclohexane label study) using a xenon arc lamp with a UV-filter to remove wavelengths of <290 nm (p. 15; Figure 2, p. 46). The solar spectrum at Richmond, CA (latitude 37° 56' N) was measured using a radiometer (1:08 p.m., 6/21/88) for comparison with the artificial light source spectrum (p. 15; Figure 3, p. 47; Appendix C, pp. 72-76). Respective mean irradiance values (300-800 nm) based on measurements at the initiation and termination of the study were 508 W/m² and 461 W/m² for the phenyl and cyclohexane label studies (p. 22); total light intensity was not reported. The determination of the intensity of the xenon arc lamp relative to natural sunlight was provided in Table II (pp. 31, 32); one nominal day of exposure to the xenon arc lamp was

equivalent to 2.4 days of exposure to natural sunlight for the phenyl label system and 2.2 days for the cyclohexane label system. Glass slides with duplicate samples of irradiated soils were removed for analysis at 0, 4.9, 11.7, 19.4, and 36.4 sunlight-equivalent days posttreatment (phenyl label study) or 0, 4.5, 13.2, 17.7, 21.9, 28.8, and 30.9 sunlight-equivalent days posttreatment (cyclohexane label study; p. 19; Tables IVa, IVb, pp. 37, 38); a single slide was removed at each interval except for sunlight-equivalent days 19.4 and 36.4, when two and three slides, respectively, were removed for analysis in the phenyl label system. Dark controls (phenyl label only) were removed for analysis at 0, 1, 4, 7, and 15 days posttreatment.

At each sampling interval, the soil disks were removed from the glass slide and transferred to separate centrifuge tubes. Soil samples were extracted by shaking three times with 0.05 M aqueous ammonium hydroxide and once with acetone; each extraction was followed by centrifugation (p. 19). Combined supernatants were neutralized (HCl) and analyzed for total radioactivity by LSC; method detection limits were not reported. Aliquots were also analyzed by HPLC (Alltima C18 5 MICRON column) using a mobile phase gradient of acetonitrile:water (both with 0.1% H₃PO₄; 0:100 to 25:75 to 100:0; v:v) with UV (wavelength not reported) and radioactive flow detection (p. 20). Samples were co-chromatographed with nonradiolabeled reference standards of the parent and suspected degradates 2-[4-methylsulfonyl]benzoyl]-1,3-cyclohexanedione (denitro-ZA1296 MBC); 6-(methylsulfonyl)-1-oxo-1,2,3,4-tetrahydro-9H-xanthen-9-one (ZA1296XanH₄); 4-(methylsulfonyl)-2-nitrobenzoic acid (MNBA); and 2-amino-4-(methylsulfonyl)benzoic acid (AMBA; Figure 12, p. 57; Table I, pp. 29, 30). HPLC eluate fractions were collected and analyzed for total radioactivity by LSC. It was unclear if an adequate confirmatory method of identification was utilized (see Comments #14, 15). Subsamples of the post-extracted soil were analyzed for total radioactivity by LSC following combustion (p. 20). Soils were extracted and analyzed within two weeks of sampling (p. 25).

Volatile traps were removed for analysis and replaced at each sampling interval. The XAD-2 column resin was extracted with ethyl acetate, and extracts were analyzed for total radioactivity by LSC (p. 17). Aliquots of the ethanolamine traps were analyzed for radioactivity by LSC. To confirm the presence of ¹⁴CO₂ in the ethanolamine solutions, ¹⁴CO₂ was volatilized by acidification (HCl) of the ethanolamine solution. The ¹⁴CO₂ was captured in 1 N NaOH solution and the presence of ¹⁴CO₂ was confirmed by precipitation with BaCl₂.

DATA SUMMARY

Uniformly phenyl ring-labeled [¹⁴C]mesotrione

Uniformly phenyl ring-labeled [¹⁴C]mesotrione (radiochemical purity 96.3-98.2%), at a nominal application rate of 300 g/ha, degraded with a registrant-calculated half-life of

5

21.3 sunlight-equivalent days ($r^2 = 0.68$; p. 23; Figure 11a, p. 55; see Comment #6) on Radford silt loam soil incubated at 20-24°C for up to 15 days while irradiated continuously with a xenon arc lamp. However, the half-life was incorrectly calculated from the reported pseudo first-order rate constant and the observed first half-life occurred between 11.7 and 19.4 sunlight-equivalent days (Table IVa, p. 37). Dissipation was observed to be biphasic, with slower dissipation occurring from 19.4 to 36.4 sunlight-equivalent days. However, data were variable between replicates at 36.4 sunlight-equivalent days posttreatment (the last sampling interval). In contrast, the parent compound was stable on the dark control soil. All data, designated as percentages of the applied radioactivity, represent percentages of the nominal application and are reviewer-calculated means of multiple replicates unless otherwise noted. In the irradiated soil, the parent was initially present at 92.4% of the applied radioactivity, decreased to 44.3% of the applied by 19.4 sunlight-equivalent days posttreatment, and was 30.4-47.6% (range of six replicates) at 36.4 sunlight-equivalent days (Table IVa, p. 37). Two minor degradates tentatively identified as 4-(methyl sulfonyl)-2-nitrobenzoic acid (MNBA) and 2-amino-4-(methylsulfonyl) benzoic acid (AMBA) and an unidentified minor degradate were each present at <9.6% of the applied radioactivity (pp. 23, 24); tabular data were not reported. Nonextractable [^{14}C]residues (reviewer-calculated) were <7% of the applied radioactivity throughout the incubation (Table IIIa, p. 33; see Comment #8). Evolved $^{14}\text{CO}_2$ accounted for 10.9% of the applied radioactivity by 31.1 sunlight-equivalent days posttreatment and 12.1% of the applied by 36.4 days (Table Va, p. 39; see Comment #9); [^{14}C]organic volatiles were negligible.

In the dark control soil, the parent compound was present at 85.7-91.5% of the applied radioactivity from 0 to 7 days posttreatment and was 84.5% of the applied at 15 days (30 sunlight-equivalent days; Table IVb, p. 38); total degradates were <7% of the applied radioactivity (tabular data were not reported; p. 25). Nonextractable [^{14}C]residues (reviewer-calculated) were <6% of the applied radioactivity and [^{14}C]volatiles were negligible (Table III d, p. 36).

The mean material balance (across all replicates) for the irradiated soil was 92.4% of the applied radioactivity (Table IIIa, p. 33; see Comment #7). The mean material balance for dark control soils was 96.5% of the applied (Table III d, p. 36).

Cyclohexane ring-labeled [2- ^{14}C]mesotrione

Cyclohexane ring-labeled [2- ^{14}C]mesotrione (radiochemical purity 98.0%), at a nominal application rate of 300 g/ha, degraded with a registrant-calculated half-life of 19.3 sunlight-equivalent days ($r^2 = 0.41$; p. 23; Figure 11c, p. 56; see Comment #6) on Radford silt loam soil incubated at 20-24°C for up to 14 days while irradiated continuously with a xenon arc lamp. However, the half-life was incorrectly calculated from the reported pseudo first-order rate and the half-life is questionable due to data variability over time (Table IVb, p. 38). In the irradiated soil, cyclohexane ring-labeled [2- ^{14}C]mesotrione was

initially present at 95.5% of the applied radioactivity, decreased to 68.7% of the applied by 4.5 sunlight-equivalent days posttreatment, and was 40.1-54.9% (range of individual replicates) from 13.2 to 30.9 sunlight-equivalent days with the exception of 25.8% (single replicate) at 21.9 days (Table IVb, p. 38). Unidentified minor degradates were present at $\leq 3.0\%$ of the applied radioactivity (pp. 24, 25); tabular data were not reported. Nonextractable [^{14}C]residues (reviewer-calculated) were $<5\%$ of the applied radioactivity throughout the incubation (Table IIIc, p. 35; see Comment #8). Evolved $^{14}\text{CO}_2$ accounted for 14.9% of the applied radioactivity at 4.5 sunlight-equivalent days posttreatment, increased to 25.4% of the applied by 13.2 sunlight-equivalent days, and was 31.8% of the applied at 30.9 days (Table Vc, p. 41); [^{14}C]organic volatiles were negligible. Dark controls were not prepared for cyclohexane ring-labeled [2- ^{14}C]mesotrione.

The mean material balance (across all replicates) for the irradiated soil was 98.4% of the applied radioactivity (Table IIIc, p. 35; see Comment #7).

COMMENTS. (Note: As stated previously, these comments do not reflect the revisions and recalculations presented in the addendum to the original study. However, the revisions do not significantly alter study conclusions given above, and have little or no current bearing on the overall environmental fate assessment for mesotrione.)

1. The results of the study (both labels) were questionable because the viability of the soil utilized in the study was not confirmed as required by Subdivision N Guidelines (also see Comment #2). The parent compound (phenyl label) was present in the dark control soil at 88.6-90.0% of the applied radioactivity from 0 to 4 days posttreatment and only decreased to 84.5% of the applied by 15 days (30 sunlight-equivalent days; Table IVb, p. 38); however, the reviewer noted that the registrant-calculated half-life of mesotrione in an aerobic soil metabolism study (MRID 44373530) was 13.5 days. The reviewer notes that dark control results (for the parent) obtained using compounds with different radiolabels may be used as checks against each other; in this study, however, dark control samples were not utilized for the cyclohexane labeled compound.
2. The soil moisture content was not maintained at 75% of 0.33 bar during the incubation period. Subsamples of a soil slurry were formed into duplicate disks (0.059 g each) on glass plates, air dried overnight, and treated with the parent (27 μL of stock solution) prior to incubation. The soil disks were incubated with a continuous flow of humidified air; however, soil moisture content was not determined following treatment or at any time during the incubation. Subdivision N Guidelines require that soil moisture be maintained at 75% of 0.33 bar moisture content throughout the incubation period to help ensure soil viability.
3. The patterns of formation and decline of degradates were not adequately addressed. The study authors stated that two minor degradates, MNBA and AMBA, and an unidentified degradate were each present at less than 9.6% of the applied radioactivity in the phenyl

label system (p. 24) and that no degradates exceeded 3% of the applied radioactivity in the cyclohexane label system (p. 25). Subdivision N Guidelines require that the patterns of formation and decline of the degradates be determined and reported in graphical and tabular formats.

4. The incubation temperature was maintained at 20-24°C (p. 15). Subdivision N Guidelines require that soil temperatures be held constant with a variation of only $\pm 1^\circ\text{C}$.
5. Total light intensity over the course of the study was not reported as required by the Subdivision N Guidelines. A comparison graph of artificial and natural light (measured at 1:08 pm on June 21, 1988 in Richmond, CA) was presented in Figure 3 (p. 47).
6. The reviewer noted that the half-lives were incorrectly calculated by the registrant from the pseudo-first order rate constants (k ; Appendix B-2, p. 70). The calculation is $t_{1/2} = \ln 2/k$. Based on the registrant-calculated rate constant for the phenyl label system ($k = 1.89 \times 10^{-2} \text{ day}^{-1}$) and cyclohexane label system ($k = 1.99 \times 10^{-2} \text{ day}^{-1}$), the reviewer calculated half-lives of 36.7 and 34.8 sunlight-equivalent days, respectively (p. 23). The registrant calculated half-lives were 21.3 and 19.3 sunlight-equivalent days for the phenyl label and the cyclohexane label systems, respectively; clarification by the registrant may be appropriate. However, the reviewer-calculated half-lives (calculated from the registrant-calculated rate constants) do not agree with the observed first half-lives. In the phenyl label system, the parent decreased from 92.4% of the applied radioactivity at time 0 to 44.3% of the applied by 19.4 sunlight-equivalent days (Table IVa, p. 37). In the cyclohexane label study, the parent decreased from 95.5% of the applied radioactivity at time 0 to 36.4-54.3% by 13.2-30.9 sunlight-equivalent days.
7. Material balances were not reported for individual replicates. Generally, laboratory radiolabel studies include material balance data for individual replicates based on the percentages of the applied radioactivity for extractable, nonextractable, and volatile residues. Instead, the study authors calculated a material balance based on the sum of radioactivity applied to all of the individual replicates, resulting in a mean material balance for the entire study. It is necessary that complete material balance data be reported for each sample and for each sampling interval so that the reviewer may determine whether material balances decreased over time and whether they remained within the acceptable range of 90-110%.
8. The reviewer determined nonextractable [^{14}C]residues by dividing the soil bound residues (reported in dpm) for individual replicates by the average radioactivity applied to an individual replicate (Table IIIa, c; pp. 33, 35); the average radioactivity applied to an individual replicate was calculated by dividing the total radioactivity applied to all samples (reported in dpm) by the total number of replicates.

8

9. Sampling intervals for the volatile traps (phenyl label study only) were inadequate to determine the pattern of the formation of $^{14}\text{CO}_2$. Volatile traps were removed for analysis at 31.1 sunlight-equivalent days posttreatment and 36.4 sunlight-equivalent days only, evolved $^{14}\text{CO}_2$ accounted for 10.9% and 12.1% of the applied radioactivity, respectively, by those sampling intervals (Table Va, p. 39).
10. Method detection limits were not reported for LSC or HPLC analyses. Limits of detection and quantitation should be reported to allow the reviewer to evaluate the adequacy of methods for determination of the test compound and its degradates.
11. The application rate was reported as 300 g/ha (p. 18). The reviewer was unable to confirm that 300 g/ha was the maximum application rate of mesotrione. Clarification by the registrant is necessary. Additionally, it is preferred that the application rate be reported in units of concentration (per sample), such as ppm.
12. The reviewer confirmed that the Radford silt loam soil was the same type of soil used in two submitted aerobic soil metabolism studies (MRIDs 44373530 with addendum MRID 44505130; and MRID 44373531).
13. An additional soil photolysis study was performed to meet EEC guidelines. Soil samples were prepared as previously described except that the samples were dried at 35°C for 7 hours prior to treatment (phenyl label only; p. 18). The parent compound was initially present at 94.4% of the applied radioactivity, decreased to 46.5% of the applied by 12.8 sunlight-equivalent days, and was 32.3% at 32.5 sunlight-equivalent days (Table IVa, p. 37). The major degradate MNBA was a maximum of 11.8% of the applied radioactivity (time not reported; p. 24); the minor degradate AMBA and an additional unidentified minor degradate were observed at <10% of the applied. Nonextractable [^{14}C]residues were $\leq 12.1\%$ of the applied radioactivity (reviewer-calculated; Table IIIb, p. 34). Evolved $^{14}\text{CO}_2$ accounted for 1.8% of the applied radioactivity at 4.7 sunlight-equivalent days posttreatment and increased to 11.8% by 32.5 sunlight-equivalent days (Table Vb, p. 40); [^{14}C]organic volatiles were negligible. The mean material balance (across all replicates) was 98.4% of the applied radioactivity (Table IIIb, p. 34).
14. Degradate confirmation was performed using soil samples from the study performed to meet EEC guidelines. Degradates were isolated from soil extracts using HPLC as previously described except phosphoric acid was replaced with trifluoroacetic acid in the mobile phase. The isolated compounds were analyzed by LC/MS in the negative electrospray ionization mode; compounds were identified as MNBA and AMBA (Figures 14, 15; pp. 59, 60).
15. The study authors described a TLC system on page 21, but did not indicate which samples, if any, were analyzed using the method. The reviewer did not report the TLC system in the methods section of this review, as it was unclear if it was used as a

confirmatory method or if it may have been used simply to test radiochemical purity of the test compounds. Clarification by the registrant is necessary.

16. The use of actinometers and the determination of quantum yield is recommended, and will become standard when internationally harmonized of guidelines are adopted.

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Pages 11 through 13 are not included in this copy.

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