# MON 52276
# (360 g/L glyphosate acid)

## Herbicide

## Application for Renewal of Approval (AIR 2) according to Commission Regulation (EC) N° 1141/2010

## ANNEX III
## Summary documentation, TIER II

## Document M:

## Point 10: Ecotoxicology

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## IIIA 10 Ecotoxicological studies on the plant protection product

Ecotoxicological studies described in this document address data requirements specified in Commission Regulation 1107/2009 of 21 October 2009 amending Council Directive 91/414/EEC. Experimental details of ecotoxicological studies done with the formulated product MON 52276 that also satisfy data requirements specified in Annex IIA, Point 8, Ecotoxicological Studies were included in Document M-II; only the conclusions will be reported here in summary form.

Details of parameters, assumptions and calculations used in the estimation of environmental exposure used for TER calculations, are discussed in the current Glyphosate EU Dossier, Annex IIIA, Document M-III, Section 5.

This document includes summaries of studies and risk assessments conducted with glyphosate, MON 52276, and the relevant metabolites AMPA and HMPA, which are the major metabolites of glyphosate in water and / or soil.

As a non-selective systemic herbicide, with no residual weed control activity, MON 52276 is applied to remove weeds, and in all weed control uses the crop, foliage or flowers are not treated. Drift reduction nozzles may be used to minimise exposure of non-target areas.

The representative application pattern that drives the risk assessments in this re-submission is summarised in the following Table, for details also see IIIA I point 3.

**Table 10-1: Proposed use pattern of MON 52276**

| Crop | Application method | Spray volume (L/ha) | Maximum individual application rate (kg a.s./ha) | Number of applications | Maximum annual application rate (kg a.s./ha) | Minimum application interval (days) | Application timing e.g. BBCH |
|---|---|---|---|---|---|---|---|
| All crops[1] | Spray | 100-400 | 2.16 | 1-2 | 4.32 | 21 | Pre-planting (post-emergence of weeds) |
| All crops[1] | Spray | 100-400 | 1.08 | 1 | 1.08 | - | Post-planting/pre emergence of crop (post-emergence of weeds) |
| Cereals (pre-harvest) | Spray | 100-400 | 2.16 | 1 | 2.16 | - | Crop maturity |
| Oilseeds (pre-harvest) | Spray | 100-400 | 2.16 | 1 | 2.16 | - | Crop maturity |
| Orchard crops, vine including citrus & tree nuts[2] | Spray | 100-400 | 2.88 | 1-3 | 4.32[3] | 28 | Post-emergence of weeds |
| Orchard crops, vines including citrus & tree nuts (spot treatment)[2] | Spray<br>Knapsack use | 0[4]-400 | 0.72-2.88<br>0.96-2.88 | 1<br>1-3 | 4.32[3] | 28 | Post-emergence of weeds |

[1] all seeded and transplanted crops (including but not restricted to root & tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables brassica vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds, cereals and sugar & fodder beet, before planting fruit crops, ornamentals, trees, nursery plants etc.
[2] ground directed / applications made round base of trunk.
[3] Annual application not to exceed 4.32 kg as/ha
[4] Including ULV (Ultra Low Volume) application of undiluted product

The potential risk from ecotoxicologically relevant metabolites have been considered, detailed discussion was provided in the EU review (glyphosate acid) and in IIIA1, point 5. The metabolites to which non-target organisms could be exposed are presented in the table below.

**Table 10-2: Glyphosate acid - metabolites**

| Parent compound | Metabolite name | Compound found in | Maximal percentage of formation % |
|---|---|---|---|
| Glyphosate acid | AMPA | Soil | 50.1 |
| | | Water | 15.7 |
| | | Sediment | 53.1 |
| | HMPA[1] | Water | 10 |

[1] Metabolite formed from AMPA in water/sediment conditions only



Guidance for conducting EU ecological risk assessments was obtained in the following references:

- Guidance of EFSA. Risk Assessment for Birds and Mammals. European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2009; 7(12):1438.
- EPPO Bulletin, Environmental risk assessment scheme for plant protection products 2010, Vol. 40.
- Draft Guidance Document on Persistence in Soil. Commission document 9188/VI/97 (rev 5 or higher)
- Guidance Document on Aquatic Ecotoxicology in the frame of Directive 91/414/EEC. Commission document Sanco/3268/2001 rev. 4 (2002)
- Guidance Document on Terrestrial Ecotoxicology. Commission document SANCO/10329/2002 rev 2 (2002)
- Candolfi M.P., Barrett K.L., Campbell P.J., Forster R., Grandy N., Huet M.C., Lewis G., Oomen P.A., Schmuck R. and Vogt H. (2001). Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. ESCORT 2 workshop (European Standard Characteristics of non-target arthropod Regulatory Testing), Wageningen, NL, March 21-23, 2000, SETAC Europe; SETAC publication August 2001
- Guidance on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated Under Council Directive 91/414/EEC, SANCO/221/2000-Rev 10 (2003)
- Council Directive 97/57/EC 22 Sept 1997, Annex VI to Directive 91/414/EEC ('Uniform Principles')

## IIIA 10.1 Effects on birds

The risk assessment was carried out according to the recent EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)[i], which follows a tiered approach to assess the effects of

plant protection products on birds based on the requirements of Regulation 544/2011 and Regulation 545/2011 for active substances and plant protection products, respectively.

The avian toxicity studies with the active substance glyphosate acid, salts of glyphosate and MON 52276 are summarised in the tables below. Detailed descriptions of ecotoxicological studies with birds are given under point 8.1 in the Annex II dossier of glyphosate.

No acute oral toxicity studies were conducted with the formulation MON 52276 since the active ingredient glyphosate shows very low acute toxicity to birds. Additionally, glyphosate and the lead formulation MON 52276 have low acute toxicity in rat gavage studies, with acute $LD_{50}$ values ≥5000 mg/kg. Further, MON 52276 is applied as a spray and, accordingly, residues on food sources are better considered in terms of the individual active ingredients rather than the formulation[1].

The acute oral toxicity of glyphosate was tested in numerous studies with three species, bobwhite quail, Japanese quail and mallard duck, and are summarized in Table 10.1-1.

**Table 10.1-1: Acute toxicity of glyphosate acid and AMPA to birds**

| Species | $LD_{50}$ (mg a.s./kg bw) | NOEL (mg a.s./kg bw) | Reference/GLP |
|---|---|---|---|
| **Glyphosate Acid** | | | |
| Bobwhite quail | > 3851 | 1785 | 139-140 [redacted] 1978/no |
| Bobwhite quail | > 2000 | 2000 | IIA 8.1.1/01 [redacted] 1997/yes |
| Bobwhite quail | > 2000 | 2000 | [redacted]48/91266 [redacted] 1991/yes |
| Japanese quail | > 2000 | 2000 | IIA 8.1.1/02 [redacted] 1996/yes |
| Japanese quail | > 2000 | 2000 | IIA 8.1.1/03 [redacted] 1999/no |
| Mallard duck | > 2000 | 2000 | IIA 8.1.1/04 [redacted] 1996/yes |
| Mallard duck | > 2000 | 2000 | [redacted]49/91843 [redacted] 1992/yes |
| **Glyphosate IPA** | | | |
| Japanese quail | >1225.4 [3] | 1225.4 | [redacted]81.186/00 [redacted] 2000/yes |
| **Glyphosate K-salt** | | | |
| Bobwhite quail | > 2241 | 484 | IIA 8.1.1/05 [redacted]2002-151 [redacted] 2003/yes |
| **AMPA** | | | |
| Bobwhite quail | > 2250 | 1350 [4] | [redacted]S95-00222 [redacted] 1991/yes |

**Studies shaded in grey have been reviewed as part of the 2001 EU evaluation.**

[1] LLD: Lowest Lethal Dose.

[2] This NOEL value is based on the observation of transient lethargy in the 3851 mg/kg bw dose group on the day of dosing. No other adverse effects were observed at 3851 mg/kg bw.

[3] Limit test; test was conducted with 2000 mg glyphosate IPA salt/kg bw (nominal), equivalent to 1225.4 mg glyphosate/kg bw.

[4] This NOEL value is based on the observation of transient lower limb weakness, a ruffled appearance, and reduced reaction to external stimuli (sound and movement) on the day of dosing.

[1] European Commission, Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO 2021/VI/98, draft of June 2002.

In all of the acute avian studies with glyphosate acid, the $LD_{50}$ was reported to be >2000 mg a.s./kg bw, with the exception of one study with 14-day-old bobwhite quail where the $LD_{50}$ was reported to be >3851 mg a.s./kg bw with transient lethargy observed only at the highest dose of 3851 mg a.s./kg bw. However, during the 2001 EU evaluation the $LD_{50}$ of >2000 mg a.s./kg bw was used for the acute assessment and provided a TER >14.

As no mortality was observed in any of the limit dose studies listed above, the relevant endpoint for the acute risk assessment of birds was determined by extrapolation of the $LD_{50}$ of >2000 mg a.s./kg bw. According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), an extrapolation factor of 2.167 may be applied when at least 20 individuals are tested at the limit dose. Considering having tested a limit dose of 2000 mg a.s./kg bw and that far more than 20 birds (actually 50 quails and 70 mallard ducks) were tested with glyphosate acid, an endpoint $LD_{50}$ = 4334 mg a.s./kg bw was determined.

For animal welfare reasons and due to the lack of acute toxicity, no study on chronic toxicity of the formulation MON 52276 to birds was conducted. However, it is anticipated that a formulation does not stay intact in the environment; therefore, evaluation of toxicity is based on the assessment of effects of the active substance. Specifically with regard to glyphosate containing formulations, it is expected that due to the post-emergence activity, treated plants will rapidly demonstrate phytotoxicity within a few days of application and consequently limit field exposures to herbivores and omnivores by consumption of treated foliage.



**Table 10.1-2: Short- and long-term toxicity of glyphosate acid and short-term toxicity of AMPA to birds**

| Test item | Species | Value (mg a.s./kg feed) | | Daily Dose [1] (mg a.s./kg bw/day) | | Reference/ GLP |
|---|---|---|---|---|---|---|
| | | NOEL/ NOAEL | $LC_{50}$ | $LC_{50}$ | NOEL/ NOAEL | |
| **Dietary studies (5-day exposure)** | | | | | | |
| Glyphosate acid | Bobwhite quail | 4640 [2] | >4640 | >1127 | 1127 | 214-106 [redacted] 1973/no |
| Glyphosate acid | Mallard duck | 4640 | >4640 | >1242 | 1242 | 241-107 [redacted] 1973/no |
| Glyphosate acid | Bobwhite quail | 5200 | >5200 | n.a. | n.a. | [redacted] 395/963857 [redacted] 1997/yes |
| Glyphosate acid | Mallard duck | 5200 | >5200 | n.a. | n.a. | [redacted] 23 [redacted] 1997/yes |
| AMPA | Bobwhite quail | 5620 [2] | >5620 | >2162 | 2162 | 139-275 [redacted] 1991/yes |
| AMPA | Mallard duck | 5620 [2] | >5620 | >1765 | 1765 | 139-276 [redacted] 1991/yes |
| MON 52276 | Bobwhite quail | 5620 [2] | >5620 | >1574 | n.a. | [redacted] 91-271 [redacted] *al.* 1991/yes |
| MON 52276 | Mallard duck | 5620 [2] | >5620 | >1629 | n.a. | [redacted] 91-270 [redacted] *al.* 1991/yes |

| Test item | Species | Value (mg a.s./kg feed) | | Daily Dose [1] (mg a.s./kg bw/day) | | Reference/ GLP |
|---|---|---|---|---|---|---|
| | | NOEL/ NOAEL | $LC_{50}$ | $LC_{50}$ | NOEL/ NOAEL | |
| **Reproduction studies** | | | | | | |
| Glyphosate acid | Bobwhite quail | 1000 [2,3] | n.a. | n.a. | 96.3 [4] | IIA 8.1.4./03<br>139-141<br>[redacted]<br>1978/no |
| Glyphosate acid | Mallard duck | 1000 | n.a. | n.a. | 125.3 [4] | IIA 8.1.4./04<br>139-143<br>[redacted]<br>1978/no |
| Glyphosate acid | Bobwhite quail | 2250 [2] | n.a. | n.a. | **201** [4] | IIA 8.1.4./01<br>123-186<br>[redacted]<br>1999/yes |
| Glyphosate acid | Mallard duck | 2250 [2] | n.a. | n.a. | 300 [4] | IIA 8.1.4./02<br>123-187<br>[redacted]<br>1999/yes |

**Studies shaded in grey have been reviewed as part of the 2001 EU evaluation.**
**Endpoints in bold are used for risk assessment**
n.a.: not available
[1] Calculation for conversion from mg/kg feed to daily dose described in Appendix I of Annex II document.
[2] Highest dose tested.
[3] The statistical NOEL of the original 1978 non-GLP bobwhite quail study was concluded to be 200 mg/kg diet, rather than 1000 mg/kg feed, based on a statistically significant finding for an endpoint (egg weight) not part of OECD 206 and that was concluded not to be biologically significant by the study director. Although there was a small difference (mean of x% lower than control) in egg weight at 1000 mg/kg feed there was no statistically significant difference from the control on the biologically relevant endpoints of initial hatchling body weight, 14 day hatchling body weight, egg shell thickness and hatchling survival. Egg weight is not a standard endpoint in avian reproduction studies and was a carryover from poultry performance studies. Consequently, the relevant NOEL from this study is considered to be 1000 mg a.e./kg diet.
[4] Since no adverse effects were observed at the highest concentrations tested in any reproduction study, the higher NOEL value of the GLP study of [redacted] 1999 on bobwhite quail has been used for the long-term risk assessment. This new study was not reviewed in the initial glyphosate monograph; however, it has been widely used to support member state re-registrations.



## Exposure

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to MON 52276 applications is considered unlikely, since at the time of application and for a short period thereafter, most birds will leave the immediate vicinity of spray operations in response to the human disturbance.

The exposure of birds to glyphosate acid was estimated following application of MON 52276 on a field containing annual weeds at:

1. A maximum single application rate of 2.16 kg a.s./ha that includes pre-planting, pre-emergence of crops and pre-harvest applications

2. A maximum single application rate for orchards of 0.96 kg a.s./ha has been used. As a worst case it is assumed that 1/3 of the area of an individual orchards or vineyard is treated, giving an overall application rate of 2.88 kg a.s./ha / 3 = 0.96 kg a.s./ha.

A repeat application of MON 52276 is only made to control new growth of weeds which would not have been exposed to the preceding application. Therefore, it is not appropriate to use a multiple application factor (MAF) for foliar residues in the case of this total herbicide.

Glyphosate residues on the relevant food items were calculated using the estimates given in the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009). For acute exposure, shortcut values for 90$^{th}$ percentile RUD values were used to calculate daily dietary dose (DDD). For long-term exposure, shortcut values for mean RUD values were used since they are considered to be more appropriate in the case of repeated applications.

The daily dietary doses (DDD) were calculated using the following equation:

DDD (mg a.s./kg diet) = Application rate (kg a.s./ha) × $RUD_{SV}$ × $f_{twa}$

Where:

- $RUD_{SV}$ is the Residue per Unit Dose (shortcut value) i.e. the residues on food items normalised to an application rate of 1 kg a.s./ha taken from the EFSA Bird and Mammal Guidance document.

- The term $f_{twa}$ is the time-weighted-average factor. This was used to calculate time-weighted average (TWA) residues on foliar material, which take into account the degradation of the active substances over time. Default TWA residues were used as an estimate of long-term exposure only, since it is considered that the use of mean initial residues provides an unrealistically extreme worst-case estimate of long-term exposure. The default $f_{twa}$ of 0.53 was used as given in the Guidance Document, assuming the averaging period of 21 days and $DT_{50}$ of 10 days. As discussed later in this section, empirical $f_{twa}$ values have been developed for grass and insects that are less than the default values and provide more realistic and relevant glyphosate exposure estimates.

- The following assumptions are used in the screening avian risk assessment

- Application rate: MON 52276 (maximum labelled rate in the EU) –2 × 2.16 kg a.s./ha on annual weeds (representing pre-planting and pre-emergence of crops), 1 × 2.16 kg a.s./ha on cereals and oilseed rape each, and 3 × 0.96 kg a.s./ha on orchards and vines

- Method of application: hydraulic sprayer overall and Knapsack spray for spot treatment on orchards and vines.

**Metabolites of glyphosate acid - AMPA**

Quantitative avian risk assessments for AMPA were not conducted because of low acute and chronic dietary field exposure levels to AMPA and equally low acute toxicity as glyphosate. Low levels of AMPA have been measured in the forage crop residue studies and numerous plant metabolism studies that are summarized in the current EU Annex II summary documentation, Section IIA 6. Measured AMPA levels were less than <10% of the total radioactive residue. Low exposure levels would also be predicted for prey items (e.g., insects) and similar to glyphosate AMPA does not possess bioaccumulation potential. Based on the combination of low exposure and low hazard, it can be concluded that risk to birds will be low and a quantitative assessment is not required.

## TER calculation

According to the EFSA (2009) guidance document, a step wise approach to the risk assessment was followed. This consisted of an initial screening assessment followed by a Tier 1 assessment (where necessary), for both acute and long term (reproductive) assessments.

## Screening step

The screening step starts by using generic 'indicator species' and is based on a multiplication of hypothetical worst-case assumptions. The crop groupings, indicator species and critical use patterns relevant to the use of MON 52276 to according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals are shown in Table 10.1-3.

The risk to birds was assessed using Toxicity: Exposure Ratio (TER), i.e. by comparing the relevant Daily Dietary Dose (DDD) with the appropriate endpoint:

$TER_A = LD_{50} / DDD_A$

$TER_{LT}$ = long-term and reproduction NOEL / $DDD_{LT}$

The Daily Dietary Dose (DDD) was estimated from parameters provided in the guidance document and the application pattern of glyphosate acid. Shortcut values are used to represent the food intake rate relative to body weight of birds (FIR/bw) and the fraction of diet obtained in the treated area.

Acute and long-term DDD are calculated according to the following formulas:

$DDD_A$ = Application rate x $RUD_{SV}$ [mg a.s./kg bw]

$DDD_{LT}$ = Application rate x $RUD_{SV}$ x TWA [mg a.s./ kg bw/d]

With:

- **SV:** To facilitate the assessment process, information on food intake rate, body weight and residue per unit doses (RUDs) is combined into a single value for a specific combination of species and crop termed as "shortcut value" (SV) and provided in the EFSA (2009) Guidance Document on Risk Assessment for Birds and Mammals. Shortcut values based on 90$^{th}$ percentile RUDs are used for acute assessments and mean residue unit doses (RUDs) for reproductive assessments.

- **TWA:** Long-term DDD values can be calculated as the time-weighted average concentration for the respective time interval. Default assumptions are based on a $DT_{50}$ of 10 days on plants and a time window of 21 days, which leads to a default TWA factor of 0.53. It should be noted that glyphosate initial residue and dissipation data from 22 trials is summarized in the 2001 EU evaluation on glyphosate for the formulation MON 2139 (360 g a.s./L) and a comparable formulation from Cheminova. MON 2139 and MON 52276 have comparable efficacy against weedy grasses which indicates comparable leaf retention and transport of the active substance. An evaluation of residue data demonstrates that glyphosate rapidly dissipates from grass with an estimated $DT_{50}$ reported in the glyphosate monograph of approximately 3 days. This estimated value is very close to the derived value of 2.8 days, which translates to a TWA factor of 0.19 (Appendix I). Additionally, a glyphosate residue field decline study was performed to evaluate decline in insects and is summarized in the Annex II document. This study provided a $DT_{50}$ value of approximately 5.5 days, which translates to a TWA factor of 0.35 (Appendix II).

SV values for the critical use pattern for each crop group and the corresponding indicator species are summarised in Table 10.1-3.

**Table 10.1-3: Screening step crop groupings, indicator species and critical use pattern relevant to the use of MON 52276**

| Crop group[1] | GAP crop species | Indicator species | Proposed /Critical use pattern | | | | $SV_{90}$ | $SV_m$ |
|---|---|---|---|---|---|---|---|---|
| | | | No. of apps | App. Interval (d) | Growth stages (BBCH) | Glyphosate acid (kg a.s./ha] | (mg a.s./kg bw/d) | |
| Annual weeds | All crops[2] | Small granivorous bird | 1- 2 | 21 | BBCH <10[3] | 2.16 | 25.3[4] | 11.4 |
| Cereals | Cereals | Small omnivorous bird | 1 | - | Pre harvest | 2.16 | 158.8 | 64.8 |
| Oilseed rape | Oilseeds | Small omnivorous bird | 1 | - | Pre harvest | 2.16 | 158.8 | 64.8 |
| Orchard | Orchard crops and vines | Small insectivorous bird | 1 - 3 | 28 | Pre harvest | 0.96 | 46.8 | 18.2 |
| Vines | | Small herbivorous bird | 1 - 3 | 28 | Pre harvest | 0.96 | 95.3 | 38.9 |

[1] Crop according to EFSA (2009) crop groupings
[2] Annual weeds in all seeded or transplanted crops, including but not restricted to: root and tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables, *Brassica* vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds cereals, and sugar- and fodder beet; before planting fruit crops, ornamentals, trees, nursery plants etc.)
[3] Not relevant for glyphosate
[4] Corrected SV values according to CRD (2011, [redacted], personal communication)

## Tier 1

A Tier 1 risk assessment is required if the TER values calculated in the screening assessment are below the Annex VI trigger of concern of 10 for acute and 5 for long-term exposure. Tier 1 uses more realistic exposure estimates along with a more representative 'generic focal species'.

A further refined risk assessment is required if the TER values calculated in Tier 1 do not reach the Annex VI TER triggers of concern as given above.

### Risk assessment to birds through drinking water

There are two scenarios provided in the EFSA (2009) Guidance Document on Risk Assessment for Birds and Mammals, for assessing the risk from drinking water.

### Leaf scenario

A leaf scenario should be considered for leaf vegetables (forming heads or with a morphology that facilitates collection of rain/irrigation) at principal growth stage 4 (BBCH 41) until harvest (BBCH 49).

Leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation are not considered in this submission. Therefore, a risk assessment for the leaf scenario is not necessary.

### Puddle scenario

This scenario is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or annual weeds. This is therefore relevant for all uses of MON 52276 and should therefore be assessed.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 ($K_{oc}$ < 500 L/kg) *or* 3000 ($K_{oc}$ ≥ 500 L/kg), as specified in the EFSA (2009) Guidance Document. For glyphosate acid and its metabolite AMPA, no specific TER calculation is required, as the ratio of effective application rate (max. single application rate 2.16 kg a.s./ha) to the relevant endpoint (lowest relevant endpoint is a NOEL of 201 mg/kg bw/d for reproductive toxicity of glyphosate acid on bobwhite quail) is 10.7.

## IIIA 10.1.1 Acute toxicity exposure ratio ($TER_A$) for birds

### Screening step

DDDs for the critical use pattern for each crop group are calculated in Table 10.1.1-1.

**Table 10.1.1-1: Screening step – Estimates of acute exposure to glyphosate acid**

| Compound | Crop group | Indicator species | App. Rate [kg a.s./ha] | $SV_{90}$ [mg a.s./kg bw/d] | $DDD_A$ [mg a.s./kg bw/d] |
|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds | Small granivorous bird | 2.16 | 25.3 | 54.6 |
| | Cereals | Small omnivorous bird | 2.16 | 158.8 | 343.0 |
| | Oilseed rape | Small omnivorous bird | 2.16 | 158.8 | 343.0 |
| | Orchards | Small insectivorous bird | 0.96 | 46.8 | 44.9 |
| | Vines | Small herbivorous bird | 0.96 | 95.3 | 91.5 |

The resulting $TER_A$ values are given in Table 10.1.1-2.

**Table 10.1.1-2: Screening step – Acute risk ($TER_A$) to birds from glyphosate acid**

| Compound | Crop group | Indicator Species | $LD_{50}$ [mg a.s./kg bw] | $DDD_A$ [mg a.s./kg bw/d] | $TER_A$ | Trigger |
|---|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds | Small granivorous bird | >4334 | 54.6 | **>79.3** | 10 |
| | Cereals | Small omnivorous bird | | 343.0 | **>12.6** | |
| | Oilseed rape | Small omnivorous bird | | 343.0 | **>12.6** | |
| | Orchards | Small insectivorous bird | | 44.9 | **>96.5** | |
| | Vines | Small herbivorous bird | | 91.5 | **>47.4** | |

**TER values in bold exceed the Annex VI trigger value of 10**

The $TER_A$ values for all application scenarios of MON 52276 are greater than the relevant trigger of 10, indicating low acute risk to birds from glyphosate acid, also covering the risk for glyphosate salts. For all limit tests on glyphosate salts, no effects were observed at the highest concentration tested (Table 10.1-1).

## IIIA 10.1.2 Short and long-term toxicity exposure ratio ($TER_{ST}$ and $TER_{LT}$)

Derivation of the short-term toxicity exposure ratio is no longer a requirement according to EFSA (2009) Guidance Document. Thus, no short-term risk assessment is presented. However, $LC_{50}$ values for glyphosate acid, glyphosate salts and MON 52276 summarized in Table 10.1-2 demonstrate low short-term toxicity with all $LC_{50}$ values >4640 mg/kg feed. For the short-term studies, $LC_{50}$ values >4640 mg/kg feed represents a no-mortality concentration.

### Long-term toxicity exposure ratio ($TER_{LT}$)

### Screening step

Long-term DDDs for the critical use pattern for each crop group are calculated in Table 10.1.2-1.

**Table 10.1.2-1: Screening step – Estimates of long term exposure to glyphosate acid**

| Compound | Crop group | Indicator species | App. Rate [kg a.s./ha] | $SV_m$ [mg a.s./kg bw/d] | $f_{twA}$ | $DDD_{LT}$ [mg a.s./kg bw/d] |
|---|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds | Small granivorous bird | 1.6 | 11.4 | 0.53 | 13.1 |
| | Cereals | Small omnivorous bird | 2.16 | 64.8 | 0.53 | 74.2 |
| | Oilseed rape | Small omnivorous bird | 2.16 | 64.8 | 0.53 | 74.2 |
| | Orchards | Small insectivorous bird | 0.96 | 18.2 | 0.53 | 9.3 |
| | Vines | Small herbivorous bird | 0.96 | 38.9 | 0.53 | 19.8 |

The resulting $TER_{LT}$ values are given in Table 10.1.2-2.

**Table 10.1.2-2: Screening step – Long term risk ($TER_{LT}$) to birds from glyphosate acid**

| Compound | Crop group | Indicator Species | NOEL [mg a.s./kg bw/d] | $DDD_{LT}$ [mg a.s./kg bw/d] | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds | Small granivorous bird | 201 | 13.1 | **15.4** | 5 |
| | Cereals | Small omnivorous bird | | 74.2 | 2.7 | |
| | Oilseed rape | Small omnivorous bird | | 74.2 | 2.7 | |
| | Orchards | Small insectivorous bird | | 9.3 | **21.7** | |
| | Vines | Small herbivorous bird | | 19.8 | **10.2** | |

**TER values in bold exceed the relevant trigger value of 5**

The $TER_{LT}$ values for application of MON 52276 on annual weeds in arable fields, i.e. pre-planting or pre-emergence of crops and for application in orchards are greater than the relevant trigger of 5, indicating a low long- term reproduction risk to birds from glyphosate acid.

For birds exposed to glyphosate acid in cereals and oilseed rape, the $TER_{LT}$ values are below the relevant trigger value. Further mitigation steps are required and are described below.

### Tier 1 Avian Risk Assessment

For mature ‘cereals', the EFSA Guidance Document defines the passerine (small insectivorous bird),

lark (small omnivorous bird) and bunting (small granivorous/insectivorous bird) as generic focal species.

For generic focal species in mature oilseed rape, dunnock (small insectivorous bird) and finch (small granivorous bird) are defined as generic focal species.

All generic focal species are considered in the following Tier 1 risk assessment. DDDs for the critical use pattern for each crop group are calculated in Table 10.1.2-3. For the purpose of conciseness, only worst case BBCH scenarios are presented.

**Table 10.1.2-3: Tier 1 – Estimates of long term exposure to glyphosate acid**

| Compound | Crop group | App. Timing (BBCH stages) | Generic focal species | App. rate [g a.s./ha] | $SV_m$ [mg a.s./kg bw/d] | $f_{TWA}$ | $DDD_{LT}$ [mg a.s./kg bw/d] |
|---|---|---|---|---|---|---|---|
| Glyphosate acid | Cereals | 71-89 | Small insectivorous bird "passerine" | 2.16 | 22.4 | 0.53 | 25.6 |
| | | ≥40 | Small omnivorous bird "lark" | 2.16 | 3.3 | 0.53 | 3.8 |
| | | Late season – seed heads | Small granivorous/ insectivorous bird "bunting" | 2.16 | 12.5 | 0.53 | 14.3 |
| | Oilseed rape | late – late (with seeds) 30-99 | Small insectivorous bird "dunnock" | 2.16 | 2.7 | 0.53 | 3.1 |
| | | late (with seeds) 80-99 | Small granivorous bird" finch" | 2.16 | 11.4 | 0.53 | 13.1 |
| | | ≥40 | Small omnivorous bird "lark" | 2.16 | 2.7 | 0.53 | 3.1 |
| | | ≥40 | Medium herbivorous/ granivorous bird "pigeon" | 2.16 | 0.9 | 0.53 | 1.0 |

The resulting Tier 1 $TER_{LT}$ values are given in Table 10.1.2-4.

**Table 10.1.2-4: Tier 1 – long term risk ($TER_{LT}$) to birds from glyphosate acid**

| Compound | Crop group | App. Timing (BBCH stages) | Generic focal species | NOEL [mg a.s./kg bw/d] | $DDD_{LT}$ [mg a.s./kg bw/d] | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|---|
| Glyphosate acid | Cereals | 71-89 | Small insectivorous bird "passerine" | 201 | 25.6 | **7.8** | 5 |
| | | ≥40 | Small omnivorous bird "lark" | | 3.8 | **53.2** | |
| | | Late season – seed heads | Small granivorous/ insectivorous bird "bunting" | | 14.3 | **14.0** | |
| | Oilseed rape | late – late (with seeds) 30-99 | Small insectivorous bird "dunnock" | | 3.1 | **65.0** | |
| | | late (with seeds) 80-99 | Small granivorous bird" finch" | | 13.1 | **15.4** | |
| | | ≥40 | Small omnivorous bird "lark" | | 3.1 | **65.0** | |
| | | ≥40 | Medium herbivorous/ granivorous bird "pigeon" | | 1.0 | **195** | |

**TER values in bold exceed the relevant trigger value of 5**

All $TER_{LT}$ values are greater than the Annex VI trigger of 5. Thus, no unacceptable long-term effects are to be expected from application of MON 52276 according to the proposed representative GAP.

## IIIA 10.1.3 In the case of baits, the concentration of active substance in the bait in mg/kg

MON 52276 is intended for use as a foliar spray, and therefore this information is not required.

## IIIA 10.1.4 In the case of pellets, granules, prills or treated seed

MON 52276 is intended for use as a foliar spray, and therefore this information is not required.

## IIIA 10.1.4.1 Amount of the active substance in or on each pellet, granule, prill or treated seed

MON 52276 is intended for use as a foliar spray, and therefore this information is not required.

## IIIA 10.1.4.2 Proportion of the $LD_{50}$ for the active substance in 100 particles and per gram of particles

MON 52276 is intended for use as a foliar spray, and therefore this information is not required.

## IIIA 10.1.5 In the case of pellets, granules and prills, their size and shape

MON 52276 is intended for use as a foliar spray, and therefore this information is not required.

### IIIA 10.1.6 Acute oral toxicity of the preparation to the more sensitive of the species identified in tests with the active substance

Avian toxicity tests with the formulation were not performed, since it is possible to extrapolate from data obtained with the active substance.

### IIIA 10.1.7 Supervised cage or field trials

The risk assessment above demonstrated that the proposed use of MON 52276 poses no unacceptable risk to birds, and therefore further studies are not considered necessary.

### IIIA 10.1.8 Acceptance of bait, granules or treated seeds by birds (palatability test)

The information concerned is not relevant since MON 52276 is intended for use as a foliar spray.

### IIIA 10.1.9 Effects of secondary poisoning

Glyphosate acid has a log $P_{OW}$ value of <-3.2 and its metabolite AMPA has a log $P_{OW}$ of -5.18. It was therefore not necessary to consider the risk from secondary poisoning further. Therefore, based on the low log $P_{OW}$ values the risk from bioaccumulation to fish-eating and worm-eating birds is negligible.

## IIIA 10.2 Effects on aquatic organisms

The toxicity of glyphosate as the acid, the isopropylamine (IPA) salt, the potassium (K)-salt and its metabolites AMPA and HMPA to aquatic organisms was investigated in a series of laboratory studies with representative species from different trophic levels of the aquatic food chain, namely fish, aquatic invertebrates, algae and aquatic plants. As algae and aquatic plants are considered most sensitive to the metabolites of glyphosate; AMPA and HMPA, for animal welfare reasons no tests on aquatic vertebrates (fish) were conducted on HMPA.

A summary of the relevant acute and long-term endpoints as listed in Annex II and the previous submission is provided below. Full details of the tests on the active substance and the metabolites AMPA and HMPA are provided in the current EU Annex II summary documentation, Section IIA 8.2 through IIA 8.6. Therefore, only studies representing the worst case for key species are presented in the tables below.

**Table 10.2-1: Acute toxicity of glyphosate acid, glyphosate IPA-salt, K-salt, AMPA and formulation MON 52276 to fish**

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment[2] | Reference/GLP |
|---|---|---|---|---|---|
| Glyphosate acid | *Oncorhynchus mykiss* | 96 h | $LC_{50}$ = 38 mg/L | $LC_{50}$ = 38 mg/L | 95-00016 [redacted] 1972 [1]/no |
| | *Lepomis macrochirus* | 96 h static | $LC_{50}$ = 47 mg/L | $LC_{50}$ = 47 mg/L | IIA 8.2.1/02 [redacted]5553/B [redacted] 1995/yes |
| | *Danio rerio* | 96 h semi static | $LC_{50}$ = 122.91 mg/L | $LC_{50}$ = 122.91 mg/L | IIA 8.2.1/03 [redacted]61.47/99 [redacted] 2000/yes |
| | *Cyprinus carpio* | 96 h semi static | $LC_{50}$ = 100 mg/L | $LC_{50}$ = 100 mg/L | IIA 8.2.1/04 2060/015 [redacted] 2006/yes |
| Glyphosate-IPA salt | *Oncorhynchus mykiss* | 96 h | $LC_{50}$ >1000 mg/L | $LC_{50}$ >1000 mg/L | 94-01161 [redacted] 1981/no |
| | *Leuciscus idus* | 96 h static | - | $LC_{50}$ >5000 mg/L | 80-91-2328-02-93 [redacted]1993/yes |
| | *Lepomis macrochirus* | 96 h | $LC_{50}$ >1000 mg/L | $LC_{50}$ >1000 mg/L | 95-00712 [redacted] 1981/no |
| Glyphosate K-salt | *Oncorhynchus mykiss* | 96 h static | - | >2573 mg/L 1227 mg a.s./L | IIA 8.2.1/07 [redacted]2002-149 [redacted], 2003/yes |

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment[2] | Reference/GLP |
|---|---|---|---|---|---|
| MON 52276 | *Oncorhynchus mykiss* | 96 h flow-through | - | >989 mg/L<br>>306 mg a.s./L | IIIA 10.2.2.1/01<br>[redacted]91-296<br>[redacted]<br>1992/yes |
| | *Cyprinus carpio* | 96 h flow-through | - | >895 mg/L<br>>277 mg a.s./L | IIIA 10.2.2.1/02<br>[redacted]91-298<br>[redacted]<br>1992/yes |
| AMPA | *Oncorhynchus mykiss* | 96 h static | - | >100 mg/L | IIA 8.2.1/05<br>232469<br>[redacted]<br>1998/yes |

**Studies shaded in grey have been reviewed as part of the 2001 EU evaluation.**
[1] Non-GLP study, considered not appropriate according to current scientific/regulatory standards
[2] Since Annex I inclusion new studies on the active substance have been performed and as a result there are new endpoints which are used in the risk assessment.

**Table 10.2-2: Chronic toxicity of glyphosate acid, glyphosate IPA salt and AMPA to fish**

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment[2] | Reference/GLP |
|---|---|---|---|---|---|
| Glyphosate acid | *Danio rerio* | 168 h Short term sac-fry exposure semi-static | - | NOEC = 3.2 mg/L | IIA 8.2.3/01<br>[redacted]61.16/99<br>[redacted]<br>2000/GLP |
| | *Oncorhynchus mykiss* | 35 d ELS flow-through | - | NOEC = 9.63 mg/L | IIA 8.2.4./01<br>1005.029.321<br>[redacted]<br>2010/yes |
| | *Pimephales promelas*[1] | 253 d FELS flow-through | NOEC = 25.7 mg/L | - | [redacted]75-129<br>[redacted], 1975/no |
| | *Lepomis macrochirus* | 56 d bio-concentration flow-through | - | BCF = 1.1 ± 0.61 steady state after 120 ± 59 d | [redacted]36440 (Part 1)<br>[redacted]<br>1989/yes<br>[redacted]9303 (Part 2)<br>[redacted]<br>1989/yes |
| Glyphosate-IPA salt | *Oncorhynchus mykiss* | 21 d semi-static | NOEC = 917 mg/L | NOEC = 235.31 mg IPA salt/L<br>NOEC = 174.36 mg a.e./L | 80-91-2328-04-93<br>[redacted]1993/yes |
| AMPA | *Pimephales promelas*[1] | 33 d ELS flow-through | | NOEC = 12.0 mg/L | IIA 8.2.4/02<br>[redacted]2010-328<br>[redacted]<br>*al.*, 2011/yes |

**Studies shaded in grey have been reviewed as part of the 2001 EU evaluation.**
[1] Limit test, highest concentration tested.
[2] Since Annex I inclusion new studies on the active substance have been performed and as a result there are new endpoints which are used in the risk assessment.

For chronic toxicity of fish exposed to glyphosate-IPA salt, in SANCO/6511/VI/99-final, a NOEC of 917 mg/L was proposed. However, at a concentration level of 917 mg test item/L, effects on behaviour of fish (all fish were mainly at the bottom of the test vessels) were observed. Therefore, the NOEC was re-determined to be 382 mg test item/L, equivalent to 235.31 mg glyphosate isopropylamine salt/L (nominal) or 174.36 mg glyphosate/L (nominal).

**Table 10.2-3: Acute toxicity of glyphosate acid, glyphosate IPA salt, K-salt, the metabolites AMPA, HMPA and formulation MON 52276 to aquatic invertebrates**

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/ VI/99-final) | Endpoints used in risk assessment[2] | Reference/GLP |
|---|---|---|---|---|---|
| **Glyphosate acid** | *Daphnia magna* | 48 h | $EC_{50}$ = 40 mg/L | $EC_{50}$ = 40 mg/L | 95-00537 [redacted] *et al.*, 1995[1]/yes |
| **Glyphosate-IPA salt** | *Daphnia magna* | 48 h | $EC_{50}$ = 930 mg/L | $EC_{50}$ = 930 mg/L | 91-01160 [redacted] 1981 |
| **Glyphosate K-salt** | *Daphnia magna* | 48 h static | - | $EC_{50}$ >2582 mg/L | 8.3.1.1/07 WL-2002-150 [redacted] *et al.*, 2003/yes |
| **MON 52276** | *Daphnia magna* | 48 h static | $EC_{50}$ = 676 mg/L<br>$EC_{50}$ = 209 mg a.s./L | | IIIA 10.2.2.2/01 [redacted]-91-295 [redacted] 1992/yes |
| **AMPA** | *Daphnia magna* | 48 h static | | $EC_{50}$ = 690 mg/L | [redacted]90-401 [redacted] 1991/yes |
| **HMPA** | *Daphnia magna* | 48 h static | - | $EC_{50}$ >100 mg/L | IIA 8.3.1.1/09 [redacted]2010-329 [redacted] *et al.*, 2011/yes |

**Studies shaded in grey have been reviewed as part of the 2002 EU evaluation.**

[1] Non-GLP study, considered invalid according to current scientific/regulatory standards.

[2] Since Annex I inclusion new studies on the active substance have been performed and as a result there are new endpoints which are used in the risk assessment.

**Table 10.2-4: Chronic toxicity of glyphosate acid, glyphosate IPA salt, and its metabolite AMPA to aquatic invertebrates**

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/ VI/99-final) | Endpoints used in risk assessment[2] | Reference/GLP |
|---|---|---|---|---|---|
| Glyphosate acid | *Daphnia magna* | 21 d semi-static | NOEC = 30 mg/L | NOEC = 30 mg/L | 250795 [redacted] 1990 [1]/yes |
| Glyphosate-IPA salt | *Daphnia magna* | 21 d semi-static | [3]NOEC = 455 mg/L | NOEC = 42.9 mg/L | 89-91-2328-05-93 [redacted] 1993/yes |
| AMPA | *Daphnia magna* | 21 d semi-static | | NOEC = 15 mg/L | IIA 8.3.2.1/02 [redacted] 2010-327 [redacted] *et al.* 2011/yes |

**Studies shaded in grey have been reviewed as part of the 2001 EU evaluation.**

[1] Non-GLP study, considered not appropriate according to current scientific/regulatory standards

[2] Since Annex I inclusion new studies on the active substance have been performed and as a result there are new endpoints which are used in the risk assessment.

[3] For chronic toxicity to Daphnids exposed to glyphosate-IPA salt, in SANCO/6511/VI/99-final, a NOEC of 455 mg/L was proposed. However, there was a significant reduction in reproduction observed in the 200 and 455 mg test item/L treatments. The NOEC value for reproduction was re-determined to be 94 mg test item/L, equivalent to 57.90 mg glyphosate isopropylamine salt/L and 42.90 mg glyphosate acid/L (nominal) (see Thun, 1993).

**Table 10.2-5: Toxicity of glyphosate acid, glyphosate IPA salt, glyphosate K-salt, the metabolites AMPA and HMPA and the formulation MON 52276 to algae**

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment[2] | Reference /GLP |
|---|---|---|---|---|---|
| Glyphosate acid | *Pseudokirchneriella subcapitata* | 72 h static | | $E_rC_{50}$ = 54 mg/L<br>$E_bC_{50}$ = 48 mg/L | 141896 [redacted] 1995/yes |
| | *Desmodesmus subspicatus* | 72 h static | | $E_bC_{50}$ (72 h) = 46 mg/L | 95-00535 [redacted] *et al.*, 1995/yes |
| | *Anabaena flos-aquae* | 120 h static | | $E_rC_{50}$ (72 h) = 22 mg/L<br>$E_bC_{50}$ (72 h) = 8.5 mg/L<br>$E_rC_{50}$ (120 h) = 38 mg/L<br>$E_bC_{50}$ (120 h) = 15 mg/L | IIA 8.4/03 [redacted] *et al.*, 1996/yes |
| | *Nitzschia palea* | 96 h static | | $E_rC_{50}$ = 11.90 mg/L<br>$E_bC_{50}$ = 4.47 mg/L | 960606 [redacted] 1996/yes |
| | *Skeletonema costatum* | 168 h static | $E_bC_{50}$ (168 h) = 0.64 mg/L[1] | - | IIA 8.4/05 1092-02-1100-3 [redacted] 1987c/no |
| | | 120 h static | | $E_rC_{50}$ (72 h) = 18 mg/L<br>$E_bC_{50}$ (72 h) =11 mg/L<br>$E_rC_{50}$ (120 h) = 24 mg/L<br>$E_bC_{50}$ (120 h) =12 mg/L | IIA 8.4/04 [redacted] et al., 1996/yes |
| | *Navicula pelliculosa* | 120 h static | | $E_rC_{50}$ (72 h) = 17 mg/L<br>$E_bC_{50}$ (72 h) =16 mg/L<br>$E_rC_{50}$ (120 h) = 17 mg/L<br>$E_bC_{50}$ (120 h) =17 mg/L | IIA 8.4/06 [redacted] *et al.*, 1996/yes |

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment[2] | Reference /GLP |
|---|---|---|---|---|---|
| Glyphosate -IPA salt | *Pseudokirchneriella subcapitata* | 96 h static | | $E_rC_{50}$ =45.68 mg/L<br>$E_bC_{50}$ = 14.69 mg/L | IIA 8.4/07<br>[redacted]99-02-03<br>[redacted]<br>2002/yes |
| | *Desmodesmus subspicatus* | 72 h static | | $E_rC_{50}$ =241 mg/L<br>$E_bC_{50}$ = 41.1 mg/L | 80-91-2328-01-93<br>[redacted]993/yes |
| Glyphosate K-salt | *Pseudokirchneriella subcapitata* | 72 h static | | $E_rC_{50}$ = 114 mg/L<br>$E_bC_{50}$ = 69 mg/L<br>$E_yC_{50}$ = 74 mg/L | IIA 8.4/09<br>[redacted]02-148<br>[redacted] *et al.*, 2003/yes |
| MON 52276 | *Pseudokirchneriella subcapitata* | 72 h static | | $E_rC_{50}$ = 284 mg/L<br>$E_rC_{50}$ = 87 mg a.s./L<br>$E_bC_{50}$ = 178 mg/L<br>$E_bC_{50}$ = 55 mg a.s./L | IIA 10.2.2.3/01<br>[redacted]91-389<br>[redacted]<br>1991/yes |
| AMPA | *Pseudokirchneriella subcapitata* | 72 h static | | $E_rC_{50}$ = 200 mg/L<br>$E_bC_{50}$ = 110 mg/L | IIA 8.4/10<br>232458<br>[redacted]<br>1998/yes |
| | *Desmodesmus subspicatus* | 72 h static | | $E_rC_{50}$ = 452 mg/L<br>$E_bC_{50}$ = 89.8 mg/L | [redacted]93006/01-<br>[redacted]<br>1994/yes |
| HMPA | *Pseudokirchneriella subcapitata* | 72 h static | | $E_rC_{50}$ (72 h) >115 mg/L<br>$E_yC_{50}$ (72 h) >115 mg/L | IIA 8.4/11<br>139[redacted]396[redacted]<br>[redacted] *et al.*<br>2011/yes |

**Studies shaded in grey have been reviewed as part of the 2001 EU evaluation.**

[1] See Appendix 2 of the Annex II document for a comprehensive critical review of this study and the summary and conclusions why the [redacted] study (study number [redacted]88-414) is not considered appropriate and acceptable for risk assessment.

**Table 10.2-6: Toxicity of glyphosate acid, glyphosate IPA salt, the metabolites AMPA and HMPA and the formulation MON 52276 to aquatic macrophytes.**

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment | Reference /GLP |
|---|---|---|---|---|---|
| Glyphosate acid | *Lemna gibba* | 14 d semi-static | $EC_{50,\ frond\ count}$ = 12 mg a.s./L | **$EC_{50,\ frond\ count}$ = 12 mg a.s./L** | AII 8.6/01<br>[redacted]5662/[redacted]<br>[redacted] *et al.*, 1996/yes |
| | *Myriophyllum aquaticum* | 14 day exposure + 7 day recovery[1] | - | $EC_{50,\ fresh\ weight,\ relative\ increase}$ = **12.3** mg a.s./L | AII 8.6/04<br>[redacted]015/4-80/[redacted]<br>[redacted]<br>2012/yes |

| Test substance | Species | Test design | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment | Reference /GLP |
|---|---|---|---|---|---|
| Glyphosate-IPA salt | *Lemna minor* | 14 d static | $EC_{50,\ frond\ count}$ = 53.56 mg a.s./L | - | AII 8.6/02 980909 [redacted] 1999/yes |
| | *Lemna minor* | 7 d semi-static | - | **$EC_{50,\ frond\ count}$ = 25.5 mg a.s./L** | IIA 8.6/03 [redacted] 1873 2002/yes |
| MON 52276 | *Lemna gibba* | 7 d semi-static | - | $EC_{50,\ frond\ count}$ = 20.57 mg a.s./L | AIII 10.8.2.1/01 [redacted] 2002-051 2002/yes |
| | *Myriophyllum aquaticum* | 14 day exposure + 7 day recovery[1] | - | **$EC_{50,\ fresh\ weight}$ = 4.44 mg a.s./L** | AIII 10.8.2.1/02 [redacted]-016/4-80/A [redacted] 2012/yes |
| AMPA | *Myriophyllum aquaticum* | 14 day exposure + 7 day recovery[1] | - | $EC_{50,\ fresh\ weight}$ =70.8 mg/L | AII 8.6/05 [redacted]-022/4-80/A [redacted], 2012/yes |
| HMPA | *Lemna gibba* | 7 d semi-static | - | $EC_{50,\ frond\ count}$ >123 mg/L<br>$EC_{50,\ dry\ weight}$ >123 mg/L | AII 8.6/06 139A-397 [redacted] *et al.* 2011/yes |

[1] $EC_{50}$ values from 14-day exposure phase and not considering the 7 day recovery.



## Summary

MON 52276 is the representative formulation in the approval renewal of glyphosate. Therefore, all risk assessments and supporting data for MON 52276 with the proposed use patterns are provided within this document. $PEC_{sw}$ are provided in MON 52276 Point IIIA 9.7, where further detail on $PEC_{sw}$ calculations are presented.

Table 10.2-7 summarizes the TERs for all the relevant aquatic organisms.

**Table 10.2-7: Summary of the risk assessment for Glyphosate acid and its metabolites AMPA and HMPA exposure to aquatic species (most sensitive species of each group)**

| Use pattern | Organism | Toxicity endpoint | FOCUS step | Max $PEC_{sw}$ [µg/L] | TER | Risk assessment trigger |
|---|---|---|---|---|---|---|
| **Glyphosate acid** | | | | | | |
| 1 × 4320 g a.s./ha | Rainbow trout *(Oncorhynchus mykiss)* | 96 hr $LC_{50}$ = 38000 µg a.s./L | 1 | 101.233 | 375 | 100 |

| Use pattern | Organism | Toxicity endpoint | FOCUS step | Max $PEC_{SW}$ [µg/L] | TER | Risk assessment trigger |
|---|---|---|---|---|---|---|
| 1 × 4320 g a.s./ha | Fathead minnow *(Pimephales promelas)* | 255 d FFLC NOEC = 25700 µg a.s./L | 1 | 101.233 | 254 | 10 |
| 1 × 4320 g a.s./ha | Rainbow trout *(Oncorhynchus mykiss)* | 85 d ELS NOEC = 9630 µg a.s./L | 1 | 101.233 | 95.1 | 10 |
| 1 × 4320 g a.s./ha | Zebra fish (*Danio rerio)* | 168 hr NOEC = 3200 µg a.s./L | 1 | 101.233 | 31.6 | 10 |
| 1 × 4320 g a.s./ha | *Daphnia magna* | 48 hr $EC_{50}$ = 40000 µg a.s./L | 1 | 101.233 | 395 | 100 |
| 1 × 4320 g a.s./ha | *Daphnia magna* | 21 d NOEC = 30000 µg a.s./L | 1 | 101.233 | 296 | 10 |
| 1 × 4320 g a.s./ha | *Nitzschia palea* | 96 hr $E_bC_{50}$ = 4470 µg a.s./L | 1 | 101.233 | 44.2 | 10 |
| 1 × 4320 g a.s./ha | *Skeletonema costatum* | 72 hr $E_bC_{50}$ = 11000 µg a.s./L | 1 | 101.233 | 108.7 | 10 |
| 1 × 4320 g a.s./ha | Common duckweed (*Lemna gibba*) | 14 d $EC_{50, frond count}$ = 12000 µg a.s./L | 1 | 101.233 | 119 | 10 |
| 1 × 4320 g a.s./ha | *Myriophyllum aquaticum* | 14 d $EC_{50}$ = 12300 µg a.s./L | 1 | 101.233 | 122 | 10 |
| **MON 52276** | | | | | | |
| 1 × 4320 g a.s./ha | Common carp *(Cyprinus carpio)* | 96 hr $LC_{50}$ > 277000 µg a.s./L | 1 | 101.233 | > 2736 | 100 |
| 1 × 4320 g a.s./ha | *Daphnia magna* | 48 hr $EC_{50}$ = 209000 µg a.s./L | 1 | 101.233 | 2065 | 100 |
| 1 × 4320 g a.s./ha | *Pseudokirchneriella subcapitata* | 72 hr $E_bC_{50}$ = 55000 µg a.s./L | 1 | 101.233 | 543 | 10 |
| 1 × 4320 g a.s./ha | Common duckweed (*Lemna gibba*) | 7 d $EC_{50, frond count}$ = 20570 µg a.s./L | 1 | 101.233 | 203 | 10 |
| 1 × 4320 g a.s./ha | *Myriophyllum aquaticum* | 14 d $EC_{50}$ = 4440 µg a.s./L | 1 | 101.233 | 43.9 | 10 |
| **AMPA** | | | | | | |
| 1 × 4320 g a.s./ha | Rainbow trout *(Oncorhynchus mykiss)* | 96 hr $LC_{50}$ >100000 µg a.s./L | 1 | 40.978 | >2440 | 100 |
| 1 × 4320 g a.s./ha | Fathead minnow *(Pimephales promelas)* | 28 d ELS NOEC = 12000 µg a.s./L | 1 | 40.978 | 293 | 10 |
| 1 × 4320 g a.s./ha | *Daphnia magna* | 48 hr $EC_{50}$ = 690000 µg a.s./L | 1 | 40.978 | 16839 | 100 |
| 1 × 4320 g a.s./ha | *Daphnia magna* | 21 d NOEC = 15000 µg a.s./L | 1 | 40.978 | 366 | 10 |
| 1 × 4320 g a.s./ha | *Desmodesmus subspicatus* | 72 hr $E_bC_{50}$ = 89800 µg a.s./L | 1 | 40.978 | 2191 | 10 |
| 1 × 4320 g a.s./ha | *Myriophyllum aquaticum* | 14 d $EC_{50}$ = 70800 µg/L | 1 | 40.978 | 1728 | 10 |
| **HMPA** | | | | | | |
| 1 × 4320 g a.s./ha | *Daphnia magna* | 48 hr $EC_{50}$ > 100000 µg a.s./L | 1 | 6.710 | >14903 | 100 |

| Use pattern | Organism | Toxicity endpoint | FOCUS step | Max $PEC_{SW}$ [µg/L] | TER | Risk assessment trigger |
|---|---|---|---|---|---|---|
| 1 × 4320 g a.s./ha | *Pseudokirchneriella subcapitata* | 72 hr $E_rC_{50}$ > 115000 µg a.s./L | 1 | 6.710 | >17139 | 10 |
| 1 × 4320 g a.s./ha | Common duckweed (*Lemna gibba*) | 7 d $EC_{50,\ frond\ count}$ > 123000 µg/L | 1 | 6.710 | >18331 | 10 |

The TER values calculated using worst-case $PEC_{SW}$ values (FOCUS Step 1) for glyphosate acid and its metabolites AMPA and HMPA (see Section 5 for full calculations) exceed the Annex VI trigger values, indicating that the risk to aquatic organisms is acceptable following use of MON 52276.

## Risk assessment

## Toxicity

The toxicity of the formulation MON 52276, glyphosate (as the acid, the isopropylamine (IPA) salt and the potassium (K)-salt) and its metabolites AMPA and HMPA to aquatic organisms was investigated in a series of laboratory studies with representative species from different trophic levels of the aquatic food chain, namely fish, aquatic invertebrates, algae and aquatic plants. Full summaries of these studies are provided under the relevant Annex Point IIIA 10.2.2.1 through 10.2.2.3 for fish, Daphnids and algae and under Annex Point IIIA 10.8.2.2 for aquatic macrophytes. The results from the tests using the formulation indicate that the toxicity of the formulation reflects the toxicity of the active ingredient (see Table 10.2-1 through Table 10.2-6).

Furthermore, in additional studies performed with glyphosate-IPA salt and glyphosate-K salt, the results indicate that toxicity from the salts of glyphosate is either substantially lower or at least in the same order of magnitude as for glyphosate acid.

### Metabolites of glyphosate acid

Aquatic organisms may be exposed to the major metabolites of glyphosate; AMPA and HMPA (see Point IIIA 9.7 and the Monograph on glyphosate, Vol. 3, B9, 2000), therefore, the risk to aquatic organisms from exposure to these metabolites also needs to be assessed. Toxicity data for these two major metabolites of glyphosate demonstrate that the toxicity to *Daphnia magna*, algae and also to aquatic plants (Table 10.2-7) is significantly less or at least at the same order of magnitude than the parent material. As algae and aquatic plants are considered most sensitive to the metabolites of glyphosate; AMPA and HMPA, for animal welfare reasons no tests on aquatic vertebrates (fish) were conducted on HMPA.

### Aquatic application conditions, exposure scenario, and risk assessment assumptions

The representative joint GAP does not include direct applications to surface water. The main routes of exposure to aquatic organisms considered in this aquatic risk assessment are via spray drift, runoff and drainage where exposure to aquatic organisms could result as a consequence of the accidental entry of the compound into the environmental compartments occupied by organisms or as a consequence of run-off and drainage events.

Further details of how the predicted environmental concentrations for glyphosate acid in surface water ($PEC_{sw}$), arising as a consequence of over-spraying, drift, drainage and run-off, are calculated, are provided in MON 52276 Point IIIA 9.7. Good Agricultural Practices are summarised in Table 10-1.

$PEC_{sw}$ values for glyphosate acid and the major metabolites were calculated using the FOCUS (2000) surface water models. PEC calculations are based on the maximum application rates of 1 × 4.32 and 2 × 2.16 kg glyphosate acid/ha that are proposed for uses in Europe.

**FOCUS Step-1:**

FOCUS Step 1 $PEC_{sw}$ values were calculated using an extreme worst-case exposure scenario. The drift rate used was the 82nd percentile at 1 m from the application site of 2.8%. The Step-1 default value of chemical loss into the water body *via* runoff/drainage is fixed at 10% of the application rate. The depth of the static water body was assumed to be 30 cm. Worst-case $PEC_{sw}$ and predicted concentrations in sediment ($PEC_{SED}$) are calculated using the FOCUS Step-1-2 calculator (ver. 1.1). The FOCUS Step-1 maximum (Day-0) $PEC_{sw}$ and $PEC_{SED}$ values for glyphosate acid were calculated to be 101.233 µg/L and 10500 µg/kg dry sediment, respectively, for worst case spring application of 2 × 2.16 kg a.s./ha, covering application rates of 1 × 4.32 kg a.s./ha and 3 applications of up to a total amount of 4.32 kg a.s./ha per annum.

FOCUS Step 1 $PEC_{SW}$ values for glyphosate acid and its metabolites are presented in Table 10.2-8.

**Table 10.2-8: FOCUS step 1 $PEC_{SW}$ values for glyphosate acid and its metabolites following application of 4.32 kg MON 52276/ha (equivalent to 12 L MON 52276/ha)**

| Test substance | FOCUS Step 1 Max $PEC_{SW}$ [µg a.s./L] | $PEC_{SED}$ [µg/kg] |
|---|---|---|
| Glyphosate acid | 101.233 | 10500 |
| AMPA | 40.978 | 3320 |
| HMPA | 6.710 | 696 |

The FOCUS Step 1 $PEC_{sw}$ values for glyphosate acid resulted in acceptable TER values for all aquatic species. The $PEC_{sw}$ for all aquatic metabolites generated TER values exceeding the Annex VI trigger values for at least a factor of 10 for all aquatic species at Step 1. Therefore, no further surface water exposure assessment was performed for these metabolites.

## IIIA 10.2.1 Toxicity exposure ratios for aquatic species

The risk assessment was conducted following the EU (2002) Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001 rev 4 (final) 17 October 2002) and considers the proposed representative uses of MON 52276 shown in Table 10-1.

The initial risk assessments were carried out by comparing the $PEC_{SW}$ values with the acute and long-term toxicity endpoints. Acute and long-term toxicity exposure ratios ($TER_A$ and $TER_{LT}$) were calculated using the following equations:

$$TER_A = \frac{EC_{50} \text{ or } LC_{50}}{\text{Maximum } PEC_{SW}}$$

$$TER_{LT} = \frac{EC_{50} \text{ or NOEC}}{\text{Maximum } PEC_{SW}}$$

### IIIA 10.2.1.1 $TER_A$ for fish

The $TER_A$ values for glyphosate acid and AMPA acute exposure to fish were calculated using 96 hour $LC_{50}$ toxicity values achieved for *Oncorhynchus mykiss* exposure to glyphosate acid and for *Cyprinus carpio* exposure to MON 52276.

The fish acute toxicity exposure ratios ($TER_A$) calculated using FOCUS Step 1 $PEC_{sw}$ values are presented in Table 10.2.1.1-1.

**Table 10.2.1.1-1: Fish acute TER values for glyphosate acid, AMPA and MON 52276 based on FOCUS Step 1 $PEC_{SW}$ values**

| Test organism | Test substance | 96 h $LC_{50}$ [µg as./L] | FOCUS Step 1 Max $PEC_{sw}$ [µg/L] | $TER_A$ | Trigger value |
|---|---|---|---|---|---|
| Rainbow trout *(Oncorhynchus mykiss)* | Glyphosate acid | 38,000 | 101.233 | 375 | 100 |
| | AMPA | >100,000 | 40.978 | >2440 | |
| Common carp (*Cyprinus carpio*) | MON 52276 | >277,000 | 101.233 | >2736 | |

The $TER_A$ values achieved for glyphosate acid, AMPA and MON 52276 (based on glyphosate acid content) all exceed the Annex VI trigger value of 100, indicating that glyphosate and its metabolite AMPA pose a low acute exposure risk to fish following application according to the uses proposed for MON 52276. HMPA is formed from AMPA in water/sediment conditions only (maximum formation rate 11% of radioactive applied glyphosate). In addition, in studies conducted with *Daphnia magna*, *Pseudokirchneriella subcapitata* and *Lemna gibba,* no adverse effects were observed at the highest concentrations tested.

## IIIA 10.2.1.2 $TER_{LT}$ for fish

The $TER_{LT}$ values for glyphosate acid chronic exposure to fish, were calculated using a 255 day NOEC value from a fish full life cycle study with *Pimephales promelas*, an 85 day NOEC value from a fish early life stage test with *Oncorhynchus mykiss* and a 168 hour NOEC value achieved in a fish short term sac fry exposure assay with *Danio rerio*.

For glyphosate's major metabolite AMPA, the $TER_{LT}$ value was calculated using a 33 day NOEC value from a fish ELS test with *Pimephales promelas*.

The fish long term toxicity exposure ratios ($TER_{LT}$) calculated using FOCUS Step 1 $PEC_{sw}$ values are presented in Table 10.2.1.2-1.

**Table 10.2.1.2-1: Fish long-term TER values for glyphosate acid and its metabolite AMPA based on FOCUS Step 1 $PEC_{SW}$ values**

| Test organism | Test substance | NOEC [µg/L] | FOCUS Step 1 Max $PEC_{sw}$ [µg/L] | $TER_{LT}$ | Trigger value |
|---|---|---|---|---|---|
| Rainbow trout *(Oncorhynchus mykiss)* | Glyphosate acid | 9630 | 101.233 | 95.1 | 10 |
| Fathead minnow *(Pimephales promelas)* | Glyphosate acid | 25,700 | 101.233 | 254 | |
| Zebra fish (*Danio rerio)* | Glyphosate acid | 3200 | 101.233 | 31.6 | |
| Fathead minnow *(Pimephales promelas)* | AMPA | 12,000 | 40.978 | 293 | |

The $TER_{LT}$ values achieved for glyphosate acid and AMPA all exceed the Annex VI trigger value of 10, indicating that glyphosate acid and AMPA pose a low chronic exposure risk to fish following application according to the uses proposed for MON 52276.

## IIIA 10.2.1.3 $TER_A$ for *Daphnia*

The $TER_A$ values based on 48 hour $EC_{50}$ (immobilisation) toxicity values achieved for *Daphnia magna* exposure to glyphosate acid, metabolites AMPA and HMPA and for MON 52276, have been calculated using FOCUS Step 1 $PEC_{sw}$ values and are presented in Table 10.1.2-3.

**Table 10.2.1.3-1: *Daphnia magna* acute TER values for glyphosate acid, its metabolites AMPA and HMPA and MON 52276 based on FOCUS Step 1 $PEC_{SW}$ values**

| Test organism | Test substance | 48 h $EC_{50}$ [µg/L] | FOCUS Step 1 Max $PEC_{sw}$ [µg/L] | $TER_A$ | Trigger value |
|---|---|---|---|---|---|
| *Daphnia magna* | Glyphosate acid | 40,000 | 101.233 | 395 | 100 |
| | MON 52276 | 209,000 | 101.233 | 2065 | |
| | AMPA | 690,000 | 40.978 | 16839 | |
| | HMPA | >100,000 | 6.710 | >14903 | |

The $TER_A$ values achieved for MON 52276 (based on glyphosate acid content), glyphosate acid, AMPA and HMPA exposure to *Daphnia magna*, all exceed the Annex VI trigger value of 100, indicating that glyphosate acid poses low acute risk to Daphnids following application according to the uses proposed for MON 52276.

## IIIA 10.2.1.4 $TER_{LT}$ for *Daphnia*

The $TER_{LT}$ values for chronic exposure of *Daphnia magna* to glyphosate acid and the metabolite AMPA were calculated using FOCUS Step 1 $PEC_{sw}$ values and are presented in Table 10.2.1.4-1.

**Table 10.2.1.4-1: *Daphnia magna* long-term TER values for glyphosate acid and its metabolite AMPA based on FOCUS Step 1 $PEC_{sw}$ values**

| Test organism | Test substance | NOEC [µg/L] | FOCUS Step 1 Max $PEC_{sw}$ [µg/L] | $TER_{LT}$ | Trigger value |
|---|---|---|---|---|---|
| *Daphnia magna* | Glyphosate acid | 30,000 | 101.233 | 296 | 10 |
| | AMPA | 15,000 | 40.963 | 366 | |

The $TER_{LT}$ values achieved for glyphosate acid and AMPA exceed the Annex VI trigger value of 10, indicating that glyphosate acid and AMPA poses low chronic exposure risk to aquatic invertebrates following application according to the uses proposed for MON 52276.

## IIIA 10.2.1.5 $TER_A$ for an aquatic insect species

As glyphosate acid is not an insecticide or insect growth regulator, $TER_A$ values for aquatic insects are not required. The risk assessments for *Daphnia magna* (Point IIIA 10.2.1.3) indicated that MON 52276 poses a low acute risk to aquatic invertebrates. In addition, this data point is not relevant since the joint representative GAP does not include direct use of MON 52276 on surface waters.

### IIIA 10.2.1.6 $TER_{LT}$ for an aquatic insect species

As glyphosate acid is not an insecticide or insect growth regulator, $TER_{LT}$ values for aquatic insects are not required. The risk assessments for *Daphnia magna* (Point IIIA 10.2.1.4) indicated that MON 52276 poses a low acute risk to aquatic invertebrates. In addition, this data point is not relevant since the joint representative GAP does not include direct use of MON 52276 on surface waters.

### IIIA 10.2.1.7 $TER_A$ for an aquatic crustacean species

$TER_A$ values for additional aquatic crustacean species are not required since the risk assessments for *Daphnia magna* (Point IIIA 10.2.1.3) indicated that MON 52276 poses a low acute risk to aquatic invertebrates.

### IIIA 10.2.1.8 $TER_{LT}$ for an aquatic crustacean species

$TER_{LT}$ values for additional aquatic crustacean species are not required since the risk assessments for *Daphnia magna* (Point IIIA 10.2.1.4) indicated that MON 52276 poses low acute risk to aquatic invertebrates.

### IIIA 10.2.1.9 $TER_A$ for an aquatic gastropod mollusc species

$TER_A$ values for aquatic gastropod molluscs are not required since the risk assessments for *Daphnia magna* (Point IIIA 10.2.1.3) indicated that MON 52276 poses a low acute risk to aquatic invertebrates. In addition, this data point is not relevant since the joint representative GAP does not include direct use of MON 52276 on surface waters.

### IIIA 10.2.1.10$TER_{LT}$ for an aquatic gastropod mollusc species

$TER_{LT}$ values for aquatic gastropod molluscs are not required since the risk assessments for *Daphnia magna* (Point IIIA 10.2.1.4) indicated that MON 52276 poses low long-term risk to aquatic invertebrates. In addition, this data point is not relevant since MON 52276 is not intended for use directly on surface waters.

### IIIA 10.2.1.11 $TER_{LT}$ for algae

$TER_{LT}$ values for algae were calculated for glyphosate acid, its metabolites AMPA and HMPA and for MON 52276, using FOCUS Step 4 PEC values and are given in Table 10.2.1.11-1.

**Table 10.2.1.11-1: Long-term TER values for alga for glyphosate acid, AMPA, HMPA and MON 52276 based on FOCUS Step 1 $PEC_{SW}$ values**

| Test organism | Test substance | $EC_{50}$ [µg/L] | FOCUS Step 1 Max $PEC_{sw}$ [µg/L] | $TER_{LT}$ | Trigger value |
|---|---|---|---|---|---|
| *Pseudokirchneriella subcapitata* | Glyphosate acid | 48,000 | 101.233 | 474 | 10 |
| *Desmodesmus subspicatus* | Glyphosate acid | 46,000 | 101.233 | 454 | |
| *Anabaena flos-aquae* | Glyphosate acid | 8,500 | 101.233 | 84.0 | |
| *Nitzschia palea* | Glyphosate acid | 4,470 | 101.233 | 44.2 | |
| *Skeletonema costatum* | Glyphosate acid | 11,000 | 101.233 | 109 | |
| *Navicula pelliculosa* | Glyphosate acid | 16,000 | 101.233 | 158 | |
| *Pseudokirchneriella subcapitata* | MON 52276 | 55,000 | 101.233 | 543 | |
| *Pseudokirchneriella subcapitata* | AMPA | 110,000 | 40.978 | 2684 | |
| *Desmodesmus subspicatus* | AMPA | 89,800 | 40.978 | 2191 | |
| *Pseudokirchneriella subcapitata* | HMPA | >115,000 | 6.710 | >17139 | |

The $TER_{LT}$ values achieved for algae exposure to MON 52276 (based on glyphosate acid content) glyphosate acid, AMPA and HMPA all exceed the Annex VI trigger value of 10, indicating that glyphosate acid, AMPA and HMPA pose a low chronic exposure risk to freshwater algae following application according to the uses proposed for MON 52276.

## IIIA 10.2.2 Acute toxicity of the formulation

The acute $TER_A$ values achieved for glyphosate acid and metabolites AMPA and HMPA and MON 52276, and the chronic $TER_A$ values achieved for glyphosate acid and metabolites AMPA and HMPA, are all greater than the Annex VI trigger values of 100 (acute) and 10 (chronic) for fish, *Daphnia magna*, algae, *Lemna* ssp. and *Myriophyllum aquaticum* (see IIA 10.8.2). These TER values relate to established risks using worst-case assumptions.

The results of the studies conducted with aquatic plants on the active substance clearly demonstrated that algae and aquatic macrophytes were the most sensitive species. Subsequent testing on the formulation with fish, Daphnids, algae, *Lemna ssp.* and *Myriophyllum aquaticum* demonstrated that the formulated product was not more toxic than glyphosate acid.

## IIIA 10.2.2.1 Fish acute toxicity $LC_{50}$ freshwater, cold-water species

The Tier II summaries presented below only include studies not reviewed previously in the 2001 EU evaluation on glyphosate.

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| IIIA 10.2.2.1/01 | [redacted] | 1992 | MON 52276: Acute toxicity to rainbow trout, *Oncorhynchus mykiss*, under flow-through test conditions<br>[redacted]<br>Report No: [redacted]-91-296<br>Date: 1992-01-23<br>GLP: yes<br>Owner: Monsanto S.A.<br>not published |

**Guideline:** US EPA FIFRA 72-1 (1982), OECD 203, and EEC Method C.1.

**Deviations:** The pH of the test system was correlated with MON 52276 concentration, and varied by more than 1 unit across the 5 dose levels. Within each test concentration, the pH variation was less than one unit. The range of temperature during the test was 2.3 °C, rather than the maximum range of 2 °C specified in the guideline. The dissolved oxygen concentration during the holding period was not reported. Fish length ranged from 3.1 – 4.1 cm, outside the recommended length of 4.0 – 8.0 cm. Fish were not inspected after the first 2 to 4 hours of the test. These deviations were not considered to have affected the outcome of the study.

**Dates of experimental work:** 1991-10-07 to 1991-10-11



## Executive Summary

The effects of MON 52276 (31% glyphosate acid) on rainbow trout (*Oncorhynchus mykiss*) were evaluated in a 96-hour flow-through toxicity test. Two groups of ten fish each were exposed for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. At 0, 48 and 96 hours, samples of test medium were taken for the analysis of glyphosate content.

Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation.

Mortality to one fish was observed at the lowest test concentration (119 mg/L), but it was judged to be not treatment-related. No mortality was observed at the higher test concentrations. No sublethal effects were observed at any test concentration. The present study is considered valid according to OECD guideline 203.

**Based on mean measured concentrations, the 96-hour $LC_{50}$ for rainbow trout (*Oncorhynchus mykiss*) exposed to MON 52276 in a flow-through test system was > 989 mg/L (> 306 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 989 mg/L (306 mg a.s./L), based on the absence of mortality and abnormal sublethal effects at this concentration.**

## I. MATERIALS AND METHODS

## A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item:: | MON 52276 |
| Active substance | Glyphosate |
| Description: | Amber liquid |
| Lot/Batch #: | LLN-9105-3135F |
| Purity: | 30.95% |

**2. Vehicle and/or positive control:** none

**3. Test organism:**

| | |
|---|---|
| Species: | Rainbow trout (*Oncorhynchus mykiss*) |
| Age: | Juveniles |
| Size: | 3.1 – 4.1 cm |
| Loading: | 10 test individual for 15 L test solution |
| Source: | [redacted] CA |
| Acclimation period: | 48 hours prior to the test initiation |
| Body weight of the animals: | 0.35 – 0.95 g |

**4. Environmental conditions:**

| | |
|---|---|
| Temperature: | 11.5 – 13.8°C |
| Photoperiod: | 16 hours, 392 – 500 lux |
| pH: | 8.1 – 8.3 (control);<br>7.3 – 7.4 (119 mg test item/L);<br>5.9 (989 mg/L) |
| Dissolved oxygen: | ≥ 7.1 mg/L |
| Conductivity: | 382 – 705 µmhos/cm |
| Hardness: | 38 - 116 mg $CaCO_3$/L |
| Alkalinity: | 57 - 77 mg $CaCO_3$/L |



## B. STUDY DESIGN AND METHODS

**1. Experimental treatments:** Two groups of ten fish each were exposed under flow-through conditions in a proportional diluter system (4.8 cycles/h) for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. During the 14-day holding period prior to test initiation, fish were fed daily and were in good health. There were two vessels per treatment, each containing ten fish (appr.24 L glass vessels containing 15 L test medium).

**2. Observations:** Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation. Water temperature in a control vessel was measured hourly throughout the test, and water pH and dissolved oxygen were measured daily in all test vessels. Hardness, total alkalinity and specific conductivity were measured at test initiation and test termination. At 0, 48 and 96 hours, samples of test medium were taken for quantification of glyphosate by HPLC.

**3. Statistical calculations:** $LC_{50}$ values were calculated along with the 95% confidence limits using non-linear interpolation.

## II. RESULTS AND DISCUSSION

### A. FINDINGS

Analytical data: The mean measured concentrations during the 96 hour exposure ranged from 119 to 989 mg test item/L and from 92 to 100% of nominal.

The $LC_{50}$ and NOEC values are given below based on mean measured concentrations.

| Endpoints (96 h) | MON 52276 [mg/L] | Glyphosate [mg/L] |
|---|---|---|
| $LC_{50}$ (95% C.I.) | >989 | >906.1 |
| NOEC | 989 | 906.1 |

### B. OBSERVATIONS

Mortality and signs of toxicity in control and treated groups are reported in Table 10.2.2.1-1. Mortality to one fish was observed at the lowest test concentration (119 mg/L), but it was judged to be not treatment-related. No mortality was observed at the higher test concentrations. No sublethal effects were observed at any test concentration.

**Table 10.2.2.1-1: Acute toxicity of MON 52276 to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions**

| MON 52276 [mg glyphosate/L] [1] | Time point [h] | Abnormalities/ Sublethal Effects | Mortality[2] | Cumulative % mortality |
|---|---|---|---|---|
| 0 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 119 | 24<br>48<br>72<br>96 | None observed | 1 | 5 |
| 208 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 362 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 581 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 989 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |

[1] Mean measured values.
[2] Number of dead fish of 20 total.

All validity criteria according to OECD 203 were fulfilled, as no mortality was observed in control group, dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

## III. CONCLUSION

**Based on mean measured concentrations, the 96-hour $LC_{50}$ for rainbow trout (*Oncorhynchus mykiss*) exposed to MON 52276 in a flow-through test system was > 989 mg/L (> 306 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 989 mg/L (306 mg a.s./L), based on the absence of mortality and abnormal sublethal effects at this concentration.**

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| IIIA 10.2.2.1/02 | [redacted] | 1992 | MON 52276: Acute toxicity to the common carp, *Cyprinus carpio,* under flow-through test conditions.<br>[redacted]<br>Report No: [redacted]-91-298<br>Date: 1992-01-24<br>GLP: yes<br>Owner: Monsanto S.A.<br>not published |

**Guideline:** US EPA FIFRA 72-1 (1982), OECD 203, and EEC Method C.1.

**Deviations:** For an estimated period of 4-6 hours, beginning at 8 hours prior to test termination, only dilution water was delivered to test chambers due to a malfunction in the diluter system. Test fish were exposed to nominal test concentrations for approximately 88 hours, followed by a slow dilution of test concentration for 4 hours, and then the exposure was adjusted to nominal concentrations for the remaining 4 hours of the test. Since there were no indications of stress or any other effects, it is unlikely that the reduction in exposure concentration for this short period had any effect on the outcome of the test. The pH of the test system was correlated with MON 52276 concentration, and varied by more than 1 unit across the 5 dose levels. Within each test concentration, the pH variation was less than one unit. The temperature range during the test was 2 °C, rather than the maximum range of 2 ºC specified in the guideline. The dissolved oxygen concentration during the holding period was not reported. During the test period, the dissolved oxygen during the test fell below 60% of the air saturation value in at least one replicate at every dose level and in both replicates at the two highest dose levels; the fish did not appear stressed as a result. Fish length ranged from 2.7 – 5 cm, outside the recommended length of 4.0 – 8.0 cm. Fish were not inspected after the first 2 to 4 hours. These deviations were not considered to have affected the outcome of the study.

**Dates of experimental work:** 1991-11-19 to 1991-11-23



## Executive Summary

The effects of MON 52276 (31% glyphosate acid) on common carp (*Cyprinus carpio*) were evaluated in a 96-hour flow-through toxicity test. Two groups of ten fish each were exposed for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test

water was a blend of treated municipal water and treated well water. At 0, 48 and 96 hours, samples of test medium were taken for the analysis of glyphosate content.

Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation.

No treatment related mortality or sublethal effects were observed in common carp at any test concentration. The present study is considered valid according to OECD guideline 203.

**Based on mean measured concentrations, the 96-hour $LC_{50}$ for common carp (*Cyprinus carpio*) exposed to MON 52276 in a flow-through test system was > 895 mg/L (> 277 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 895 mg/L (277 mg a.s./L).**

## I. MATERIALS AND METHODS

### A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item:: | MON 52276 |
| Active substance | Glyphosate |
| Description: | Amber liquid |
| Lot/Batch #: | LLN-9105-3135F |
| Purity: | 30.95% |

**2. Vehicle and/or positive control:** None

**3. Test organism:**

| | |
|---|---|
| Species: | Common carp (*Cyprinus carpio*) |
| Age: | Juveniles |
| Size: | 2.7 – 5.0 cm |
| Loading: | 10 test individuals for 15 L test solution (0.93 g fish/L) |
| Source: | [redacted] |
| Acclimation period: | 48 hours prior to the test initiation |
| Body weight of the animals: | 0.57 – 2.97 g |

**4. Environmental conditions:**

| | |
|---|---|
| Temperature: | 21.7 – 23.8°C |
| Photoperiod: | 16 hours light, 350 - 425 lux |
| pH: | 7.2 – 8.1 (control); 7.1 (98 mg test item/L); 5.7 (895 mg/L) |
| Dissolved oxygen: | 6.7 – 8.7 mg/L |
| Conductivity: | 1614 - 1688 µmhos/cm |
| Hardness: | 184 - 192 mg $CaCO_3$/L |
| Alkalinity: | 34 - 45 mg $CaCO_3$/L |

## B. STUDY DESIGN AND METHODS

**1. Experimental treatments:** Two groups of ten fish each were exposed under flow-through conditions using a proportional diluter system (3.8 daily volume turnover) for 96 hours to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600 and 1000 mg/L. The test water was a blend of treated municipal water and treated well water. During the 14-day holding period prior to test initiation, fish were fed daily and were in good health. There were two vessels per treatment, each containing ten fish (appr.24 L glass vessels containing 15 L test medium).

**2. Observations:** Mortality and signs of toxicity were recorded at 24, 48, 72 and 96 hours after test initiation. Water temperature in a control chamber was measured hourly throughout the test, and water pH and dissolved oxygen were measured daily in all test chambers. Hardness, total alkalinity and specific conductivity were measured at test initiation and test termination. At 0, 48 and 96 hours, samples of test medium were taken for quantification of glyphosate by HPLC.

**3. Statistical calculations:** $LC_{50}$ values were calculated along with the 95% confidence limits using non-linear interpolation.

# II. RESULTS AND DISCUSSION

## A. FINDINGS

Analytical data: The mean measured concentrations during the 96 hour exposure ranged from 98 to 895 mg test item/L and from 75 to 90% of nominal.

The $LC_{50}$ and NOEC values are given below based on mean measured concentrations.

| Endpoints (96 h) | MON 52276 [mg/L] | Glyphosate [mg/L] |
|---|---|---|
| $LC_{50}$ (95% C.I.) | >895 | >277 |
| NOEC | 895 | 277 |

## B. OBSERVATIONS

Mortality and signs of toxicity in control and treated groups are reported in Table 10.2.2.1-2. Mortality to one fish was observed at the lowest test concentration (119 mg/L), but it was judged to be not treatment-related. No mortality was observed at the higher test concentrations. No sublethal effects were observed at any test concentration.

**Table 10.2.2.1-2: Acute toxicity of MON 52276 to Common carp (*Cyprinus carpio*) under flow-through conditions**

| MON 52276 (mg/L) [1] | Time point (h) | Abnormalities/ Sublethal Effects | Mortality[2] | Cumulative % mortality |
|---|---|---|---|---|
| 0 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 98 | 24<br>48<br>72<br>96 | None observed | 1 | 5 |
| 176 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 340 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 552 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |
| 895 | 24<br>48<br>72<br>96 | None observed | 0 | 0 |

[1] Mean measured values.
[2] Number of dead fish of 20 total.

All validity criteria according to OECD 203 were fulfilled, as no mortality was observed in control group, dissolved oxygen concentration was ≥ 60% of air saturation and constant exposure conditions have been maintained.

## III. CONCLUSION

**Based on mean measured concentrations, the 96-hour $LC_{50}$ for common carp (*Cyprinus carpio*) exposed to MON 52276 in a flow-through test system was > 895 mg/L (> 277 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 895 mg/L (277 mg a.s./L).**

## IIIA 10.2.2.2 Acute toxicity (24 & 48 h) for *Daphnia* preferably *Daphnia magna*

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| IIIA 10.2.2.2./01 | [redacted] | 1992 | MON 52276: Acute toxicity to the water flea, *Daphnia magna*, under flow-through test conditions [redacted]<br>Report No: [redacted]-91-295<br>Date: 1992-01-23<br>GLP: yes<br>Owner: Monsanto S.A.<br>Not published |

**Guideline:** US EPA FIFRA 72-2 (1982), OECD 202 (1984), and EEC Method C.2 (1992).

**Deviations:** The pH of the test system was correlated with MON 52276 concentration, and varied by more than 1 unit across the 5 dose levels. Within each test concentration, the pH variation was less than one unit. The temperature range during the test was 3.8 °C, rather than the maximum range of 2 °C specified in the guideline. These deviations were not considered to have affected the outcome of the study.

**Dates of experimental work:** 1991-10-16 to 1991-10-18



### Executive Summary

The effects of MON 52276 (30.95% w/w glyphosate acid) on *Daphnia magna* were evaluated in a 48-hour flow-through toxicity test. Neonates of *Daphnia magna* were exposed to nominal concentrations of MON 52276 at 130, 216, 360, 600, and 1000 mg/L and a negative control consisting of dilution water. The test consisted of two replicates per treatment group and control. 10 Daphnids were exposed per replicate and were not fed during the test. Total number of *Daphnia magna* exhibiting immobility and other clinical signs of toxicity was recorded at 24 and 48 hours after test initiation.

Temperature, pH-values and dissolved oxygen concentrations were measured at the beginning, at approximately 24 hours during the test and at the end of the test. At 0 and 48 hours, samples of test medium were taken for quantification of glyphosate by HPLC. The analysed test concentrations ranged between 95 and 105% of the nominal values.

No mortality to *Daphnia magna* from exposure to MON 52276 was observed at test concentrations ≤ 356 mg/L. At 580 mg/L, 20% mortality was observed at 48 hours, with 100% mortality observed at 948 mg/L. Sublethal effects were observed only at the 580 mg/L concentration. All validity criteria according to the OECD guideline 202 were fulfilled.

**Based on mean measured concentrations, the 48-hour $EC_{50}$ for *Daphnia magna* exposed to MON 52276 in a flow-through test system was 676 mg/L (95% confidence limits of 580 and 948 mg/L), (equivalent to 209 mg a.s./L). The corresponding no observed effect concentration (NOEC) was 356 mg/L (110 mg a.s./L), based on the lack of mortality and sublethal effects at this concentration.**

# I. MATERIALS AND METHODS

## A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item:: | MON 52276 |
| Active substance: | Glyphosate |
| Lot/Batch #: | Amber liquid |
| Purity: | LLN-9105-3135F |

**2. Vehicle and/or positive control:** None

**3. Test organism:**

| | |
|---|---|
| Species: | *Daphnia magna* Straus |
| Age: | Neonates (< 24 h old) |
| Loading: | 1 daphnid per 30 mL test medium |
| Source: | [redacted] |
| Diet/Food: | none |
| Acclimation period: | Not stated |

**4. Environmental conditions**:

| | |
|---|---|
| Temperature: | 20.0 – 23.8 °C |
| Photoperiod: | 16 hours light, 384 - 517 lux |
| pH: | 7.1 – 8.6 |
| Dissolved oxygen: | 7.4 – 8.7 mg O$_2$/L |
| Conductivity: | 436 – 644 µS/cm |
| Hardness: | 60 – 96 mg $CaCO_3$/L |



## B: STUDY DESIGN AND METHODS

**1. Experimental treatments:** The effects of MON 52276 (30.95% w/w glyphosate acid) on neonates of *Daphnia magna* were evaluated in a 48-hour flow-through toxicity test using a proportional diluter system (1.6 cycles/h). Twenty Daphnids (2 replicates of 10 animals per test beaker) were exposed to nominal concentrations of MON 52276 at 0 (controls), 130, 216, 360, 600, and 1000 mg/L dissolved in a blend of treated municipal water and treated well water. In addition, a control group was exposed to test water without test substance (blank control).

**2. Observations:** Total number of immobile *Daphnia magna* was recorded 24 h and 48 h after test initiation. In addition, specimens were observed for clinical signs of toxicity.

Water temperature was measured at 0 and 48 hours in each test chamber, as well as hourly in one negative control replicate. Water pH and dissolved oxygen were recorded at test start then every 24 hours. Hardness, alkalinity and specific conductance were measured once in the dilution water at test initiation.

At 0 and 48 hours, samples of test medium were taken for quantification of glyphosate by HPLC.

**3. Statistical calculations:** $EC_{50}$ values including 95% confidence limit were determined by non-linear interpolation.

## II. RESULTS AND DISCUSSION

### A. FINDINGS

The analysed test concentrations ranged between 86 and 103% of the nominal values. The $EC_{50}$ and NOEC values given below are based on mean measured concentrations.

| Endpoints | MON 52276 [mg/L] | Glyphosate [mg a.s./L] |
|---|---|---|
| $EC_{50}$ (48 h) | 676 mg/L (580 – 948 mg/L) | 209 mg/L |
| NOEC (48 h) | 356 mg/L | 110 mg/L |

### B. OBSERVATIONS

No mortality to *Daphnia magna* from exposure to MON 52276 was observed at test concentrations ≤ 356 mg/L. At 580 mg/L, 20% mortality was observed at 48 hours with 100% mortality observed at 948 mg/L. Sublethal effects were observed only at the 580 mg/L concentration.

**Table 10.2.2.2-1: Acute toxicity of MON 52276 to *Daphnia magna* under flow-through conditions**

| MON 52276 (mg/L) [1] | Time point (h) | Abnormalities/ Sublethal Effects | No. of *Daphnia* immobilised or dead[2] | Cumulative % mortality |
|---|---|---|---|---|
| 0 | 24<br>48 | None observed | 0<br>0 | 0<br>0 |
| 133 | 24<br>48 | None observed | 0<br>0 | 0<br>0 |
| 227 | 24<br>48 | None observed | 0<br>0 | 0<br>0 |
| 356 | 24<br>48 | None observed | 0<br>0 | 0<br>0 |
| 580 | 24<br>48 | None observed<br>3 lethargic | 0<br>4 | 0<br>20 |
| 948 | 24<br>48 | None observed<br>-- | 11<br>20 | 55<br>100 |

[1] Mean measured values.
[2] Of 20 total *Daphnia* in group.

All validity criteria according to the OECD 202 were fulfilled, as no immobility of Daphnids was observed in control groups and dissolved oxygen concentration was ≥ 3 mg/L in all test vessels.

## III. CONCLUSION

**Based on mean measured concentrations, the 48-hour $EC_{50}$ for *Daphnia magna* exposed to MON 52276 in a flow-through test system was 676 mg/L (95% confidence limits of 580 and 948 mg/L), (equivalent to 209 mg a.s./L). The corresponding no observed effect concentration**

**(NOEC) was 356 mg/L (110 mg a.s./L), based on the lack of mortality and sublethal effects at this concentration.**

## IIIA 10.2.2.3 Effects on algal growth and growth rate

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| IIIA 10.2.2.3./01 | [redacted] | 1992 | A1ga, Growth Inhibition Test Effect of MON 52276 on The Growth Of *Selenastrum capricornutum* [redacted]<br>Report No: [redacted]-91-389<br>Date: 1992-01-10<br>Owner: Monsanto S.A.<br>GLP: yes<br>Not published |

**Guideline:** OECD Guideline 201 (2006)

**Deviations to OECD 201:** No analysis of glyphosate in the test system was conducted. However, no degradation of glyphosate is expected to have occurred over the course of the study, since glyphosate concentrations have been demonstrated to be stable over a 72-hour period in other formulation studies. The pH of the test system was correlated with MON 52276 concentration, and varied by more than 1.5 units across the 5 dose levels. Within each test concentration, the pH variation was less than 1.5 units. These deviations were not considered to have affected the outcome of the study.

**Dates of experimental work:** 1991-10-15 to 1991-10-18



**Executive Summary**

The effects of the test item MON 52276 on *Pseudokirchneriella subcapitata* were evaluated in a 72-hour static toxicity test. After a range-finding test, suspensions of *P. subcapitata* were exposed to five nominal concentrations (50, 90, 160, 290 and 500 mg test item/L). In addition, algae were exposed to test medium without test substance (negative control).

For each concentration, three replicates with an initial cell density adjusted to $10^4$ cells/mL were prepared. For the control group, six replicates were prepared. The culture vessels were incubated on a shaking plate over several generations for 72 h.

After 24, 48, and 72 hours, mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements. The inhibition of cell growth and reduction of cell growth rate were thereafter calculated. The concentrations resulting in 50% reduction of growth rate ($E_rC_{50}$) and 50% inhibition of cell growth ($E_bC_{50}$) were determined, as well as the associated NOEC values.

At or above the nominal concentration of 160 mg test item/L, the cell density decreased continuously at increasing test concentrations. Inhibition of cell growth and reduction of algal growth rate increased

with increasing concentration of MON 52276 from a nominal concentration of 160 mg test item/L upwards. All validity criteria according to the OECD guideline 201 were fulfilled.

**Based on absorbance, the 72 h $E_rC_{50}$ and the 72 h $E_bC_{50}$ for *Pseudokirchneriella subcapitata* exposed to MON 52276 were calculated to be 393 mg test item/L and 150 mg test item/L, equivalent to 121.8 mg glyphosate acid/L and 46.5 mg glyphosate acid/L, respectively. The NOEC was determined to be 90 mg test item/L equivalent to 27.9 mg glyphosate acid/L. For the cell counting method, the 72 h $E_rC_{50}$ and the 72 h $E_bC_{50}$ for *Pseudokirchneriella subcapitata* exposed to MON 52276 were calculated to be 284 mg test item/L and 178 mg test item/L, equivalent to 88 mg glyphosate acid/L and 55.2 mg glyphosate acid/L respectively. The NOEC was determined to be 90 mg test item/L equivalent to 27.9 mg glyphosate acid/L.**



## I. MATERIALS AND METHODS

### A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item:: | MON 52276 |
| Description: | Light amber-brown liquid formulation |
| Lot/Batch #: | LLN 260491 B |
| Purity: | 31% glyphosate acid equivalent, as 41% isopropylamine salt of glyphosate |
| Density: | 1.16 mg/cm$^3$ |

**2. Vehicle and/or positive control:** None

**3. Test organism:**

| | |
|---|---|
| Species: | *Pseudokirchneriella subcapitata* |
| Initial cell concentration | $10^4$ cells/ml |
| Source: | [redacted] |
| Acclimatisation period: | The pre-culture, which was used for the inoculation of the test cultures, was incubated 4 days under the conditions of the test. |

**4. Environmental conditions**:

| | |
|---|---|
| Temperature: | 20.9 – 23.1°C |
| Photoperiod: | 24 h light |
| Light intensity | 8875 ± 125 Lux |
| pH: | 8.31 – 8.97 (control), 5.88 – 5.98 (highest test concentration) |
| Conductivity: | Not stated |
| Hardness: | Not stated |

### B. STUDY DESIGN AND METHODS

**1. Experimental treatments:** Based on the range finding test, the definitive algal growth inhibition test was performed with five concentrations (50, 90, 160, 290 and 500 mg test item/L). In addition, algae were exposed to test medium without test substance (blank control). The algal medium

recommended in OECD Guideline 201 (1981) was used. For each concentration, three parallel cultures were prepared in 150 mL Erlenmeyer glass flasks covered with cotton wool. To each test vessel, 100 mL of the algal suspension containing the test item preparation were added, with an initial cell density adjusted to $10^4$ cells/mL. For the control group, six parallel test vessels were prepared. The culture vessels were incubated on a shaking plate over several generations for 72 h. During the incubation, the algal cells were kept in suspension by continuous shaking.

**2. Observations:** After 24, 48, and 72 hours, aliquots of media from each culture were taken and mean cell densities for each test concentration and control were determined based on spectrophotometrical measurements (absorbance measurement). Algal cell concentrations were also determined by microscopic counting at 48 h and 72 h. The endpoints were calculated for the absorbance and cell counting method. Temperature and the light intensity were recorded daily during the test, while the pH was measured in one replicate of each test concentration at test initiation and test termination.

**3. Statistical calculations:** Inhibition of cell growth and reduction of cell growth rate were derived from growth curves obtained by plotting the average algal cell concentrations for each test concentration against time. Concentrations resulting in 50% reduction of growth rate ($E_rC_{50}$) and 50% inhibition of cell growth ($E_bC_{50}$) were determined, with corresponding NOEC values. The median effect concentrations ($EC_{50}$ values) were determined using the Logit model of Chou and Chou (1985).

## II. RESULTS AND DISCUSSION

### A. FINDINGS

The $E_rC_{50}$, $E_bC_{50}$ and NOEC values are given below based on nominal concentrations.

| Endpoint | MON 52276 [mg/L] | | Glyphosate acid [mg/L] | |
|---|---|---|---|---|
| | absorbance | cell counting | absorbance | cell counting |
| $E_rC_{50}$ (72 hours) | 303 | 284 | 121.8 | 88.0 |
| $E_bC_{50}$ (72 hours) | 150 | 178 | 46.5 | 55.2 |
| NOEC (72 hours) | 90 | | 27.9 | |

### B. OBSERVATIONS

Mean cell densities*:* At or above the nominal concentration of 160 mg test item/L, the cell density decreased continuously as the test concentrations increase, reaching 33 x $10^3$ cells/mL at the highest test concentration, against 644 x $10^3$ cells/mL observed in blank control. However, the mean cell numbers observed for the two lowest test concentrations were numerically higher that those observed in control.

Inhibition of cell growth: Inhibition of cell growth increased with increasing concentration of MON 52276 from a nominal concentration of 160 mg test item/L upwards. For the two lowest test concentration of 50 mg test item/L and 90 mg test item/L, increases in cells growth of 36.9% and 27.7%, respectively, compared to the control were observed.

Reduction of growth rate: Reduction of algal growth rate increased with increasing concentration of MON 52276 from a nominal concentration of 160 mg test item/L upwards. For the two lowest test concentration of 50 mg test item/L and 90 mg test item/L, increases of algal growth rate of 13.6% and 8.4%, respectively, compared to the control were observed.

**Table 10.2.2.3-1: Percentage reduction of growth rate and inhibition of cell growth of *Pseudokirchneriella subcapitata* exposed for 72 hours to MON 52276**

| MON 52276 [mg/L] | Control | 50 | 90 | 160 | 290 | 500 |
|---|---|---|---|---|---|---|
| **Glyphosate acid [mg/L]** | **-** | **15.5** | **27.9** | **49.6** | **89.9** | **155** |
| Mean cell densities [x 1000 cells/mL] | 644 | 741 | 663 | 315 | 45 | 33 |
| Cell growth rate reduction [%] | | -13.6 | -8.4 | 10.9 | 42.8 | 58.2 |
| Cell growth inhibition [%] | | -36.9 | -27.7 | 50.3 | 81.5 | 89.6 |

The biomass in the control cultures increased by a factor of >16, the coefficient of variance for section specific growth rates was ≤35%, for the whole test period it was ≤7%. The validity criteria according to guideline OECD 201 were therefore fulfilled.



## III. CONCLUSION

**The 72-hour $E_rC_{50}$ (growth rate) and $E_bC_{50}$ (biomass) of MON 52276 to *Pseudokirchneriella subcapitata* under static test conditions were determined to be 284 mg/L (87 mg a.s./L) and 178 mg/L (55 mg a.s./L), respectively. The corresponding 72-hour no observed effect concentration (NOEC) for both parameters was 90 mg/L (28 mg a.s./L).**

## IIIA 10.2.2.4 Marine or estuarine organisms acute toxicity $LC_{50}/EC_{50}$

This is not a data requirement according to Regulation 1107/2009/EC.

Based on the use of MON 52276, exposure to a marine or estuarine organism environment is unlikely. A marine or estuarine organism acute toxicity study on the formulation was not required and was therefore not conducted.

## IIIA 10.2.2.5 Marine sediment invertebrates, acute toxicity $LC_{50}/EC_{50}$

This is not a data requirement according to Regulation 1107/2009/EC.

Based on the use of MON 52276, exposure to a marine or estuarine organism environment is unlikely. A marine or estuarine organism long term toxicity study on the formulation was not required and was therefore not conducted.

## IIIA 10.2.3 Microcosm or mesocosm study

Microcosm or mesocosm studies are not required for MON 52276 since all $TER_A$ and $TER_{LT}$ values for glyphosate acid and its metabolites and for MON 52276 all exceed the Annex VI trigger values of 100 (acute) and 10 (chronic) respectively, (see Point IIIA 10.2.1 in this document).

## IIIA 10.2.4 Residue data in fish (long-term)

The octanol/water partition coefficient of glyphosate acid, expressed as log Pow, is -3.2. Values less than 3 indicate a low potential for bioaccumulation, therefore no further assessment is necessary. However, although the potential for bio-concentration is low for glyphosate acid, residues in fish were measured for glyphosate acid and are presented in IIA 8.2.3 (IIA 8.2.3/01 and IIA 8.2.3/02).

## IIIA 10.2.5 Chronic fish toxicity data

The $TER_{LT}$ values achieved in the long-term fish risk assessment indicate that the active substance glyphosate acid of MON 52276 does not pose an unacceptable chronic risk to fish. Long-term fish testing with the formulation MON 52276 is not required.

### IIIA 10.2.5.1 Chronic toxicity (28 day exposure) to juvenile fish

The studies conducted with glyphosate acid can be used to predict the toxicity of the formulated product (see IIA 8.2.2). Due to the low chronic toxicity of the active ingredient and the low acute toxicity of the metabolites, no juvenile fish studies were conducted with the formulated product.

### IIIA 10.2.5.2 Fish early life stage toxicity test

Glyphosate acid and its metabolite AMPA have been shown to have low chronic toxicity in fish ELS studies. Additionally, the studies conducted with glyphosate acid can be used to predict the toxicity of the formulated product (see IIA 8.2.2). Therefore, additional early life stage toxicity tests are not required for the formulated product.

### IIIA 10.2.5.3 Fish life cycle test

Glyphosate acid has been shown to have the low chronic toxicity in a fish full life cycle study. Additionally, the studies conducted with glyphosate acid can be used to predict the toxicity of the formulated product (see IIA 8.2.2). Therefore, a fish full life cycle study is not required for the formulated product.

## IIIA 10.2.6 Chronic toxicity to aquatic invertebrates

As risk assessments indicate that the active substance glyphosate acid of MON 52276 does not pose an unacceptable chronic risk to aquatic invertebrates, tests with the formulation MON 52276 are not required.

### IIIA 10.2.6.1 Chronic toxicity in *Daphnia magna* (21-day)

Glyphosate acid and its metabolite AMPA have been shown to have the low chronic toxicity in *Daphnia* 21 day reproduction studies. Additionally, the studies conducted with glyphosate acid can be used to predict the toxicity of the formulated product. Therefore, additional chronic *Daphnia magna* studies with the formulated product are not required.

### IIIA 10.2.6.2 Chronic toxicity for a representative species of aquatic insects

MON 52276 is not an insecticide or insect growth regulator and is not applied directly to water. Therefore, determination of chronic toxicity to aquatic insects is not required under 1107/2009/EC.

### IIIA 10.2.6.3 Chronic toxicity for a representative species of aquatic gastropod molluscs

MON 52276 is not an insecticide or insect growth regulator, has demonstrated a low toxicity to aquatic invertebrates and according to the proposed uses, will not be applied directly to surface water. Therefore, determination of chronic toxicity to aquatic gastropod molluscs is not required under Regulation 1107/2009/EC.

## IIIA 10.2.7 Accumulation in aquatic non-target organisms

Bioaccumulation of any of the active substances under natural conditions is not expected to occur (refer to Section IIIA 10.2.4).

A fish-bioconcentration study is not required, due to the low $Log_{POW}$, which is below the trigger value of 3 ($Log_{POW}$ = -3.2). However, a fish bioconcentration study has been conducted which achieved a bioconcentration factor of 1.1± 0.61, which is far below the Annex VI BCF trigger value of 1000. Therefore, a study is not necessary to determine bioaccumulation in aquatic non-target organisms.

## IIIA 10.3 Effects on terrestrial vertebrates other than birds

The wild mammal risk assessment was carried out according to the EFSA (2009) Guidance Document on Risk Assessment for Birds and Mammals [i], which follows a tiered approach to assess the effects of plant protection products based on the requirements of Regulation 544/2011 and Regulation 545/2011 for active substances and plant protection products, respectively.

The mammalian toxicity studies with the active substance glyphosate acid, the formulation MON 52276 and the metabolite AMPA are summarised in the table below. Detailed descriptions of ecotoxicological studies with mammals are given under point 5 in the Annex II dossier of glyphosate.

**Table 10.3-1: Toxicity of glyphosate acid, MON 52276 and AMPA to mammals**

| Study type | Species | Endpoint | Value | Reference |
|---|---|---|---|---|
| **Glyphosate Acid** | | | | |
| Acute oral | Rat | $LD_{50}$ [mg/kg bw] | >8000[1] | IIA 5.2 [redacted] 1990 |
| Two generation | Rat | NOEAEL [mg/kg feed] | **30000**[1] | IIA 5.6.1/03 [redacted] 96-0031 [redacted] 1997 |
| | | NOEAEL [mg/kg bw/d] | **2150** (male) 2532 (female) | |
| **MON 52276** | | | | |
| Acute oral | Rat | $LD_{50}$ [mg/kg bw] | >5000[1] | IIIA 7.1.1/01 [redacted]-91-261 [redacted] 1991 |
| **AMPA** | | | | |
| Acute oral | Mouse | $LD_{50}$ [mg/kg bw] | >5000[1] | IIA 5.8/01 [redacted] 96-0075 [redacted] 1996 |
| 13 week oral | Rat | NOAEL [mg/kg bw/d] | >1000[1] | IIA 5.8 [redacted] 1993 |

**Endpoints in bold are used for risk assessment**
[1] Highest dose tested
[2] NOEAEL = no observed ecologically adverse effect level (based on no ecologically significant effects)



A full assessment of the toxicity to assess the risk to wild mammals has been performed. Table 10.3-1 summarises the endpoints used in the assessment. These data for the active substance, glyphosate are summarised in IIA 5.2.1 of Annex II for glyphosate and therefore further details are not provided here. In total, 24 studies on acute oral toxicity of glyphosate acid have been previously conducted and are summarised in Table IIA 5.2-1. The highest concentrations tested ranged from 2000 to 8000 mg/kg bw. Glyphosate can be considered non-toxic to mammals, as there were no deaths in these toxicological studies, and all the end points quoted are for the highest concentration tested. Consequently the risk assessments are conservative, in comparison to data where lethal endpoints were measured.

There is no agreed EU endpoint for long term toxicity in mammals. In line with the acute oral toxicity of glyphosate acid to mammals, a substantial number of reproductive studies are available. Considering that in none of the studies available an ecological relevant effect was observed, an endpoint is proposed based on the multigenerational rat reproduction study by [redacted], 1997 (see IIA 5.6.1/03). For this ecotoxicological risk assessment the highest rate in the study (30000 mg/kg feed (2150 - 2532 mg/kg bw/d; male-female)), is used as the NOEAEL based on ecologically significant effects. For none of the studies available (see IIA 5.6), continuous feeding exhibited any

effect on the propagation of two to three generations in the rat. The fertility and reproductive performance of each generation of parental animals and the clinical condition and survival of their offspring were not adversely affected. The only effect of treatment observed on four out of nine studies was a slight reduction in the bodyweight of the F1A pups at concentrations of 10000 to 30000 mg/kg feed in the glyphosate acid group with a subsequent reduction in bodyweight of the selected F1 parent males for the duration of the pre-mating period. This is not considered to be ecologically relevant.

Therefore, for glyphosate the lowest NOEAEL value of 2150 mg/kg bw/d is used for this risk assessment (Table 10.3-1). Mammalian toxicological studies have shown that there is no evidence for cumulative glyphosate toxicity and that it is not genotoxic, oncogenic, teratogenic or a reprotoxin. Thus, acute oral dosing and the reproduction study are considered to be appropriate for this risk assessment.

The mammalian toxicity endpoints for glyphosate that are most appropriate for acute and long-term ecological risk assessment are summarised in Table 10.3-1. Further details can be found in the corresponding EU dossier for glyphosate, Annex IIA, Section 5, Point 5.



**Exposure**

Exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Direct exposure of mammals to MON 52276 applications is considered unlikely, since at the time of application and for a short period thereafter, most mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

The exposure of mammals to glyphosate acid was estimated following application of MON 52276 on a field containing annual weeds at:

1. A maximum single application rate of 2.16 kg a.s./ha that includes pre-planting, pre-emergence of crops and pre-harvest applications

2. A maximum single application rate for orchards a 0.96 kg a.s./ha has been used. As a worst case it is assumed that 1/3 of the area of an individual orchards or vineyard is treated, giving an overall application rate of 2.88 kg a.s./ha / 3 = 0.96 kg a.s./ha.

A repeat application of MON 52276 is only made to control new growth of weeds which would not have been exposed to the preceding application. Therefore, it is not appropriate to use a multiple application factor (MAF) for foliar residues in the case of this total herbicide.

Glyphosate residues on the relevant food items were calculated using the estimates given in the EFSA Guidance Document on Risk Assessment for Birds and Mammals. For acute exposure, shortcut values for 90$^{th}$ percentile RUD were used to calculate daily dietary dose (DDD), and for long-term exposure, shortcut values for mean RUD were used since they are considered to be more appropriate in the case of repeated applications.

The daily dietary doses (DDD) were calculated using the following equation:

$$DDD\ (mg\ a.s./kg\ diet) = Application\ rate\ (kg\ a.s./ha) \times RUD_{SV} \times f_{twa}$$

Where:

- $RUD_{SV}$ is the Residue per Unit Dose (shortcut value) i.e., the residues on food items normalised to an application rate of 1 kg a.s./ha taken from the EFSA Bird and Mammal Guidance document.

- The term $f_{twa}$ is the time-weighted-average factor. Default TWA residues were used as an estimate of long-term exposure only, since it is considered that the use of mean initial residues provides an unrealistically extreme worst-case estimate of long-term exposure. The default $f_{twa}$ of 0.53 was used as given in the Guidance Document, assuming the averaging period of 21 days and $DT_{50}$ of 10 days. As discussed later in this section, empirical $f_{twa}$ values have been developed for grass and insects that are less than the default values and provide more realistic and relevant glyphosate exposure estimates.

- The following assumptions are used in the screening mammal risk assessment

- Application rate: MON 52276 (maximum labelled rate in the EU) –2 × 2.16 kg a.s./ha on annual weeds (representing pre-planting and pre-emergence of crops), 1 × 2.16 kg a.s./ha on cereals and oilseed rape each, and 3 × 0.96 kg a.s./ha on orchards and vines.

- Method of application: hydraulic sprayer overall and Knapsack spray for spot treatment on orchards and vines.

**Metabolites of glyphosate acid - AMPA**

Quantitative wild mammalian risk assessments for AMPA were not conducted because of AMPA's low acute toxicity profile and because of the low acute and chronic dietary field exposure levels to AMPA and low toxicity. Low levels of AMPA have been measured in the forage crop residue studies and numerous plant metabolism studies that are summarized in the EU Annex II summary documentation, Section IIA 6. Measured AMPA levels were <10% of the total radioactive residue. Low exposure levels would also be predicted for prey items (e.g., insects) and similar to glyphosate AMPA does not possess bioaccumulation potential. Based on the combination of low exposure and low hazard, it can be concluded that risk to mammals will be low and a quantitative assessment was not required.

**TER calculation**

According the guidance document, a step wise approach was followed. This consisted of an initial screening assessment followed by a Tier 1 assessment (where necessary), for both acute and long term (reproductive) assessments.

**Screening step**

The screening step starts by using generic 'indicator species' and is based on a multiplication of hypothetical worst-case assumptions. The crop groupings, indicator species and critical use patterns relevant to the use of MON 52276 according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals are shown in Table 10.3-2.

The risk to mammals was assessed using Toxicity: Exposure Ratio (TER), i.e. by comparing the relevant Daily Dietary Dose (DDD) with the appropriate endpoint:

$TER_A = LD_{50} / DDD_A$

$TER_{LT}$ = long-term and reproduction NOEAEL / $DDD_{LT}$

The Daily Dietary Dose (DDD) was estimated from parameters provided in the guidance document and the application pattern of glyphosate acid. Shortcut values are used to represent the food intake rate relative to body weight of mammals (FIR/bw) and the fraction of diet obtained in the treated area.

Acute and long-term DDD are calculated according to the following formulas:

$DDD_A$ = Application rate x $RUD_{SV}$ [mg a.s./kg bw]

$DDD_{LT}$ = Application rate x $RUD_{SV}$ x TWA [mg a.s./ kg bw/d]

With:

- **SV:** To facilitate the assessment process, information on food intake rate, body weight and residue per unit doses (RUDs) is combined into a single value for a specific combination of species and crop termed as 'shortcut value' (SV) and provided in the guidance document EFSA (2009) Guidance Document on Risk Assessment for Birds and Mammals.. Shortcut values based on 90$^{th}$ percentile RUDs are used for acute assessments and mean residue unit doses (RUDs) for reproductive assessments.

- **TWA:** Long-term DDD values can be calculated as the time-weighted average concentration for the respective time interval. Default assumptions are based on a $DT_{50}$ of 10 days on plants and a time window of 21 days, which leads to a default TWA factor of 0.53. It should be noted that glyphosate initial residue and dissipation data from 22 trials is summarized in the previous glyphosate monograph for the formulation MON 2139 (360 g a.s./L) and a comparable formulation from Cheminova A/S. MON 2139 and MON 52276 have comparable efficacy against weedy grasses which indicates comparable leaf retention and transport of the active substance. An evaluation of residue data demonstrates that glyphosate rapidly dissipates from grass with an estimated $DT_{50}$ reported in the glyphosate monograph of approximately 3 days. This estimated value is very close to the derived value of 2.8 days, which translates to a TWA factor of 0.19 (Appendix I). Additionally, a glyphosate residue field decline study was performed to evaluate decline in insects and is summarized in the Annex 2 document. This study provided a $DT_{50}$ value of approximately 5.5 days, which translates to a TWA factor of 0.35 (Appendix II).

SV values for the critical use pattern for each crop group and the corresponding indicator species are summarised in Table 10.3-2.

**Table 10.3-2: Screening step crop groupings, indicator species and critical use pattern relevant to the use of MON 52276**

| Crop group [1] | GAP crop species | Indicator species | Proposed /Critical use pattern | | | | $SV_{90}$ | $SV_m$ |
|---|---|---|---|---|---|---|---|---|
| | | | No. of apps | App. Interval [d] | Growth stages (BBCH) | Glyphosate acid [kg a.s./ha] | [mg a.s./kg bw/d] | |
| Annual weeds | All crops [2] | Small granivorous mammal | 1- 2 | 21 | BBCH <10 | 2.16 | 14.4 | 6.6 |
| Cereals | Cereals | Small herbivorous mammal | 1 | - | Pre harvest | 2.16 | 118.4 | 48.3 |
| Oilseed rape | Oilseeds | Small herbivorous mammal | 1 | - | Pre harvest | 2.16 | 118.4 | 48.3 |
| Orchards | Orchard crops and vines | Small herbivorous mammal | 1 - 3 | 28 | Pre harvest | 0.96 | 136.4 | 72.3 |
| Vines | | Small herbivorous mammal | 1 - 3 | 28 | Pre harvest | 0.96 | 136.4 | 72.3 |

[1] Crop according to EFSA (2009) crop groupings.
[2] All seeded or transplanted crops, including but not restricted to: root and tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables, *Brassica* vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds cereals, and sugar- and fodder beet; before planting fruit crops, ornamentals, trees, nursery plants etc.)



## Tier 1

A Tier 1 risk assessment is required, if the TER values calculated in the screening assessment are below the Annex VI trigger of concern of 10 for acute and 5 for long-term exposure. Tier 1 uses more realistic exposure estimates along with a more representative 'generic focal species'.

A further refined risk assessment is required if the TER values calculated in Tier 1 do not reach the Annex VI TER triggers of concern as given above.

### Risk assessment to mammals through drinking water

There are two scenarios provided in the EFSA (2009) Guidance Document on Risk Assessment for Birds and Mammals for assessing the risk from drinking water.

### Leaf scenario

According to the EFSA Guidance Document, this scenario is not relevant for mammals.

### Puddle scenario

This scenario is relevant for mammals drinking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or annual weeds. This is therefore relevant for all uses of MON 52276 and should therefore be assessed.

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to acute and long-term endpoint (in mg/kg bw/d) does not exceed 50 ($K_{oc}$ < 500 L/kg) *or* 3000 ($K_{oc}$ ≥ 500 L/kg), as specified in the ***EFSA Guidance Document***. For glyphosate acid and its metabolite AMPA, no specific TER calculation is required, as the ratio of effective application rate (2160 g a.s./ha) to the relevant endpoints (lowest relevant endpoint is a NOAEC of 2150 mg/kg bw/d for reproductive toxicity of glyphosate acid on rats) is 1.0.

## IIIA 10.3.1 Toxicity exposure ratios for terrestrial vertebrates other than birds

### IIIA 10.3.1.1 Acute toxicity exposure ratio ($TER_A$)

**Screening step**

DDDs for the critical use pattern for each crop group are calculated in Table 10.3.1.1-1.

**Table 10.3.1.1-1: Screening step – Estimates of acute exposure to glyphosate acid**

| Compound | Crop group | Indicator species | App. Rate [kg a.s./ha] | $SV_{90}$ [mg a.s./kg bw/d] | $DDD_A$ [mg a.s./kg bw/d] |
|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds | small granivorous mammal | 2.16 | 14.4 | 31.1 |
| | Cereals | small herbivorous mammal | 2.16 | 118.4 | 255.7 |
| | Oilseed rape | small herbivorous mammal | 2.16 | 118.4 | 255.7 |
| | Orchards | small herbivorous mammal | 0.96 | 136.4 | 130.9 |
| | Vines | small herbivorous mammal | 0.96 | 136.4 | 130.9 |

The resulting $TER_A$ values are given in Table 10.3.1.1-2.

**Table 10.3.1.1-2: Screening step – Acute risk ($TER_A$) to mammals from glyphosate acid**

| Compound | Crop group | Indicator Species | $LD_{50}$ [mg a.s./kg bw/d] | $DDD_A$ [mg a.s./kg bw/d] | $TER_A$ | Trigger |
|---|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds (pre-planting and pre-emergence) | small granivorous mammal | >8000 | 31.1 | **>257** | 10 |
| | Cereals | small herbivorous mammal | | 255.7 | **>31.3** | |
| | Oilseed rape | small herbivorous mammal | | 255.7 | **>31.3** | |
| | Orchards | small herbivorous mammal | | 130.9 | **>61.1** | |
| | Vines | small herbivorous mammal | | 130.9 | **>61.1** | |

**TER values in bold exceed the relevant trigger value of 10**

All $TER_A$ values for application of MON 52276 are greater than the relevant trigger of 10, indicating low acute risk to mammals from glyphosate acid.

### IIIA 10.3.1.2 Short-term toxicity exposure ratio ($TER_{ST}$)

According to the EU guidance document, short-term risk to mammals is not presented as it is covered by the long-term risk assessment.

## IIIA 10.3.1.3 Long-term toxicity exposure ratio ($TER_{LT}$)

### Screening step

DDDs for the critical use pattern for each crop group are calculated in Table 10.3.1.3-1.

**Table 10.3.1.3-1: Screening step – Estimates of long term exposure to glyphosate acid**

| Compound | Crop group | Indicator species | App. Rate [kg a.s./ha] | $SV_m$ [mg a.s./kg bw/d] | $f_{TWA}$ | $DDD_{LT}$ [mg a.s./kg bw/d] |
|---|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds | small granivorous mammal | 2.16 | 6.6 | 0.53 | 7.6 |
| | Cereals | small herbivorous mammal | 2.16 | 48.3 | 0.53 | 55.3 |
| | Oilseed rape | small herbivorous mammal | 2.16 | 48.3 | 0.53 | 55.3 |
| | Orchards | small herbivorous mammal | 0.96 | 72.3 | 0.53 | 36.8 |
| | Vines | small herbivorous mammal | 0.96 | 72.3 | 0.53 | 36.8 |

The resulting $TER_{LT}$ values are given in Table 10.3.1.3-2.

**Table 10.3.1.3-2: Screening step – Long term risk ($TER_{LT}$) to mammals from glyphosate acid**

| Compound | Crop group | Indicator Species | NOEAEL [mg a.s./kg bw/d] | $DDD_{LT}$ [mg a.s./kg bw/d] | $TER_{LT}$ | Trigger |
|---|---|---|---|---|---|---|
| Glyphosate acid | Annual weeds (pre-planting and pre-emergence) | small granivorous mammal | 2150 | 7.6 | **285** | 5 |
| | Cereals | small herbivorous mammal | | 55.3 | **38.9** | |
| | Oilseed rape | small herbivorous mammal | | 55.3 | **38.9** | |
| | Orchards | small herbivorous mammal | | 36.8 | **58.4** | |
| | Vines | small herbivorous mammal | | 36.8 | **58.4** | |

**TER values in bold exceed the relevant trigger value of 5**

The $TER_{LT}$ values for application of MON 52276 are greater than the relevant trigger of 5, indicating low long term risk to mammals from glyphosate acid.

## IIIA 10.3.2 Effects on terrestrial vertebrates other than birds, where the required information is not provided by testing in accordance with points IIA 5 and IIIA 7, and where exposure is likely

The risk assessment above demonstrated that the proposed use of MON 52276 poses no unacceptable risk to mammals, and therefore further information is not considered necessary.

### IIIA 10.3.2.1 Acute oral toxicity of the preparation

Mammal toxicity tests with the formulation were not performed, since it is possible to extrapolate from data obtained with the active substance.

### IIIA 10.3.2.2 Acceptance of bait, granules or treated seeds by terrestrial vertebrates (palatability test)

MON 52276 is intended for use as a foliar spray, and therefore this information is not required.

### IIIA 10.3.2.3 Effects of secondary poisoning

Glyphosate acid has a log $P_{OW}$ value of -3.2 and its metabolite AMPA has a log $P_{OW}$ of -5.18. It was therefore not necessary to consider the risk from secondary poisoning further. Therefore, based on the low log $P_{OW}$ values the risk from bioaccumulation to fish-eating and worm-eating mammals is negligible.

## IIIA 10.3.3 Supervised cage or field trials or other appropriate studies

The risk assessment above demonstrated that the proposed use of MON 52276 poses no unacceptable risk to mammals, and therefore further studies are not considered necessary.

## IIIA 10.4 Effects on bees

Acute oral and contact toxicity studies on honey bees have been carried out with glyphosate (technical), glyphosate IPA-salt, glyphosate K-salt and the formulation MON 52276. Additionally, a study to address the toxicity of glyphosate (tested as the IPA salt) to bee brood was carried out. A summary of the relevant acute and long-term endpoints is depicted in IIA 8.3.1 and IIA 8.3.2. Full details of the tests are provided in the EU Annex II summary documentation.

**Table 10.4-1: Toxicity of glyphosate, its salts and formulation MON 52276 to bees**

| Active substance | EU agreed endpoints (SANCO/6511/VI/99-final) | Endpoints used in risk assessment [a] |
|---|---|---|
| Glyphosate (technical) | Oral (48 h) $LD_{50}$ >100 µg a.s./bee<br>Contact (48 h) $LD_{50}$ >100 µg a.s./bee | - |
| Glyphosate IPA salt | - | Contact (48 h) $LD_{50}$ > 100 µg a.s./bee |
| Glyphosate K salt | - | Oral (48 h) $LD_{50}$ > 104 µg a.s./bee<br>Contact (48 h) $LD_{50}$ > 100 µg a.s./bee |
| MON 52276 | - | Oral (48 h) $LD_{50}$ >254 µg MON 52276/bee (>177 µg a.s./bee)<br>Contact (48 h) $LD_{50}$ >330 µg MON 52276/bee (>100 µg a.s./bee) |
| Glyphosate IPA salt | - | Bee brood test NOAEL 266 mg a.s./kg |

[a] Since previous Annex I inclusion studies have been performed with the product MON 52276 and the active substance glyphosate and as a result there are new endpoints which are used in the risk assessment.



### Summary

MON 52276 is the representative formulation in the Annex I re-registration dossier of glyphosate. Therefore, all risk assessments and supporting data for MON 52276 with the proposed representative use patterns are provided within this section.

The risk assessment performed with MON 52276 data results in hazard quotients (HQ) considerably lower than the trigger value of 50, indicating that MON 52276 poses no unacceptable risk to bees when used according to the label recommendations.

### Risk assessment

### Toxicity

Table 10.4-2 presents the results of bee toxicity study with MON 52276. Further details regarding the test with the formulation MON 52276 are provided in Section 6, Point IIIA 10.4.2.

**Table 10.4-2: Toxicity to bees of MON 52276**

| Substance | Endpoint | Value | Reference/GLP |
|---|---|---|---|
| MON 52276 | 48 h contact $LD_{50}$ | >330 µg MON 52276/bee<br>>100 µg a.s./bee | IIIA 10.4.2/01<br>[redacted]00-2<br>[redacted]2001/yes* |
| | 48 h oral $LD_{50}$ | >254 µg MON 52276/bee<br>>77 µg a.s./bee | |

* Study not reviewed in the 2001 EU evaluation on glyphosate

**Exposure**

Applications of pesticides can potentially result in exposure of honeybees either through direct over-spray, or by contact with residues on plants whilst bees are foraging for food. In order to consider a worst-case scenario which also covers off-field exposure caused by spray drift, a maximum application rate of 2.88 kg a.s./ha is used for risk assessment purposes.

## IIIA 10.4.1 Hazard quotients for bees

**Acute risk to honeybees**

The acute risk to honeybees from use of MON 52276 was assessed using the maximum single application rate and the $LD_{50}$ values to calculate hazard quotients (***EPPO 2010***)[ii] as follows:

$$\text{Hazard Quotient} = \frac{\text{Maximum application rate (g a.s./ha)}}{\text{Acute } LD_{50} \text{ (µg a.s./bee)}}$$

Hazard quotients were calculated for oral exposure ($Q_{HO}$) and contact exposure ($Q_{HC}$) to MON 52276 with the highest individually applied dose of 2880 g a.s./ha and the toxicity endpoint of the formulation. A hazard quotient of less than 50 indicates a low risk to bees in the field. The results are shown in Table 10.4.1-1.

**Table 10.4.1-1: Risk to bees from exposure to MON 52276**

| Substance | Application rate [g a.s./ha] | $LD_{50}$ [µg a.s./bee] | Hazard quotient |
|---|---|---|---|
| MON 52276 | 2880 | Contact > 100 | <28.8 |
| | | Oral > 77 | <37.4 |

Both hazard quotients are less than 50 indicating that the active substances pose a low risk to bees. Therefore a low risk to bees is expected from the application of MON 52276.

**Chronic risk to honeybees**

**Qualitative risk assessment**

The potential effect of glyphosate on the development of honey bee brood was determined in a bee brood feeding study (summarised in IIA 8.7.4/01) performed in the field and in which colonies were exposed to glyphosate by feeding colonies treated sucrose solution. Conservative exposure doses for glyphosate were based on measured residues that were determined in a glasshouse residue study (summarised in IIA 8.7.3/01) following application of 8 L MON 52276/ha, equivalent to 2.88 kg a.s./ha onto flowering *Phacelia* and considering food requirements of bee colonies. Exposure estimates are regarded as conservative and worst case for foraging bees, since information was

derived from an enclosed greenhouse and the bees could only forage on highly attractive treated *Phacelia* flowers. Residue findings were adjusted to the spray application rate of 2.16 kg a.s./ha because this rate is the maximum single application rate in field crops and cereals. The rate of 2.88 kg a.s./ha is reserved for spot treatments in orchards along the base of trees and does not represent a worst-case exposure. Three dose levels were tested in the bee brood study. The lowest dose was based on the mean pollen and nectar residue concentrations measured over the first 3 days following the spray application (75 mg glyphosate a.e./L), the mid-dose was based on the highest residue concentrations measured in pollen and nectar following the spray application (150 mg glyphosate a.e./L) and the highest dose was twice this latter dose (301 mg glyphosate a.e./L).

In the test colonies, comb cells containing eggs, young and old larvae were selected and marked using the acetate overlay method prior to feeding of the colonies with the treated sucrose solution. Development and fate of the marked brood cells was observed throughout the study.

No adverse toxicological or behavioural effects on adult bees or bee brood development were observed in any of the glyphosate treated colonies. The NOAEL (No Observed Adverse Effect Level) for brood development was the highest dose tested, 301 mg glyphosate a.s./L nominal (245 mg glyphosate a.s./kg nominal; 266 mg glyphosate a.s./kg actual measured), indicating low chronic risk to honeybee colonies from the application of MON 52276 according to the proposed GAP.

**Quantitative risk assessment**

The chronic risk to honeybee colonies from use of MON 52276 was assessed by comparing the NOAEL determined in the bee brood feeding study with colony intake over the exposure period to calculate toxicity exposure ratios (***EPPO 2010***) as follows:

$$TER = \frac{\text{NOAEL (mg a.s./kg food)}}{\text{Intake } (mg\ a.s./colony)}$$

Toxicity exposure ratios were calculated for the potential exposure of bee colonies foraging on MON 52276 treated crops and the toxicity endpoint of the bee brood feeding test. A TER of more than 1 indicates a low risk to bees in the field (EPPO 2010). The results are shown in Table 10.4.1-2.

As already described, the lowest dose was based on the mean pollen and nectar residue concentrations measured over the first 3 days following the spray application (75 mg glyphosate a.s./L), the mid-dose was based on the highest residue concentrations measured in pollen and nectar following the spray application (150 mg glyphosate a.s./L) and the highest dose was twice this latter dose (301 mg glyphosate a.s./L). For additional details on the exposure study and development of the scenarios for dose setting see IIA 8.7.4/01.

The risk assessment is based on scenario 1, which represents the mean exposure levels from the worst-case green-house study with treated *Phacelia*.

**Table 10.4.1-2: Risk to honeybee colonies from exposure to MON 52276**

| Substance | NOAEL [mg a.s./kg food] | Glyphosate intake/colony [mg a.s.] | $TER_{LT}$ |
|---|---|---|---|
| Glyphosate acid | 266 | 75 | 3.5 |

The resulting $TER_{LT}$ value for application of MON 52276 is greater than the relevant trigger of 1, indicating low risk to honeybee brood development from exposure to glyphosate.

### IIIA 10.4.1.1 Oral exposure $Q_{HO}$

The hazard quotient for oral exposure of honeybees based on the maximum recommended field use rate is presented in Table 10.4.1-1 above.

### IIIA 10.4.1.2 Contact exposure $Q_{HC}$

The hazard quotient for contact exposure of honeybees based on the maximum recommended field use rate is presented in Table 10.4.1-1 above.

## IIIA 10.4.2 Acute toxicity of the preparation to bees

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| IIIA 10.4.2./01 | [redacted] | 2001 | Laboratory bioassays to determine acute oral and contact toxicity of MON 52276 to the honeybee, *Apis mellifera*<br>[redacted]<br>Report No: [redacted]00-2 version 2<br>Date: 2001-01-08<br>Monsanto S.A.<br>GLP: yes<br>not published |

**Guideline:** OECD Guidelines 213 (1998) and 214 (1998)
EPPO Guideline on test methods for evaluating the side-effects of plant protection products on honeybees. No. 170. (1992).

**Deviations to OECD 213 and 214:** In the oral test, the bees were deprived of food for 3-4 hours, instead of the 2 hours as specified in the guideline. The range of relatively humidity of the test chamber was larger than the guideline-recommended range of 60 – 70%. The condition of the bees in the control treatment indicated that these minor deviations from the protocol did not affect the survivor ship of the bees. Thus, these deviations were not considered to have affected the outcome of the study.

**Dates of experimental work:** 2000-08-14 to 2000-08-24



**Executive Summary**
In a single dose limit test, oral and contact toxicity of the formulated product MON 52276 to young adult worker bees (*Apis mellifera* L.) were tested using a single nominal dose of 134 µg glyphosate isopropylamine/bee, equivalent to100 µg glyphosate acid equivalent/bee for contact exposure and 103 µg glyphosate isopropylamine/bee, equivalent to 77 µg glyphosate acid equivalent/bee for oral exposure. 5 replicate cages, each containing 10 bees, were used for the test item treatments and control. Additionally, honeybees were treated with dimethoate as reference standard at concentrations ranging from 0.075 to 0.3 µg dimethoate/bee or with aqueous sucrose solution as a control (negative control). 3 replicate cages containing each 10 bees were used for the reference standard. Assessment of mortality was done after 1, 3, 24 and 48 hours.

The mortality in control varied between 2% and 4% in the 48-hour exposure. For both oral and contact exposures, the mortality did not reach or exceed 50%. The highest mortality was recorded for the contact toxicity test with 6% after test initiation. All validity criteria according to OECD guidelines 213 and 214 were fulfilled.

**The $LD_{50}$ (48 h) for honey bees exposed to MON 52276 were determined to be > 134 µg glyphosate isopropylamine/bee, equivalent to > 100 µg glyphosate acid equivalent/bee for contact exposure and 103 µg glyphosate isopropylamine/bee, equivalent to 77 µg glyphosate acid equivalent/bee for oral exposure, respectively.**

### I. MATERIALS AND METHODS

### A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item: | MON 52276 |
| Formulation type | Soluble concentrate (SL) |
| Description: | Dark yellow-coloured fluid |
| Active substance | glyphosate isopropylamine salt |
| Lot/Batch #: | 100399 |
| Purity: | 41.5% w/w glyphosate isopropylamine |
| | 30.3% w/w glyphosate acid equivalent (measured) |
| Density: | 1.168 g/cm$^3$ (nominal) |

**2. Vehicle and/or positive control:** [redacted] Dimethoate 40 (400 g dimethoate/L)

**3. Test organisms:**

| | |
|---|---|
| Species: | Honey bee (*Apis mellifera* L.) |
| Age: | Young adult worker bees |
| Source: | [redacted] UK |

**4. Environmental conditions**:

| | |
|---|---|
| Temperature: | 24 - 26°C |
| Humidity: | 46-83% |
| Photoperiod: | 24 h dark |



## B. STUDY DESIGN AND METHODS

**1. Experimental treatments:** Specimens were exposed to the formulated product MON 52276 in stainless steel coated 2 – 2.5 mm wire mesh tubes measuring 140 mm deep by 40 mm in diameter, closed by corks at both ends with bungs of polyurethane foam. Bees were placed in each cage and fed with 50% sucrose.

For the oral application, bees were deprived of food for 3-4 hours prior to dosing. Each cage (10 bees) was presented with approximately a 50% w/v aqueous sucrose solution (200 µL/cage) containing nominally 16.1 µg MON 52276/µL (5 µg a.e./µL). This corresponded to a target dose of 100 µg/bee. Control bees were presented with untreated sucrose solution. Toxic reference groups were presented with Dimethoate (as [redacted] Dimethoate 40) in 50% sucrose solution at three concentrations, equivalent to 0.3, 0.15, and 0.075 µg/bee. The bees were allowed to feed for five hours, at which time the feeding tube was replaced with tubes containing untreated sucrose solution. The original feeding tubes were weighed to quantify the test solution consume by the bees. Based on the test solution consumption, the mean dose corresponded to 254 µg MON 52276/bee (77 µg a.e./bee).

For the contact application, MON 52276 was diluted in solvent (water with 1% Farmon Blue[2]), and a 1-µL droplet was placed on the ventral thorax of lightly anaesthetized bees to give an exposure of 330 µg MON 52276/bee (100 µg a.e./bee). As a control, a separate group of bees was treated with 1µL of 1% aqueous Farmon Blue solution. A third group of bees was treated with a toxic reference (Dimethoate, as [redacted] Dimethoate 40) in 1% aqueous Farmon blue, applied at levels of 0.3, 0.15, and 0.075 µg/bee (1µL droplet per bee).

[2] Farmon Blue (nominally 87.3% w/w alkyl phenol ethylene oxide) is used to improve the adhesion of the droplets to the bee body and is non-toxic to bees.

For both oral and contact tests, there were five cages per treatments (5 replicates) containing each 10 worker bees, for a total of 50 bees per treatment. For the toxic reference group, there were 30 bees (3 replicates of 10 bees) per dose level. The toxic reference tests were conducted in two parts: the first part (conducted concurrently with the MON 52276 treatments) included the highest toxic reference treatment and a control group, while the second part was conducted later and included two lower treatments and a second control group. Bees were kept in groups of 10 at 24 – 26°C and 46 – 83% (mean 71%) relative humidity. Mortality and behavioural abnormalities were assessed 1,3,24, and 48 hours after dosing.

**2. Observations:** In all bioassays, an assessment of the condition of the bees was made 1, 3, 24 and 48 hours after treatment. The bees were classified as being live, affected, or moribund/dead.

**3. Statistical calculations:** The $LD_{50}$ was determined by visual observation of the data.

## II. RESULTS AND DISCUSSION

### A. FINDINGS AND OBSERVATIONS

The oral and contact $LD_{50}$ values are given below based on nominal concentrations.

| Endpoints (48 h) | glyphosate acid equivalent [µg/bee] | glyphosate isopropylamine [µg/bee] |
|---|---|---|
| $LD_{50}$ oral | > 77 | >103 |
| $LD_{50}$ contact | > 100 | >134 |

The mortality in control varied between 2% and 4% in the 48-hour exposure. For both oral and contact exposures, the mortality did not reach or exceed 50%. The highest mortality was recorded for the contact toxicity test with 6% after test initiation.

**Table 10.4.2-1: Oral toxicity of MON 52276 to honey bees (*Apis mellifera*)**

| Exposure | Mortality [%] | | Corrected mortality[b] [%] |
|---|---|---|---|
| | Control | 77µg a.e./bee [a]<br>103 µg a.s./bee [a] | |
| 24 h | 0 | 0 | |
| 48 h | 4 | 4 | 0 |

[a] based on mean weight of test solution of 5µg/µL consumed per cage of 10 bees, corrected for the density of the 50% w/w sugar solution
[b:] Corrected mortality according to Abbott (1925)
a.e = glyphosate acid equivalent, a.s.= glyphosate isopropylamine

**Table 10.4.2-2: Contact toxicity of MON 52276 to honey bees (*Apis mellifera*)**

| Exposure | Mortality [%] | | Corrected mortality[b] [%] |
|---|---|---|---|
| | Control | 100 µg a.e./bee<br>134 µg a.s./bee | |
| 24 h | 0 | 0 | - |
| 48 h | 2 | 6 | 4 |

[b:] Corrected mortality according to Abbott (1925)
a.e. = glyphosate acid equivalent, a.s. = glyphosate isopropylamine

For the reference product [redacted] Dimethoate 40, 100% mortality was observed in both contact and oral exposures for the highest test con concentration of 0.3 µg a.s./bee.

The mortality in the control treatments did not exceed 10%. The validity criteria according to guideline OECD 213 and 214 are therefore fulfilled.

## III. CONCLUSION

**The $LD_{50}$ (48 h) for honey bees exposed to MON 52276 were determined to be > 134 µg glyphosate isopropylamine/bee, equivalent to > 100 µg glyphosate acid equivalent/bee for contact exposure and 103 µg glyphosate isopropylamine/bee, equivalent to 77 µg glyphosate acid equivalent/bee for oral exposure, respectively.**

### IIIA 10.4.2.1 Acute oral toxicity

Acute oral and contact toxicity testing for bees is conducted in the same study. The acute oral and contact study endpoints are summarised in Point IIIA.10.4.2 in this document.

### IIIA 10.4.2.2 Acute contact toxicity

Acute oral and contact toxicity testing for bees is conducted in the same study. The acute oral and contact study endpoints are summarised in Point IIIA.10.4.2 in this document.

### IIIA 10.4.3 Effects on bees of residues on crops

As MON 52276 does not pose an unacceptable risk to honey bees, further testing is not required.

### IIIA 10.4.4 Cage tests

As MON 52276 does not pose an unacceptable risk to honey bees, further testing is not required.

### IIIA 10.4.5 Field tests

As MON 52276 does not pose an unacceptable risk to honey bees, further testing is not required.

### IIIA 10.4.6 Investigation of special effects

### IIIA 10.4.6.1 Larval toxicity

A bee brood study was undertaken to determine the potential for toxicity to developing honey bee larvae and pupae to glyphosate (tested as the IPA salt) and is summarised in IIA 8.7.4/01. Colonies of honey bees were fed with 75, 150 and 301 mg a.e./L of glyphosate, a toxic reference and a non-toxic control in 1 litre of 50% w/v sucrose. Dose levels for glyphosate were based on residues in pollen and nectar as measured in a glasshouse residues study (IIA 8.7.3/01) after application of 2.88 kg a.e./ha onto flowering *Phacelia* while considering food requirements of bee colonies and residue concentrations being adjusted to 2.16 kg a.e./ha. In the brood feeding study the lowest dose (75 mg glyphosate a.s./L) was based on the mean pollen and nectar residue concentrations over the first 3 days following the spray application, the mid-dose (150 mg glyphosate a.s./L) was based on the maximum mean residue concentrations determined in pollen and nectar following the spray application and the highest dose (301 mg glyphosate a.s./L) was twice this latter dose.

- In the test colonies, cells containing eggs, young and old larvae were selected and marked using the acetate overlay method prior to feeding of the colonies with the treated sucrose solution.

Development and fate of the marked brood cells was observed throughout the study. Over a 16 day observation period after dosing no adverse effects on adult mortality, development of eggs, young larvae and old larvae were observed in any treatment group. As the colonies consumed 1 L of sucrose solution over the exposure period, the overall NOAEL for brood development of honey bees was the highest dose tested – 301 mg glyphosate acid equivalent/L sucrose solution (nominal) equivalent to 245 mg glyphosate acid equivalent/kg nominal, when considering the density of the sucrose solution and 266 mg glyphosate acid equivalent/kg sucrose solution actually measured.

For details on the residue determination study as well as the bee brood feeding study please refer to AII 8.7.4/01.

### IIIA 10.4.6.2 Long residual effects

As MON 52276 does not pose an unacceptable long term risk to honey bees, further testing is not required.

### IIIA 10.4.6.3 Disorienting effects on bees

As MON 52276 does not pose an unacceptable risk to honey bees, further testing is not required.

### IIIA 10.4.7 Tunnel testing to investigate effects of feeding on contaminated honey dew or flowers

As MON 52276 does not pose an unacceptable risk to honey bees, further testing is not required.

## IIIA 10.5 Effects on arthropods other than bees

To assess the effects of MON 52276 on terrestrial non-target arthropods other than bees, six species of different arthropod taxa (*Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Chrysoperla carnea*, *Aleochara bilineata*, *Poecilus cupreus*, and *Pardosa spp.*) were exposed to MON 52276. Several of the studies covering a decade of testing were performed under the guidance and a risk assessment scheme which preceded ESCORT II (Candolfi et al., 2001). The new tests were chosen according to the recommendations of ESCORT II and represent different ecological groups. The tests cover different levels of exposure from laboratory trials on inert substrate to extended laboratory trials. The results of the studies are summarized in Table 10.5-1.

The studies summarised in Table 10.5-1 have been evaluated as part of the current Annex I listing and are therefore not submitted with this dossier. Existing data on non-target arthropods were assessed during the 2001-EU Evaluation and are summarised in SANCO/6511/VI/99-final. In support of that, additional studies with the lead formulation MON 52276 were conducted with *Aphidius rhopalosiphi*, *Typhlodromus pyri* and *Aleochara bilineata* and are summarised in IIA 8.8.2.

**Table 10.5-1: Toxicity of MON 52276 to terrestrial non-target arthropods other than bees**

| Species | Test type | Treatment rates [L/ha] | Results | Reference/GLP |
|---|---|---|---|---|
| *Typhlodromus pyri* | Laboratory (inert substrate) | 10 | $LR_{50}$ < 3.6 kg a.s./ha<br>100% mortality | 95 10 48 056<br>[redacted]1995/yes |
| *Typhlodromus pyri* | Extended laboratory (leaf discs) | 36% mortality at 3 L/ha;<br>> 75% mortality at 6 and 12 L/ha;<br>No effects on fecundity at 3 L/ha after 21 d | | [redacted]98-195<br>[redacted]1998/yes |
| *Typhlodromus pyri* | Extended laboratory (leaf discs) | No effect on mortality at 16 L/ha<br>No effects on fecundity at 8 L/ha | | IIA 8.8.2.2/01<br>[redacted]2009-404<br>[redacted]2010/yes |
| *Typhlodromus pyri* | Extended laboratory (whole leaf) | $LR_{50}$ > 4.32 kg a.s./ha<br>36% and 30% mortality at 6 and 12 L/ha, respectively;<br>21% mortality at 3 L/ha;<br>No mortality at 0.6 L/ha;<br>No effects on fecundity at 12 L/ha or less | | [redacted]99-092<br>[redacted]1999/yes |
| *Aphidius rhopalosiphi* | Laboratory (inert substrate) | 10 | $LR_{50}$ < 3.6 kg a.s./ha<br>100% mortality | 95 10 48 054<br>[redacted]1995/yes |
| *Aphidius rhopalosiphi* | Extended laboratory (whole plant) | $LR_{50}$ > 4.32 kg a.s./ha<br>No effect on mortality at 12 L/ha<br>No effects on fecundity at 12 L/ha | | 98 10 48 066<br>[redacted]1999/yes |
| *Aphidius rhopalosiphi* | Extended laboratory (whole plant) | No effect on mortality at 16 L/ha<br>No effects on fecundity at 16 L/ha | | IIA 8.8.2.1/01<br>[redacted]2009-405<br>[redacted]2010/yes |
| *Chrysoperla carnea* | Laboratory (inert substrate) | $LR_{50}$ > 2.16 kg a.s./ha<br>59% mortality and 20% reduction on fecundity at 12 L/ha (no dose-response effect on fecundity);<br>No effects on survival or fecundity at 0.6 and 6 L/ha | | [redacted]99-3<br>[redacted]1999/yes |
| *Aleochara bilineata* | Extended Laboratory (soil) | $LR_{50}$ > 4.32 kg a.s./ha<br>No effect on mortality at 12 L/ha<br>No effects on fecundity at 12 L/ha | | IIA 8.8.2.3/01<br>[redacted]2009-403<br>[redacted]2010/yes |
| *Poecilus cupreus* | Laboratory (inert substrate) | 10 | $LR_{50}$ > 3.6 kg a.s./ha<br>No effects on survival or feeding activity | [redacted]2000-203<br>[redacted]1995/yes |

| Species | Test type | Treatment rates [L/ha] | Results | Reference/GLP |
|---|---|---|---|---|
| *Pardosa sp.* | Laboratory (inert substrate) | 10 | $LR_{50}$ > 3.6 kg a.s./ha<br>No effects on survival or feeding activity | [redacted] 2000-204<br>[redacted] 1995/yes |

**Studies shaded in grey have been reviewed as part of the 2001 EU evaluation.**

Two further non-GLP studies were conducted exposing the carabid beetles (*Trechus quadristriatus* and *Bembidion lampros*) to different glyphosate formulations (Roundup and PMG 360) at rates of 3.6 kg a.s./ha and 4.89 kg a.s./ha. No effect was observed in any of these studies.

## Exposure

### In-field exposure

Non-target arthropods living in the crop can be exposed to residues from MON 52276 by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items. MON 52276 is applied at a maximum single application rate of 2.88 kg a.s./ha, equivalent to 8 L MON 52276/ha.

The in-field exposure (predicted environmental residues [PER]) is calculated according to ESCORT 2 using the following equation:

$$PER_{in-field} = Application\ rate\ (g\ product/ha) \times MAF$$

The MAF is a generic multiple application factor which is used to take into account the potential build-up of applied substances between applications based on the application interval, $DT_{50}$ value, and number of applications. Default foliar and soil MAF values are given in the ESCORT 2 Guidance Document but have not been used for foliar exposures. As explained in the birds and mammal assessment, a repeat application of MON 52276 is only made to control new growth of weeds which would not have been exposed to the preceding application. Therefore, it is not appropriate to use a multiple application factor (MAF) for foliar residues in the case of this total herbicide. It is also to be emphasized, as reviewed in the birds and mammals assessments that glyphosate residues rapidly decline on foliage, with a $DT_{50}$ value significantly less than the default values making this a conservative assessment.

The maximum predicted environmental residues (PER) occurring within the field after application of MON 52276 at the maximum application rate are presented in Table 10.5-2.

**Table 10.5-2: In-field exposure calculation for MON 52276**

| Crop | Max. single application rate [kg a.s./ha] | Max. Number of Applications | PER (foliar) [kg a.s./ha] | PER (soil) [kg a.s./ha] |
|---|---|---|---|---|
| All crops | 2.16 | 2 | 2.16 | 4.320 [1] |
| Cereals | 2.16 | 1 | 2.160 | 2.160 |
| Oilseed rape | 2.16 | 1 | 2.160 | 2.160 |
| Orchard crops, vines | 2.88 | 3 | 2.88 [1] | 4.320 [1] |

[1] a maximum total annual application rate of 4.32 kg a.s./ha is not to be exceeded

The maximum in-field exposure (predicted environmental rate [PER]) to foliar-dwelling and soil-dwelling organisms are 2.88 kg a.s./ha (equivalent to 8 L MON 52276/ha) and 4.32 kg a.s./ha (equivalent to 12 L MON 52276/ha), assuming the worst-case (contradiction) of 100% crop interception and 0% crop interception, respectively.

**Off-field exposure**

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration, and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to MON 52276 mainly is due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure via soil residues in off-field areas is not considered. Off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the BBA (2000) [iii] as shown in the following equation:

$$Off-field\ \ foliar\ PER = \frac{maximum\ in-field\ foliar\ PER \times \left(\frac{\%\ drift}{100}\right)}{vegetation\ distribution\ factor}$$

**Vegetation distribution factor**

The model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERS to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies (*i.e.* where spray treatment is applied onto whole plants) the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design. Due to the fact that the ecotoxicologically relevant studies on *Typhlodromus pyri* and *Aphidius rhopalosiphi* were conducted by application of spray on whole plants, the vegetation distribution factor is not considered in the following risk assessment.

The resulting off-field PER values are shown in Table 10.5-3.

**Table 10.5-3: Off-field exposure calculation for MON 52276**

| Crop | Study type | Maximum in-field foliar PER [kg a.s./ha] | Drift factor (% drift/100) | Off-field foliar PER [kg a.s./ha] |
|---|---|---|---|---|
| All crops | 3-dimensional | 2.160 | 0.0238 | 0.0514 |
| Cereals, oilseed rape | 3-dimensional | 2.160 | 0.0277 | 0.0598 |
| Orchard crops, vines [1] | 3-dimensional | 2.880 | 0.0201 | 0.0579 |

[1] Due to the fact that only ground directed application is intended, drift values for vegetables, ornamentals and small fruits <50 cm are considered.

## Risk assessment

Risk assessments were conducted to examine the potential effects of MON 52276 on non-target arthropods following the guidance of ESCORT 2 (2001). According to the guidance, a tiered approach is proposed, whereby Tier 1 testing and risk assessments should be carried out using dose-response data for the representative sensitive indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi*.

The detailed Tier 1 and Tier 2 data for the indicator species as well as the crop relevant species are presented in Table 10.5-1. The following equations were used to calculate the in-field and off-field hazard quotients (HQs):

$$In\ field\ HQ = \frac{In\text{-}field\ PER\ (g/ha)}{LR_{50}\ (g/ha)} \quad \text{and}$$

$$Off\text{-}field\ HQ = \frac{PER_{off\text{-}field}\ (g/ha)}{LR_{50}\ (g/ha)} \times Correction\ factor$$

Due to the fact that in the two Tier 1 worst-case laboratory studies, no $LR/LC_{50}$ was determined for *Typhlodromus pyri* and *Aphidius rhopalosiphi*, the risk assessment is based on Tier 2 extended laboratory studies. The HQ trigger for Tier 2 extended laboratory studies is 1.

The resulting HQ in-field values are presented in Table 10.5-4.

**Table 10.5-4: In-field HQs for non-target arthropods (*T. pyri and A. rhopalosiphi;* Tier 2, 3D exposure scenario) exposed to MON 52276**

| Crop scenario | $LR_{50}$ [kg a.s./ha] | In-field foliar | | In-field soil | | Trigger value |
|---|---|---|---|---|---|---|
| | | PER [kg a.s./ha] | HQ | PER [kg a.s./ha] | HQ | |
| All crops | >4.32 | 2.16 | <0.50 | 4.32 | <1.0 | 1 |
| Cereals, oilseed rape | >4.32 | 2.16 | <0.50 | 2.16 | <0.50 | |
| Orchard crops, vines | >4.32 | 2.88 | <0.67 | 4.32 | <1.0 | |

The off-field HQ values are given in Table 10.5-5.

**Table 10.5-5: Off-field HQs for non-target arthropods (*T. pyri and A. rhopalosiphi;* Tier 2, 3D exposure scenario) exposed to MON 52276**

| Crop scenario | $LR_{50}$ [kg a.s./ha] | Off-field foliar PER [kg a.s./ha] | Correction factor | Off-field foliar HQ | Trigger value |
|---|---|---|---|---|---|
| All crops | >4.32 | 0.0514 | 5 | <0.101 | 1 |
| Cereals, oilseed rape | >4.32 | 0.0598 | 5 | <0.069 | |
| Orchard crops, vines | >4.32 | 0.0579 | 5 | <0.101 | |

HQ = Off-field foliar PER *Correction factor / $LR_{50}$

For *Aphidius rhopalosiphi* and *Typhlodromus pyri*, the trigger value of HQ = 1 demonstrates that no risk or unacceptable effects are expected from the use of MON 52276 considering in-field or off-field habitats.

In addition to the laboratory and extended laboratory studies conducted with *T. pyri* and *A. rhopalosiphi*, laboratory and extended laboratory studies are available with the foliage-dweller *Chrysoperla carnea* and the soil-dwelling predators *Aleochara bilineata, Poecilus cupreus* and *Pardosa spp*. The additional species tested represent different specific ecological compartments and therefore provide additional information for use in the risk assessment.

**Foliage-dwelling arthropod species**

**a) *Chrysoperla carnea***

In a worst case laboratory study conducted on inert substrate (i.e. glass plates) ([redacted] 1999), the foliage dwelling species *Chrysoperla carnea* was exposed to fresh air-dried spray residues of 0.6 to 12 L MON 52276/ha. The $LR_{50}$ was determined to be > 6.0 and < 12 L MON 52276/ha. A reduction of 20% of fecundity was observed at the highest rate tested, 12 L MON 52276/ha. No unacceptable effects on survival and reproduction were observed at any of the lower application rates tested, concluding in an $ER_{50}$ > 12 L MON 52276/ha; equivalent to 4.32 kg a.s./ha.

The $ER_{50}$ exceeds the worst-case in-field exposure of 2.88 kg a.s./ha. Also, the $ER_{50}$ is 72-fold the worst case off-field exposure of 59.8 g a.s./ha. The $LR_{50}$ of > 6.0 L MON 52276/ha, equivalent to 2.16 kg a.s./ha equals the maximum single application rate for all pre-plantings and pre-emergence applications and the application on cereals and oilseed rape. With regard to the application in the understorey of orchards and vines, due to the fast degradation of glyphosate on foliar material ($DT_{50}$, foliar = 2.8 days), a rapid re-colonization from untreated adjacent plots is anticipated. Therefore, no risk or unacceptable effects are expected for *Chrysoperla carnea* resulting from the intended use of MON 52276.

**The results of the worst-case laboratory study indicate that no risk or unacceptable effects on foliage dwelling arthropods will be anticipated assuming in-field and off- field exposure scenarios. No risk or unacceptable effects on non-target arthropods will be anticipated for both in- and off-field habitats, resulting from the use of MON 52276 according to the proposed use pattern.**

**Soil dwelling arthropod species**

Studies on three representative species of this group are available; *Aleochara bilineata*, *Poecilus cupreus*, and *Pardosa* spp. (see Table 10.5-1).

**a)** ***Aleochara bilineata***

An extended laboratory study was conducted with MON 52276 on the reproduction capacity of the staphylinid beetle *Aleochara bilineata* (IIA 8.8/03, [redacted] 2010). The beetles were exposed to rates of up to 12.0 L MON 52276/ha applied on a soil substrate. No effects on mortality and reproduction capacity of *Aleochara bilineata* were observed. Accordingly, the resulting $LR/ER_{50}$ was determined to be >12.0 L MON 52276/ha, equivalent to 4.32 kg a.s./ha. As already shown by the HQ-approach for the indicator species, no in-field effects for *A. bilineata* are expected from the use of MON 52276. The $ER_{50}$ exceeds the worst-case in-field exposure and is 72-fold the worst case off-field exposure of 59.8 g a.s./ha. Therefore, no risk or unacceptable effects are expected for *A. bilineata* resulting from the intended use of MON 52276.

**b)** ***Poecilus cupreus***

In a worst-case laboratory study, adults of *Poecilus cupreus* were exposed to residues of MON 52276 on inert substrate (i.e. quartz sand), applied via direct overspray ([redacted] 1995). No mortality or effects on feeding activity occurred at the tested rate of 10 L MON 52276/ha, resulting in an $LR/ER_{50}$ of >10 L MON 52276/ha, equivalent to 3.6 kg a.s./ha.

The $LR/ER_{50}$ are not covered by the maximum worst case in-field exposure rate of 4.32 kg a.s./ha. However, due to the high mobility of *P. cupreus* a rapid recolonisation is expected. The $LR/ER_{50}$ is about 60-fold the worst case off-field exposure of 59.8 g a.s./ha. Therefore, no risk or unacceptable effects are expected for *P. cupreus* resulting from the intended use of MON 52276. However, as the highest in-field PER is not covered by the highest rate tested, the results of this study may be regarded as supplemental information.

**c)** ***Pardosa spp.***

In a worst-case laboratory study, adults of *Pardosa spp.* were exposed to residues of MON 52276 on inert substrate (i.e. quartz sand), applied via direct overspray ([redacted] 1995). No mortality or effects on feeding activity occurred at the tested rate of 10 L MON 52276/ha, resulting in an $LR/ER_{50}$ of >10 L MON 52276/ha, equivalent to 3.6 kg a.s./ha.

The $LR/ER_{50}$ is about 60-fold the worst case off-field exposure of 59.8 g a.s./ha. Therefore, no risk or unacceptable effects are expected for *Pardosa spp.* resulting from the intended use of MON 52276. However, as the highest in-field PER is not covered by the highest rate tested, the results of this study may be regarded as supplemental information.

**The results of the worst case laboratory studies indicate that no risk or unacceptable effects on soil dwelling arthropods will be anticipated assuming in-field and off- field exposure scenarios. No risk or unacceptable effects on non-target arthropods will be anticipated for both in- and off-field habitats, resulting from the use of MON 52276 according to the proposed use pattern.**

**On account of the available laboratory and extended laboratory studies and based on the respective assessment schemes, no risk or unacceptable effects from MON 52276, applied according to the recommended use pattern, are to be expected for non-target arthropods other than bees in-field and off-field exposure scenarios.**

## IIIA 10.5.1 Effects on sensitive species already tested, using artificial substrates

Testing for effects on arthropod species other than bees was carried out using the formulated product MON 52276 rather than the active substance. Laboratory studies were conducted to assess effects on the sensitive indicator species; the Phytoseiid mite (*Typhlodromus pyri*) and parasitic wasp (*Aphidius*

*rhopalosiphi*). Further studies were conducted on the green lacewing (*Chrysoperla carnea*), the carabid beetles *Poecilus cupreus* and the lycosiid spider *Pardosa ssp.* Summaries of these studies are given in the current Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.8 and the 2001 EU evaluation on glyphosate (Annex B, B 8, 2000).

## IIIA 10.5.2 Effects on non-target terrestrial arthropods in extended laboratory tests

Summaries of extended laboratory non-target arthropod studies performed on MON 52276 are given in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.8 and the monograph on glyphosate (Annex B, B 8, 2000).

## IIIA 10.5.3 Effects on non-target terrestrial arthropods in semi-field tests

The results presented on non-target arthropods other than bees indicate low risk of glyphosate and the formulation MON 52276 when applied at maximum recommended application rate and according to good agricultural practices. Therefore, no further studies are required.

## IIIA 10.5.4 Field tests on arthropod species

The results presented on non-target arthropods other than bees indicate low risk of glyphosate and the formulation MON 52276 when applied at maximum recommended application rate and according to good agricultural practices. Therefore, no further studies are required.

## IIIA 10.6 Effects on earthworms and other soil macro-organisms

Acute and chronic earthworm toxicity studies have been carried out with glyphosate acid, glyphosate IPA salt, the formulation MON 52276 and the metabolite AMPA.

Further details of the acute study with the formulation are given in Point IIIA 10.6.2 below. Further details of the acute and chronic studies with glyphosate, its salts and its metabolite AMPA are given in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6. The acute and chronic earthworm toxicity endpoints are summarised in Table 10.6-1.

**Table 10.6-1: Acute and chronic toxicity of glyphosate acid, IPA-salt, its metabolite AMPA and formulation MON 52276 to earthworms, soil mites and springtails**

| Species | Test design | $LC_{50}$ (mg a.s./kg dry soil) | NOEC (mg a.s./kg dry soil) | EU agreed endpoints (SANCO/6510/VI/99-final) [3] | Reference/GLP |
|---|---|---|---|---|---|
| **Glyphosate acid** | | | | | |
| *Eisenia fetida* | 14 d acute | **>1000** | 1000 [1] | - | [redacted]502 [redacted] 1995/yes |
| *Eisenia fetida* | 14 d acute | | >480 [4] | | Glyphosate monograph, 1998 p51 |
| *Eisenia fetida* | 14 d acute | >1000 | - | | [redacted]96-00099 [redacted] 1990/yes |
| *Eisenia fetida* | 14 d acute | >1000 | 1000 [1] | - | [redacted]96-00095 [redacted]et al., 1995/yes |
| *Eisenia fetida* | 14 d acute | 5600 – 10000 | <5600 | - | IIA 8.9.1/01 [redacted]1875 [redacted] 2002/yes |
| **Glyphosate-IPA salt** | | | | | |
| *Eisenia fetida* | 14 d acute | >1000 | 1000 [1] | - | [redacted]92-0024-01 [redacted] *et al.*, 1992/yes |
| *Eisenia fetida* | 14 d acute | >5000 | 158.2 | - | 80-91-2078-06-91 [redacted]1995/yes |
| *Eisenia fetida* | 56 d chronic | - | 28.79 [1] 21.31 mg a.e. /kg dry soil | 28.79 [1] 21.31 mg a.e. /kg dry soil | IIA 8.9.2/02 [redacted]1173 [redacted] 2000/yes |
| *Eisenia fetida* | 56 d chronic | >638.1 | 638.1 [1] **472.8 mg a.e. /kg dry soil** | - | IIA 8.9.2/01 09 10 48 056 [redacted] 2009/yes |
| *Hypoaspis aculeifer* | 14 d chronic | >1000 mg/kg >472.8 mg a.e./kg | 1000 mg/kg **472.8 mg a.e./kg** | - | IIA 8.9.2/05 09 10 48 058 [redacted] [redacted]2009/yes |
| *Folsomia candida* | 28 d chronic | >1000 mg/kg >587 mg a.e./kg | 1000 mg/kg **587 mg a.e./kg** | - | IIA 8.9.2/06 09 10 48 057 [redacted] [redacted] 2010/yes |

| Species | Test design | $LC_{50}$ (mg a.s./kg dry soil) | NOEC (mg a.s./kg dry soil) | EU agreed endpoints (SANCO/6511/VI/99-final) [2, 3] | Reference/GLP |
|---|---|---|---|---|---|
| **MON 52276** | | | | | |
| *Eisenia fetida* | 14 d acute | >1250 mg MON 52276/kg >388 mg a.e./kg | 270 mg MON 52276/kg 83.7 mg a.e./kg | - | IIIA 10.6.2/01 139-306 [redacted] 1992/yes |
| **AMPA** | | | | | |
| *Eisenia andrei* | 14 d acute | **>1000** | 100 | - | IIA 8.9.1/02 [redacted] 2000/yes |
| *Eisenia fetida* | 56 d chronic | - | 28.12 [1] | - | IIA 8.9.2/02 [redacted] 2000/yes |
| *Eisenia fetida* | 56 d chronic | - | 10.7 [1] | - | IIA 8.9.2/03 011120 [redacted] 2000/yes |
| *Eisenia fetida* | 56 d chronic | - | **100.1** | - | IIA 8.9.2/04 01-64-077 [redacted] 2003/yes |
| *Hypoaspis aculeifer* | 14 d chronic | >320 | **320** | - | IIA 8.9.2/07 10 10 48 053 [redacted] 2010/yes |
| *Folsomia candida* | 28 d chronic | >315 | **315** | - | IIA 8.9.2/08 10 10 48 054 [redacted] 2010/yes |

[1] Highest concentration tested
[2] Since the 2001 EU evaluation on glyphosate new studies on the active substance have been performed and as a result there are new endpoints which are used in the risk assessment.
[3] The proposed new endpoints are highlighted with bold type.
[4] There appears to be a minor error in the Glyphosate Monograph endpoint list; despite having reviewed the study by Hänisch et al. (1991), no agreed endpoint was listed in the quoted reference as study was conducted with a formulation containing glyphosate trimesium, not with glyphosate acid.



For evaluation of acute risk of glyphosate acid on *Eisenia fetida*, a new endpoint ($LC_{50}$ >1000 mg a.s./kg dry soil) is proposed (see Table 10.6-1). The original endpoint of $LC_{50}$ > 480 mg a.s./kg dry soil of the Glyphosate 2001 monograph was derived from a formulation study conducted with glyphosate trimesium ([redacted] *et al.* 1991, erroneously listed as 360 SL formulation), which is not considered in the current submission. In addition, the study conducted by [redacted] *et al.* (1991) was a limit test, i.e. no effect was observed at the highest concentration tested.

**Summary**

MON 52276 is the representative formulation in the Annex I re registration dossier of glyphosate. Therefore, all risk assessments and supporting data for MON 52276 with the proposed use patterns are provided within this document. $PEC_{soil,\ plateau}$ values are provided in MON 52276, Point IIIA 9.4. Full details are provided in that section of how the $PEC_{soil}$ are calculated.

Table 10.6-2 summarizes the TERs for all the relevant organisms.

**Table 10.6-2: Toxicity data for soil macro-organisms**

| Use pattern | Organism | Proposed toxicity endpoint[1] [mg/kg dry soil] | Maximum $PEC_{soil, plateau}$ [mg/kg dry soil] | TER | Risk assessment trigger |
|---|---|---|---|---|---|
| **Glyphosate acid/Glyphosate IPA** | | | | | |
| 1 × 4320 g a.s./ha | *Eisenia fetida* | 14 d $LC_{50}$ >1000 | 8.065 | >124 | 10 |
| 1 × 4320 g a.s./ha | *Eisenia fetida* | 56 d NOEC = 472.8 | 8.065 | 58.6 | 5 |
| 1 × 4320 g a.s./ha | *Hypoaspis aculeifer* | 14 d NOEC = 472.8 | 8.065 | 58.6 | 5 |
| 1 × 4320 g a.s./ha | *Folsomia candida* | 28 d NOEC = 587 | 8.065 | 72.8 | 5 |
| **AMPA** | | | | | |
| 1 × 4320 g a.s./ha | *Eisenia andrei* | 14 d $LC_{50}$ >1000 | 5.345 | >187 | 10 |
| 1 × 4320 g a.s./ha | *Eisenia fetida* | 56 d NOEC = 198.1 | 5.345 | 37.1 | 5 |
| 1 × 4320 g a.s./ha | *Hypoaspis aculeifer* | 14 d NOEC = 320 | 5.345 | 59.9 | 5 |
| 1 × 4320 g a.s./ha | *Folsomia candida* | 28 d NOEC = 313 | 5.345 | 58.9 | 5 |

[1] Since the 2001 EU evaluation on glyphosate new studies on the active substance have been performed and as a result these are proposed endpoints which are used in the risk assessment.

In the study conducted with the formulation MON 52276 it is clearly demonstrated that the toxicity of the formulation is within the same range as for the active substance glyphosate, i.e. within one order of magnitude.

The TER values calculated using worst-case $PEC_{soil, plateau}$ values for glyphosate and its metabolite AMPA (see Section 5 for full calculations) exceeded the relevant triggers, indicating that the risk to soil macro organisms is acceptable following use of MON 52276.

## IIIA 10.6.1 Toxicity exposure ratios for earthworms, $TER_A$ and $TER_{LT}$

The TER values were determined for MON 52276, and the glyphosate metabolite AMPA based on the ratio of the $LC_{50}$ or NOEC values to the maximum $PEC_{soil, plateau}$.

The maximum $PEC_{soil}$ values were calculated following the recommendations of the FOCUS soil working group (FOCUS, 1997) assuming a soil depth of 5 cm, a bulk density of 1.5 g/cm$^3$ and an application rate of 4.32 kg glyphosate/ha following single application of MON 52276. For field crops, in addition to the worst case soil depth of 5 cm an accumulation following ploughing in 20 cm is considered, for orchard crops, vines and other no-tillage crop scenarios a $PEC_{plateau, max}$ for a soil depth of 5 cm was calculated. A detailed description of $PEC_{soil}$ calculations for glyphosate and its metabolite AMPA is provided in the Glyphosate EU Dossier, Annex IIIA, Document M-III, Section 5.

**Acute toxicity: Exposure**

The potential acute risk of MON 52276 and AMPA to earthworms was assessed by comparing the maximum instantaneous $PEC_s$ with the 14-day $LC_{50}$ value to generate acute TER values. The studies performed with glyphosate acid, glyphosate IPA-salt and AMPA were conducted in soils with 10% organic matter. However, as the log $P_{ow}$ value for glyphosate and AMPA is <2, there was no need to reduce the $LC_{50}$ by a factor of 2 in order to account for the organic matter content of the artificial test soil. The $TER_A$ was calculated as follows:

$$TER_a = \frac{LC_{50}\,(mg/kg)}{PEC_S\,(mg/kg)}$$

The resulting $TER_A$ values are shown in Table 10.6.1-1.

**Table 10.6.1-1: Acute TER values for earthworms**

| Parent compound | Test substance | $LC_{50}$ [mg a.s./kg dry soil] | Maximum $PEC_{soil, plateau}$ [mg/kg dry soil] | $TER_A$ |
|---|---|---|---|---|
| Glyphosate | Glyphosate acid | 5600 | 8.065 | 694 |
| | Glyphosate IPA salt | >1000 | 8.065 | >124 |
| | MON 52276 | >388 | 8.065 | >48 |
| | AMPA | >1000 | 5.345 | >187 |

All the acute TER values are much higher than the Annex VI acute trigger value of 10, indicating that MON 52276 poses low acute risk to earthworms when applied according to the proposed use rates.

**Chronic toxicity: Exposure/Long –term risk**

The potential long-term risk of MON 52276 and AMPA to earthworms was assessed by calculating long-term TER ($TER_{LT}$) values by comparing the NOEC values and the maximum instantaneous $PEC_s$ using the following equation

$$TER_{LT} = \frac{NOEC(mg/kg)}{PEC_s(mg/kg)}$$

The resulting $TER_{LT}$ values are presented in Table 10.6.1-2.

**Table 10.6.1-2: Long-term TER values for earthworms**

| Test substance | NOEC [mg a.s./kg dry soil] | Maximum $PEC_{soil, plateau}$ [mg/kg dry soil] | $TER_{LT}$ |
|---|---|---|---|
| Glyphosate IPA salt | 472.8 | 8.065 | 58.6 |
| AMPA | 198.1 | 5.345 | 37.1 |

The $TER_{LT}$ values exceed the relevant Annex VI decision-making criteria of 5 for earthworms. Therefore, it can be concluded that chronic risk to earthworms for glyphosate from the use of MON 52276 in all crops according to the proposed good agricultural practice will be low.

## IIIA 10.6.2 Acute toxicity to earthworms

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| IIIA 10.6.2/01 | [redacted] | 1992 | MON 52276: An acute study with the earthworm in an artificial soil substrate.<br>[redacted]<br>Report No: 139-306<br>Date: 1992-09-18<br>GLP: yes<br>Owner: Monsanto S.A.<br>Not published |

**Guideline:** OECD Guideline No. 207

**Deviations to OECD 207:** Light intensity measurements were not taken during the exposure period. The test chambers were covered with a metal lid with ventilation holes, rather than a glass plate or plastic film. The average temperature was slightly higher than specified in the guideline. These deviations were not considered to have affected the outcome of the study because conditions for the validity of the study were met.

**Dates of experimental work:** 1991-08-22 to 1991-09-18



**Executive Summary**

The effects of MON 52276 on the earthworm *Eisenia fetida andrei* were tested in a 14 days acute laboratory test with regard to the parameters mortality, development of body weight and alteration in behaviour and appearance. The test was conducted with five test concentrations (162, 270, 450, 750 and 1250 mg test item/kg dry soil) and a negative control in OECD soil containing 10% peat moss.

After 14 days, no mortality was observed in any of the treatment groups and in the control. Furthermore, no treatment related effect on worm body weight was observed. However, at a concentration level of 450 mg test item/kg dry soil and higher, earthworms showed effects on behaviour and appearance. All validity criteria according to the OECD guideline 207 were fulfilled.

**In conclusion, the 14-day $LC_{50}$ of MON 52276 for earthworms is considered to be > 388 mg glyphosate acid/kg dry soil. The no observed effect concentration (NOEC) was determined to be 83.7 mg glyphosate acid/kg dry soil.**

### I. MATERIALS AND METHODS

### A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item:: | MON 52276 (Roundup) |
| Description: | Clear yellow liquid |
| Lot/Batch #: | LLN-9105-3135F |
| Purity: | 30.95 % |

**2. Vehicle and/or positive control:** None

**3. Test organism:**

| | |
|---|---|
| Species: | Earthworm (*Eisenia fetida andrei*) |
| Age: | Adults, with clitellum |
| Weight: | 320 - 410 mg (test initiation) |
| Source: | [redacted] USA |
| Food: | None |
| Acclimation period: | Earthworms were acclimatised to the artificial soil for a period of around 24 h. |

**4. Environmental conditions:**

| | |
|---|---|
| Temperature: | 23 ± 1°C |
| Photoperiod: | 24 h light |
| Relative humidity: | 20 ± 11% |
| Soil pH: | 5.73 (test initiation); 5.22 – 5.59 (test termination) |
| Soil moisture content: | 19 % (test initiation); 14.70 – 16.75 (test termination) |



## B. STUDY DESIGN AND METHODS

**1. Experimental treatments:** The test was conducted with five test concentrations: 162, 270, 450, 750 and 1250 mg test item/kg dry soil and a negative control. The test item was suspended into deionised water and the suspension was mixed into the artificial soil substrate (10% sphagnum peat; 20% kaolin clay, 70% quartz sand and 0.2% calcium carbonate). The moisture content was adjusted to 19% (35% of the water holding capacity) using deionised water. Four replicate test containers (quart size glass jars containing approximately 750 g soil, wet weight) were prepared for each treatment group. 10 adult earthworms per replicate (a total of 40 worms) were exposed for 14 days. The negative control was treated with deionised water only. Temperature and relative humidity in the study room were recorded twice a day during the test period. Soil water content and pH were determined at the beginning and the end of the test.

**2. Observations:**

Mortality: The replicates were examined for live and dead earthworms after 7 and 14 days.

Behaviour and appearance: Effects on behaviour and appearance were noted after 7 and 14 days, when mortality was determined.

Mean Body Weights: All surviving earthworms per replicate were weighed as a group and average individual weights were calculated prior to test initiation and at day 14 after application.

**3. Statistical calculations:** A statistical evaluation was not conducted due to a lack of observed mortality in this test. Descriptive statistics were used to determine toxicity endpoints.

## II. RESULTS AND DISCUSSION

## A. FINDINGS

The 14 d $LC_{50}$ and NOEC values are given below based on nominal concentrations.

| Endpoints | Test item [mg/kg dry soil] |
|---|---|
| $LC_{50}$ (14 d) | > 1250 |
| NOEC (14 d) | 270 |

## B. OBSERVATIONS

**Table 10.6.2-1: Mortality and observations of the earthworm (*Eisenia fetida andrei*) exposed to MON 52276**

| Nominal MON 52276 [mg/kg dry soil] | % Mortality Day 14 | Observations[1] | | Day 14 mean bodyweight as % of Day 0 value |
|---|---|---|---|---|
| | | Day 7 | Day 14 | |
| 0 | 0 | 40 AN | 40 AN | + 10.1 |
| 162 | 0 | 40 AN | 40 AN | + 3.7 |
| 270 | 0 | 40 AN | 40 AN | + 1.1 |
| 450 | 0 | 36 AN/4 SS | 40 AN | - 4.0 |
| 750 | 0 | 24 AN/16 SS | 39 AN/ 1 SS | + 1.4 |
| 1250 | 0 | 28 AN/12 SS | 34 AN/ 6 SA | + 0.2 |

[1]AN – appeared normal; SS – shortened and stiff; SA – shortened & abnormally coloured.

Mortality: No mortality was observed in any of the treatment groups and in the negative control.

Mean Body Weight: There appeared to be no treatment related effect on worm body weight but worms in the treatment groups did not show the 10% weight increase observed in the control group.

Behaviour and appearance: All worms at the 162 and 270 mg/kg dry soil treatment levels were normal in appearance and behaviour at each observation interval. At the 450 mg/kg dry soil concentration, four worms were noted as shortened and stiff at day 7, but all worms in this group appeared normal at day 14. In the 750 mg/kg dry soil group, sixteen worms were found to be shortened and stiff on day 7, with only one worm from this group remaining shortened and stiff by day 14. In the 1250 mg/kg dry soil group, twelve worms were noted as shortened and stiff on day 7, with six worms found to be shortened and abnormally coloured by day 14.

The validity criteria according to guideline OECD 207 are fulfilled as no mortalities were observed in the control group.



## III. CONCLUSION

**The 14-day $LC_{50}$ for earthworms (*Eisenia fetida andrei*) exposed to the test item MON 52276 in an artificial soil substrate was determined to be > 1250 mg/kg dry soil >388 mg a.s./kg dry soil), the highest dose tested. The corresponding no-mortality concentration was 1250 mg/kg dry soil (388 mg a.s./kg dry soil). The corresponding NOEC was determined to be 270 mg/kg dry soil (83.7 mg a.s./kg dry soil), based on abnormalities noted at higher dose levels.**

## IIIA 10.6.3 Sub lethal effects on earthworms

The earthworm $TER_A$ value for glyphosate and AMPA was much higher than the Annex VI criterion of 10 (Point IIIA 10.6.1 in this document). Therefore, testing of sublethal effects using the formulated

product was not necessary. A summary of earthworm TERs for glyphosate and AMPA is presented under Point IIIA 10.6.1.

## IIIA 10.6.4 Field test (effects on earthworms)

The earthworm $TER_A$ value for glyphosate and AMPA was much higher than the Annex VI criterion of 10 (Point IIIA 10.6.1 in this document). Therefore, field-testing using the formulated product was not necessary. A summary of earthworm TERs for glyphosate and AMPA is presented in Point IIIA 10.6.1.

## IIIA 10.6.5 Residue content of earthworms

Glyphosate is very unlikely to bio-accumulate in earthworms, as indicated by its low $log_{POW}$ value (<<3) in combination with its low toxicity. Therefore, studies determining residue content of earthworms are not necessary.

## IIIA 10.6.6 Effects of other soil non-target macro-organisms

Studies not reviewed during the 2001 EU evaluation on other soil non-target organisms have been carried out exposing *Hypoaspis aculeifer* and *Folsomia candida* to glyphosate IPA salt and AMPA, respectively.

Details of the studies are given in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6. The toxicity endpoints for *Hypoaspis aculeifer* and *Folsomia candida* are summarised in Table 10.6.6-1.

**Table 10.6.6-1: Toxicity of glyphosate IPA salt and glyphosate metabolite AMPA**

| Species | Test design | $EC_{50}$ [mg/kg dry soil] | NOEC [mg/kg dry soil] | Reference/GLP |
|---|---|---|---|---|
| **Glyphosate IPA salt** | | | | |
| *Hypoaspis aculeifer* | 14 d chronic | >1000 mg/kg >472.8 mg a.s./kg | 1000 mg/kg 472.8 mg a.e./kg | IIA 8.10/01 09 10 48 058 [redacted] 2009/yes |
| *Folsomia candida* | 28 d chronic | >1000 mg/kg >587 mg a.s./kg | 1000 mg/kg 587 mg a.e./kg | IIA 8.10/02 09 10 48 057 [redacted] 2010/yes |
| **AMPA** | | | | |
| *Hypoaspis aculeifer* | 14 d chronic | >320 | 320 | IIA 8.10/03 10 10 48 053 [redacted] 2010/yes |
| *Folsomia candida* | 28 d chronic | >315 | 315 | IIA 8.10/04 10 10 48 054 [redacted] 2010/yes |

The resulting $TER_{LT}$ values are shown in Table 10.6.6-2.

**Table 10.6.6-2: Chronic TER values for other soil non target organisms**

| Test substance | Species | NOEC [mg/kg dry soil] | Maximum $PEC_{soil, plateau}$ [mg/kg dry soil] | $TER_{LT}$ |
|---|---|---|---|---|
| Glyphosate IPA salt | *Hypoaspis aculeifer* | 472.8 | 8.065 | 58.6 |
| | *Folsomia candida* | 587 | 8.065 | 72.8 |
| AMPA | *Hypoaspis aculeifer* | 320 | 5.345 | 59.9 |
| | *Folsomia candida* | 315 | 5.345 | 58.9 |

The TER values calculated using worst-case $PEC_{SOIL}$ values for glyphosate IPA-salt and its metabolite AMPA (see IIIA 9.4 for full calculations) exceeded the relevant triggers, indicating that the risk to soil macro organisms other than earthworms is acceptable following use of MON 52276.

## IIIA 10.6.7 Effect on organic matter breakdown

The potential toxicity of glyphosate and its soil metabolite AMPA has been assessed in tests with earthworms (acute and chronic exposure), springtails (*Collembola*), soil mites (*Hypoaspis*) and soil microflora and the risk assessments indicate low risk to these soil organisms. Therefore, testing the effects of glyphosate, its salts and soil metabolite AMPA on organic matter breakdown in litterbag studies is not triggered according to SANCO/10329/2002 rev. 2 final.

## IIIA 10.7 Effects on soil microbial activity

Studies on nitrogen and carbon mineralisation have been carried out with glyphosate technical, MON 52276 and the metabolite AMPA.

Further details of the study with MON 52276, which was not included in the monograph on glyphosate (2000), are given in Point IIIA 10.7.1 below. Further details of the studies with glyphosate technical and its metabolite AMPA are given in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6. The soil nitrogen and carbon mineralisation endpoints are summarised in Table 10.7-1.

**Treatment rates conversions for Table 10.7.1:**
The predicted environmental concentrations in soil (PEC$_s$) were calculated using the following equation:

$$PEC_s\ (mg/kg) = \frac{Application\ rate\ (g/ha)}{100 \times soil\ depth\ (cm) \times Soil\ dry\ bulk\ density\ (g/cm^3)}$$

It was assumed that the soil had a bulk density of 1.5 g/cm$^3$, that the pesticide is homogenously distributed within a depth of 5 cm. Consequently the maximum seasonal application rate of 4320 g glyphosate acid/ha is equivalent to 5.76 mg glyphosate acid/kg soil and 60 L/ha of MON 52276 is equivalent to 28.8 mg glyphosate acid/kg soil (Hutcheson, 2012).

**Table 10.7-1: Effects on soil micro-organisms**

| Compound | Study design | Endpoint (units as reported) | Maximum NOEC | Reference |
|---|---|---|---|---|
| MON 52276 | Nitrogen- and Carbon-mineralisation 28-day study | No significant effects (>25%) on carbon transformation and nitrogen mineralisation by day 28 at 18.8 and 94 mg MON 57726/kg dry soil, (12 and 60 L MON 52276/ha) in loamy sand | **28.8 mg glyphosate acid/kg dry soil** | IIIA 10.7.1/01 [redacted] 5259 [redacted] 2012 |
| AMPA | Nitrogen- and Carbon-mineralisation 28/56-day study | No significant effects (>25%) on carbon transformation and nitrogen mineralisation by day 28 at concentrations of up to 160 mg/kg dry soil. | **160 mg AMPA/kg dry soil** | IIA 8.10.1/02 10 10 48 010 [redacted] 2010 |

**The proposed new endpoints are highlighted with bold type.**

**Table 10.7-2: EU Conclusions – Effects on soil microorganisms**

| Test substance | EU agreed endpoint (glyphosate; EU Review Report, 6511/VI/99 final, 2002) | Proposed EU endpoint* |
|---|---|---|
| Glyphosate | No effects up to 18 kg a.s./ha | No effects up to 28.8 mg a.s./kg dry soil |
| AMPA | - | NOEC = 160 mg AMPA/kg dry soil |

*Since Annex I inclusion, new studies were provided in the post Annex I dossier, and also additional studies have been performed and as a result these new endpoints are used in the risk assessment (further details provided below)

## Summary

MON 52276 is the representative formulation in the current EU review of glyphosate. The intended use pattern for national registration is within the representative use pattern considered for EU review. Therefore appropriate assessments for effects on soil microbial activity from MON 52276 are evaluated as part of the EU review of glyphosate, where all study references and conclusions can be found. The MON 52276 data are also provided here.

## Toxicity

The toxicity of MON 52276 (Glyphosate 360 SL) and its soil metabolite AMPA to soil micro-organisms is summarised in Table 10.7-1 and Table 10.7-2. Further details of the study on MON 52276 (Glyphosate 360 SL) is provided in Point IIIA 10.7.1/01 below, details of the studies on glyphosate technical and its soil metabolite AMPA are given in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.6.

## Exposure

The maximum predicted environmental concentrations of glyphosate and its soil metabolite AMPA in soil (PEC) were calculated as described in the earthworm section above (Point IIIA 10.6).

**Table 10.7-3: Predicted Peak Environmental Concentrations in soil (PECs) of glyphosate and AMPA**

| Compound | Maximum $PEC_s$ [mg/kg] |
|---|---|
| Glyphosate | 5.76[1] |
| AMPA | 5.345 |

[1] Maximum initial PEC assuming application rate of 4.32 kg a.s./ha, a soil incorporation depth of **5 cm** and a soil bulk density of 1.5 g/cm$^3$.

The maximum instantaneous predicted environmental concentrations of MON 52276 in soil ($PEC_s$) were calculated as described in the earthworm section above (Point 10.6). The resulting maximum instantaneous $PEC_S$ values for the 1× and 5× application rate were determined to be 5.76 mg a.s./kg and 28.8 mg a.s./kg, respectively.

## Risk assessment

Based on laboratory testing with MON 52276, the Annex VI trigger value of > 25% effects after 28 days was not exceeded at concentrations of 1× and 5× the maximum recommended annual use rate for 4.32 kg a.s./ha. As no significant effects on soil micro-organisms were observed at these rates, the use of MON 52276 at the proposed field rates poses **no unacceptable long-term risk to non-target soil micro-organisms.**

Additionally, the NOEC value of 160 mg a.s./kg dry soil for the metabolite AMPA is approximately 30 times higher than the maximum $PEC_{soil,\ plateau}$ of 5.345 mg/kg for AMPA.

## IIIA 10.7.1 Laboratory test to investigate impact on soil microbial activity

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| IIIA 10.7.1/01 | [redacted] | 2012 | MON 52276: Effect on Soil Microbial Activity, Carbon and Nitrogen Transformations<br>[redacted]<br>Report No: [redacted]5259<br>Date: 2012-01-26<br>GLP: yes<br>Owner: Glyphosate Task Force<br>not published |

**Guideline:** OECD 216 (2000)
OECD 217 (2000)

**Deviations for OECD 216 and 217:** Temperature went out of specification for 4 hours during study (minimum 17.93 °C).

**Dates of experimental work:** 2011-11-11 to 2011-12-15



**Executive summary**

The effects of MON 52276 on soil microflora respiration and soil nitrogen transformation were investigated in a sandy loam soil (LUFA standard soil type 2.3). The test item was applied at two test item treatment concentrations, 18.8 and 94 mg MON 57726/kg dry soil, equivalent to 12 and 60 L MON 52276/ha (1× and 5×), using three replicates per treatment. In addition a negative control (deionised water) was tested. Substrate-induced (glucose) respiration and nitrogen transformation measurements were made on Days 0, 7, 14 and 28.

The results showed that MON 52276 did not result in ecotoxicologically significant effects for soil microflora respiration and soil nitrogen transformation 28 days after treatment. The deviation from the control was below the trigger of 25% at 5× the maximum seasonal application rate during the course of the study for both treatment levels. All validity criteria according to OECD 216 and 217 were fulfilled.

**MON 52276, when applied at 18.8 and 94 mg MON 57726/kg dry soil (5.76 and 28.8 mg a.s./kg dry soil), equivalent to 12 and 60 L MON 52276/ha, respectively, is not expected to cause significant effects on soil microflora respiration and soil nitrogen transformation processes.**

### I. MATERIALS AND METHODS

### A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item: | MON 52276 |
| Description: | Soluble liquid |
| Lot/Batch #: | A9K0106104 |
| Purity: | 358.8 g/L glyphosate acid equivalent<br>30.68% w/w |
| Density: | 1.17 g/mL |

**2. Vehicle and/or positive control:** none

**3. Test system:**

| | |
|---|---|
| Soil | LUFA standard soil type 2.3 (sandy loam soil) |
| Source: | [redacted], batch no. F2.34011 |
| Water content of soil: | 35 ± 5% |
| Water holding capacity | 35.6 %w/w |
| pH: | 7.5 |
| Total Org. C: | 0.94% |
| Microbial biomass | 1.91% of TOC |
| Clay (< 0.002 mm): | 8.7 ± 1.7% |
| Silt (0.002 mm - 0.05 mm): | 27.6 ± 3.8% |
| Sand (0.05 – 2.00 mm): | 63.7 ± 4.4% |

**4. Environmental conditions:**

| | |
|---|---|
| Temperature: | 20 ± 2°C |
| pH: | 7.5 – 7.9 |
| Water content: | 38.9 of WHC |



## B. STUDY DESIGN AND METHODS

**1. Experimental treatments:** MON 52276 was applied at two test item treatment concentrations, 18.8 and 94 mg MON 57726/kg dry soil, equivalent to 12 and 60 L MON 52276/ha, using three replicates per treatment.. In addition a negative control (deionised water) was tested. Moisture content of the soil was maintained at 40% (± 5%) of the MWHC and at each time point the weight of the moisture control vessel was determined as a guide to test vessel water loss. For soil nitrogen transformation, each replicate was amended with Lucerne meal (0.5%) as a nitrogen source. The Lucerne had a carbon content of 41.67% w/w and a nitrogen content of 2.54% w/w giving a C/N ratio of 16.4:1. For soil microflora respiration, a 8.9 mg glucose amendment/g dry soil was used. Soil was taken from one replicate from each treatment from the carbon test for pH (water) determination at the start and end of the study, soil was taken from one replicate from each treatment from the carbon test for moisture and dry matter content determination at the end of the study.

**2. Observations:**

Soil microflora respiration: As soon as possible after dosing and after 7, 14, 28 and 43 days, 100g soil sample (based on dry weight) was removed from each replicate for the determination of glucose-induced respiration rates in soil respiration chamber. Carbon dioxide evolution was measured using an infrared carbon dioxide analyser and automatic column switching unit (GHU) (ADC 2250 and GHU). The total $CO_2$ evolution over a period of at least 12 hours was calculated for each replicate.

Soil nitrogen transformation: As soon as possible after dosing and after 7, 14, 28 and 43 days, 50 g soil sample (based on dry weight) was removed for determination of $NH_4^+$, $NO_2^-$ and $NO_3$. Soil extracts were prepared by extracting soil samples with 250 ml 2 M KCl and shaking for two hours and then centrifuged for 15 minutes. The supernatant was analysed using a Bran + Luebbe Autoanalyzer AA3 system.

**3. Statistical calculations:** ANOVA followed by Dunnett's two-tailed test ( $\alpha = 0.05$).

## II. RESULTS AND DISCUSSION

### A. FINDINGS AND OBSERVATIONS

Statistical analysis showed there was a significant difference (α = 0.05, Dunnett's two-tailed test) between the treatment rate of 94 mg MON 52276/kg dry soil and the control treatment for soil carbon.

As the difference in respiration rates between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long-term influence on carbon transformation in soils.

Statistical analysis showed there was a significant difference (α = 0.05, Dunnett's two-tailed test) between the treatment rate of 94 mg MON 52276/kg dry soil and the control treatment for nitrate production from Day 14 to 28.

As the difference in nitrate production between the treatment rates of MON 52276 (18.8 and 94 mg MON 52276/kg dry soil) and control is less than 25% at Day 28, the test item can be evaluated as having no long term influence on nitrogen transformation in soils.

**Table 10.7.1-1: Effects of MON 52276 on soil nitrogen transformation in sandy loam soil**

| | | Nitrogen concentration [mg N/kg soil] | | % deviation from control | |
|---|---|---|---|---|---|
| | **Control** | **18.8 mg/kg** (1×) | **94 mg/kg** (5×) | **18.8 mg/kg** | **94 mg/kg** |
| **Nitrate transformation rates** | | | | | |
| Day 0-7 | -3.20 | -3.48 | -3.59 | +8.84 | +12.24 |
| Day 7-14 | +3.69 | +4.69 | +6.04 | +27.14 | +63.72 |
| Day 14-28 | +3.54 | +3.69 | +3.81 | +4.31 | +7.85 |
| **Nitrate ($NO_3^-$)** | | | | | |
| Day 0 | 22.4 | 24.4 | 25.1 | +8.93 | +12.05 |
| Day 7 | 0.0 | 0.0 | 0.0 | - | - |
| Day 14 | 25.8 | 32.8 | 42.3 | +27.13 | +63.95 |
| Day 28 | 75.3 | 84.5 | 95.7 | +12.22 | +27.09 |
| **Ammonium ($NH_4^+$)** | | | | | |
| Day 0 | 10.3 | 10.7 | 11.2 | +3.88 | +8.74 |
| Day 7 | 3.0 | 2.9 | 2.8 | -3.33 | -6.67 |
| Day 14 | 1.6 | 1.6 | 1.6 | +0.00 | +0.00 |
| Day 28 | 1.1 | 1.1 | 1.0 | +0.00 | -9.09 |

- = inhibition, + = stimulation

**Table 10.7.1-2: Effects of MON 52276 on soil microflora respiration**

| | $CO_2$ [mg $CO_2$/kg soil/h] | | | % deviation from control | |
|---|---|---|---|---|---|
| | **Control** | **18.8 mg/kg** (1×) | **94 mg/kg** (5×) | **18.8 mg/kg** | **94 mg/kg** |
| Day 0 | 16.08 | 16.16 | 17.24 | +0.47 | +7.19 |
| Day 7 | 15.42 | 16.64 | 18.73 | +7.97 | +21.52 |
| Day 14 | 15.42 | 16.93 | 18.77 | +9.78 | +21.71 |
| Day 28 | 16.49 | 17.15 | 18.90 | +3.96 | +14.57 |

- = inhibition, + = stimulation

All validity criteria according to OECD 216 and 217 were fulfilled, as the variation between replicate control samples was less than ± 15%.

## III. CONCLUSION

**MON 52276, when applied at 18.8 and 94 mg MON 57726/kg dry soil (5.76 and 28.8 mg a.s./kg dry soil), equivalent to 12 and 60 L MON 52276/ha, respectively, is not expected to cause significant long-term effects on soil microflora respiration and soil nitrogen transformation processes.**

## IIIA 10.7.2 Further laboratory, glasshouse or field testing to investigate impact on soil microbial activity

No additional testing is required since it can be predicted that glyphosate and its soil metabolite AMPA will have no significant effect on soil microflora.

## IIIA 10.8 Effects on non-target plants

### IIIA 10.8.1 Effects on non-target terrestrial plants

The effects of glyphosate on the seedling emergence, vegetative vigour, and phytotoxicity with a range of terrestrial non-target plants were assessed in laboratory studies.

Glyphosate is a non-selective herbicide that is absorbed by foliage, translocated into the roots from where it affects the treated plants. For glyphosate, existing GLP studies (see Annex IIA, Document M-II, Section 6, Point IIA 8.12) investigated the potential for effect on seedling emergence (pre-emergence) and effects on vegetative vigour (post-emergence) with a wide range of monocotyledonous and dicotyledonous plants. Following a pre-emergence application at 4480 g a.s./ha (above the recommended maximum single use rate of 2160 g a.s./ha for row crops), no adverse effects were observed on any non-target plant species tested. This result was expected, as glyphosate binds strongly to soil (it is regarded as 'immobile' according to the McCall classification scheme). Therefore, no effects on pre-emergent non-target plants are anticipated given the proposed glyphosate use patterns. Consequently, only the vegetative vigour data is considered in the non-target plant assessment below.

Following post-emergent applications at rates of up to 1480 g a.s./ha to 17 non-target plant species, adverse effects were observed for all the plants tested. The lowest $ER_{50}$ value generated from data on vegetative vigour was 146 g a.s./ha for tomato (see tables below).

All of the vegetative vigour studies had comparable range of $ER_{50}$ values including the study performed with MON 52276. The range of the $ER_{50}$ values from the four vegetative vigour studies is summarized below.



**Summary of range of $ER_{50}$ values from the vegetative vigour studies**

| Test Material | Range of $ER_{50}$ value [kg a.s./ha] | Reference/GLP |
|---|---|---|
| Glyphosate acid | 0.2488 to >0.7422 | IIA 8.12/01<br>236 GLY, [redacted] 1994/yes |
| Glyphosate acid with Triton surfactant | 0.146 to 1.792 | IIA 8.12/02<br>[redacted] 13320, [redacted]<br>1994/yes |
| Glyphosate acid formulated as a wettable powder | 0.149 to 1.253 | IIA 8.12/03<br>[redacted] 2009B, [redacted] et al., 1996/yes |
| MON 52276 | 0.252 to 0.597 | IIA 8.12/05<br>[redacted].104, [redacted] 2005/no |

A summary of the effects on vegetative vigour on individual non-target terrestrial plants species for each study is provided in Table 10.8.1-1 and Table 10.8.1-3. The results from the seedling emergence study are summarized in Table 10.8.1-3.

**Table 10.8.1-1: Effects of glyphosate on terrestrial non-target plants – vegetative vigour**

| Test species | Test duration | $ER_{50}$ [kg a.s./ha] | Effect parameter | Reference [a] |
|---|---|---|---|---|
| Onion (*Allium cepa*) | 21-day | > 0.7442 | all parameters | IIA 8.12/01<br>236 GLY, [redacted] 1994 |
| | 21-day | 1.792 | dry weight | IIA 8.12/02<br>[redacted] 13320, [redacted], 1994 |
| Corn (*Zea mays*) | 21-day | 0.64 | dry weight | IIA 8.12/01<br>236 GLY, [redacted], 1994 |
| | 21-day | 0.750 | dry weight | IIA 8.12/02<br>[redacted]-13320, [redacted], 1994 |
| | 28-day | 0.386 | damage assessment | IIA 8.12/03<br>[redacted] 2009[redacted] [redacted] *et al.*, 1996 [2] |
| Oat (*Avena sativa*) | 21-day | > 0.7442 | all parameters | IIA 8.12/01<br>236 GLY, [redacted] 1994 |
| | 21-day | 0.874 | dry weight | IIA 8.12/02<br>[redacted]-13320, [redacted], 1994 |
| | 28-day | 0.376 | dry weight | IIA 8.12/03<br>[redacted] 2009B, [redacted] *et al.*, 1996 [2] |
| Wheat (*Triticum aestivum*) | 21-day | 0.6478 | dry weight | IIA 8.12/01<br>236 GLY, [redacted] 1994 |
| | 28-day | 0.242 | dry weight | IIA 8.12/03<br>[redacted] 2009B, [redacted] *et al.*, 1996 [2] |
| Ryegrass (*Lolium perenne*) | 21-day | 1.344 | dry weight | IIA 8.12/02<br>[redacted]-13320, [redacted], 1994 |
| Purple nutsedge (*Cyperus rotundus*) | 28-day | 1.253 | dry weight | IIA 8.12/03<br>[redacted] 2009B, [redacted] *et al.*, 1996 [2] |
| Soybean (*Glycine max*) | 21-day | 0.6590 | plant height | IIA 8.12/01<br>236 GLY, [redacted] 1994 |
| | 21-day | 0.974 | dry weight | IIA 8.12/02<br>[redacted]-13320, [redacted], 1994 |
| | 28-day | 0.358 | dry weight | IIA 8.12/03<br>[redacted] 2009B, [redacted] *et al.*, 1996 [2] |
| Radish (*Raphanus sativus*) | 21-day | 0.2488 | survival | IIA 8.12/01<br>236 GLY, [redacted] 1994 |
| | 21-day | 0.246 | dry weight | IIA 8.12/02<br>[redacted]-13320, [redacted], 1994 |
| | 28-day | 0.417 | damage assessment | IIA 8.12/03<br>[redacted] 2009B, [redacted] *et al.*, 1996 [2] |
| Cucumber (*Cucumis sativus*) | 21-day | > 0.7442 | all parameters | IIA 8.12/01<br>236 GLY, [redacted] 1994 |
| | 21-day | 0.896 | dry weight | IIA 8.12/02<br>[redacted] 13320, [redacted] 1994 |
| | 28-day | 0.254 | damage assessment | IIA 8.12/03<br>[redacted] 2009B, [redacted] *et al.*, 1996 [2] |
| Sunflower (*Helianthus annuus*) | 21-day / | 0.2959 | dry weight | IIA 8.12/01<br>236 GLY, [redacted] 1994 |

| Test species | Test duration | $ER_{50}$ [kg a.s./ha] | Effect parameter | Reference [a] |
|---|---|---|---|---|
| Tomato (*Lycopersicon esculentum*) | 21-day | 0.5335 | dry weight | IIA 8.12/01<br>236 GLY, [redacted] 1994 |
| | 21-day | 0.146 | dry weight | IIA 8.13/02<br>[redacted]-13320, [redacted], 1994 [1] |
| Carrot (*Daucus carota*) | 21-day | 0.6512 | dry weight | IIA 8.12/01<br>236 GLY, [redacted], 1994 |
| Lettuce (*Lactuca sativa*) | 21-day | 0.762 | dry weight | IIA 8.12/02<br>[redacted]13320, [redacted], 1994 [1] |
| | 28-day | 0.402 | dry weight | IIA 8.12/03<br>[redacted] 2009[redacted] [redacted] *et al.*, 1996 [2] |
| Cabbage (*Brassica oleracea*) | 21-day | 0.739 | dry weight | IIA 8.12/02<br>[redacted]13320[redacted], 1994 [1] |
| Oilseed rape (*Brassica napus*) | 28-day | 0.149 | damage assessment | IIA 8.12/03<br>[redacted] 2009[redacted] [redacted] *et al.*, 1996 [2] |
| | 22-day | 0.511 | fresh weight | IIA 8.12/05<br>[redacted].104, [redacted] 2005 [2] |
| Sugar beet (*Beta vulgaris*) | 28-day | 0.377 | dry weight | IIA 8.12/03<br>[redacted] 2009[redacted], [redacted] *et al.*, 1996 [2] |
| Okra (*Abelmoschus esculentus*) | 28-day | 0.346 | dry weight | IIA 8.12/03<br>[redacted] 2009[redacted], [redacted] *et al.*, 1996 [2] |

[a] Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.13
[1] Study conducted with glyphosate acid and the addition of Triton surfactant, see Annex IIA, Document M-II, Section 6, Point IIA 8.12
[2] Study conducted with glyphosate acid formulated as a wettable powder; see Annex IIA, Document M-II, Section 6, Point IIA 8.12

**Table 10.8.1-2: Effect of MON 52276 on terrestrial non-target plants in a 22-day vegetative vigour study**

| Test species | $ER_{50}$ [kg/ha] | Effect parameter | Reference |
|---|---|---|---|
| Wheat (*Triticum aestivum*) | 0.344 | Fresh weight | IIA 8.12/05<br>[redacted].104, [redacted], 2005 |
| Ryegrass (*Lolium perenne*) | 0.307 | Fresh weight | IIA 8.12/05<br>[redacted].104, [redacted] 2005 |
| Oilseed rape (*Brassica napus*) | 0.511 | Fresh weight | IIA 8.12/05<br>[redacted].104, [redacted] 2005 |
| Sugar beet (*Beta vulgaris*) | 0.307 | Fresh weight | IIA 8.12/05<br>[redacted].104, [redacted], 2005 |
| Garden cress (*Lepidum sativum*) | 0.252 | Fresh weight | IIA 8.12/05<br>[redacted].104, [redacted], 2005 |
| Pea (*Pisum sativum*) | 0.597 | Fresh weight | IIA 8.12/05<br>[redacted].104, [redacted] 2005 |

**Table 10.8.1-3: Effects of glyphosate technical on terrestrial non-target plants – seedling emergence**

| Test species | Test duration | $ER_{50}$ [kg/ha] | Effect parameter | Reference[a] |
|---|---|---|---|---|
| Onion (*Allium cepa*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Corn (*Zea mays*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Oat (*Avena sativa*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Wheat (*Triticum aestivum*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Purple nutsedge (*Cyperus rotundus*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Soybean (*Glycine max*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Cucumber (*Cucumis sativus*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Lettuce (*Lactuca sativa*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Oilseed rape (*Brassica napus*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Sugar beet (*Beta vulgaris*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Okra (*Abelmoschus esculentus*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |
| Rhubarb (*Rheum rhoponticum*) | 28-day | >4.48 | all parameters | IIA 8.12/04 [redacted] 2008 [redacted] *et al.*, 1996 |

[a] Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.12 ; study conducted with formulated product (WP), see Annex IIA, Document M-II, Section 6, Point IIA 8.12.



There are no EU agreed endpoints for glyphosate for terrestrial plants. The following endpoints are proposed.

**Table 10.8.1-4: Proposed endpoints to evaluate toxicity of glyphosate to non-target terrestrial plants.**

| Substance | Vegetative vigour | Proposed Endpoint |
|---|---|---|
| **Glyphosate acid** | **Most sensitive species Tomato (*Lycopersicon esculentum*)** | **$ER_{50}$ of 146 g a.s./ha** |
| **Glyphosate acid** | **Species sensitivity distribution 17 species** | **$HC_5$ of 206.35 g a.s./ha** |

## Assessment of risk to non-target terrestrial plants

Non-target plant testing with glyphosate and glyphosate formulations evaluating potential effects following pre-emergent (soil) exposure and post-emergent (foliar) exposure indicated that the compound demonstrated no activity in the seedling emergence study. For vegetative vigour, the $ER_{50}$

for shoot fresh weight for the most sensitive monocotyledon species, wheat, was 242 g a.s./ha; the $ER_{50}$ for shoot fresh weight for the most sensitive dicotyledonous species, tomato, was 146 g a.s./ha. In a study conducted with the lead formulation MON 52276 it is clearly demonstrated that the toxicity is comparable to the other vegetative vigour studies.

$PER_{drift}$ values at 1, and 5, meters were calculated based on the maximum application rate of 2.16 kg a.s./ha (equivalent to 6 L product/ha) for row crops in the EU. The resulting TER values are given in Table 10.8.1-5. A maximum allowable single rate of 2.88 kg a.s./ha was not assessed because this application rate is reserved for ground directed applications only that are made around the base of tree trunks.

**Table 10.8.1-5: Glyphosate: TERs for terrestrial non-target plants (vegetative vigour)**

| Buffer distance [meters] | Application rate [g a.s./ha] | Drift value[a] [%] | Drift reduction [%] | $PEC_{drift}$ [g a.s./ha] | $ER_{50}$[b] [g a.s./ha] | TER |
|---|---|---|---|---|---|---|
| **Field crops, vegetables** | | | | | | |
| 1 | 2160 | 2.77 | 0 | 59.832 | 146 | 2.4 |
| | | | 75 | 14.958 | | **9.8** |
| 5 | | 0.57 | 0 | 12.312 | | **11.9** |

[a] Drift estimates are based on 90th percentile values (BBA 2000).
[b] $ER_{50}$ is based on shoot fresh weight for the most sensitive plant tomato
**Values in bold exceed trigger value of 5**

Based on this assessment, a TER trigger of 5 according to SANCO/10329/2002 rev. 2 final is achieved when applying 75% drift reduction and a buffer of 1 m. Without drift reduction, a TER >5 is achieved with a buffer of 5 m.

The results from the study with MON 52276 provide confirmatory data and comparable and slightly greater TER values, with the estimated $ER_{50}$ value for the most sensitive of 0.252 kg a.s./ha.

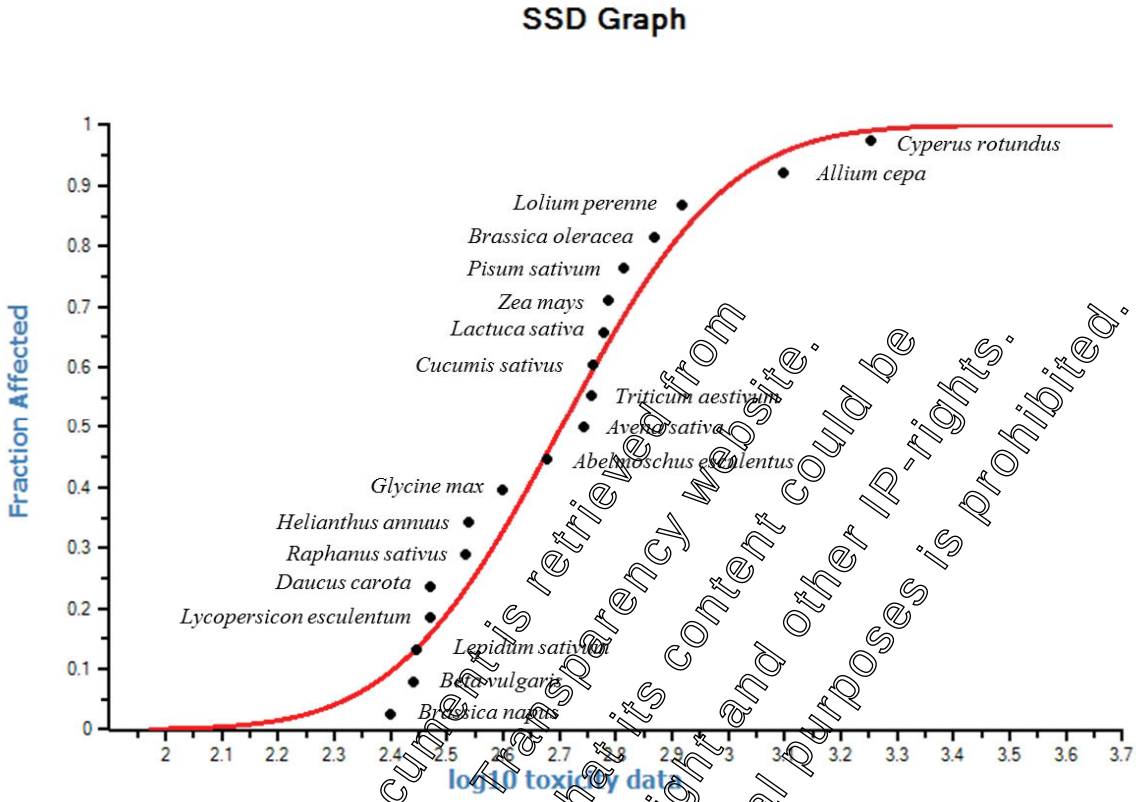
**Refinement of exposure level protective of 95% of species ($HC_5$ evaluation)**

The effect endpoints used in the terrestrial non-target plant risk assessment (*i.e.* $ER_{50}$ for the 17 plant species for vegetative vigour studies (not including data from Mon 52276) were re-evaluated with a species sensitivity distribution from which an $HC_5$ was obtained. The $HC_5$ was calculated based on the recommendations of EUFRAM (EUFRAM, 2006 [v]) using the software package ETX 2.0, developed by the RIVM, the Netherlands, based on Aldenberg and Jaworska (2000) [vi] and Aldenberg and Luttik (2002) [vii]. In case more than one endpoint was available for each plant species, the $ER_{50}$ was recalculated using the geometric mean. "Greater than" data were not included in the analysis, i.e. not considered for the $HC_5$ evaluation. However, excluding the three "greater than" values did not reduce the number of species considered for the $HC_5$ evaluation nor significantly impact the results of this analysis.

Figure 10.8.1-1 is a graphical representation of the species distribution. Based on this methodology, the $HC_5$ value was estimated to be 206.35 g a.s./ha with a lower 95% confidence limit of 138.21 and an upper 95% confidence limit of 270.73 g a.s./ha.

For the MON 52276 vegetative vigour study, a comparable $HC_5$ value was estimated to be 220.2 g a.s./ha with a lower confidence limit of 112.9 and an upper confidence limit of 297.0 g a.s./ha.

**Figure 10.8.1-1: Species sensitivity distribution for the $HC_5$ estimation of Non-target Terrestrial Plants (NTPs) exposed to glyphosate; Log distribution of $ER_{50}$ values**






According to the Guidance Document on Terrestrial Ecotoxicology SANCO/10329/2002 rev.2 (final), 17 October 2002 [viii], risk to terrestrial plants is considered to be acceptable if the $ER_{50}$ for less than 5% of the species is below the highest predicted exposure level. As this is the case for terrestrial non-target plants, for the refined risk assessment the TER values considering the $HC_5$ are compared to a trigger of 1.

**Table 10.8.1-4: Glyphosate: TERs using an $HC_5$ based on a collection of non-target plant $ER_{50}$s (vegetative vigour at 21 and 28 days)**

| Buffer distance [meters] | Application rate [g a.s./ha] | Drift value[a] [%] | Drift reduction [%] | $PER_{drift}$ [g a.s./ha] | $HC_5$ [g a.s./ha] | TER |
|---|---|---|---|---|---|---|
| **Field crops, vegetables** | | | | | | |
| 1 | 2160 | 2.77 | 0 | 59.832 | 206.35 | **3.4** |
| 5 | | 0.57 | 0 | 12.312 | 206.35 | **16.8** |

[a] Drift estimates are based on 90th percentile values (BBA 2000).
**Values in bold exceed trigger value of 1**

In the environment, there is generally a large seed bank contained in the soil. This means that even if some individual plants of sensitive species were affected, populations would be able to recover within one season due to the soil reserve of viable seeds.

Not all seeds of a given species will germinate at the same time within the same year due to various environmental and biological parameters (soil conditions, moisture, seed coats, etc.). Therefore, at any given time, plants will be of different ages and sensitivities to glyphosate. Also, part of the seeds contained in the seed bank will remain dormant, with plants emerging only one to several years after application of the herbicide. This contributes to the population recovery potential of affected species. Many types of plant seeds are transported by wind, plants and animals. Recolonisation of damaged areas is therefore also possible via this route.

Further evidence supporting the fact that glyphosate formulations will not cause irreversible effects on non-target plants outside the treated field can be found in a study by Zwerger and Pestemer (2000)[3] where four different species (oilseed rape, oat, *Chenopodium album* and *Alopecurus myosuroides*) were exposed to a glyphosate formulation (MON 52276, containing 360 g a.s./L with a different surfactant system) at different rates up to a maximum of 450 g a.s./ha to assess effects on the plants' life cycle. The results indicate that, although some effects could be seen on vegetative endpoints (plant biomass) at rates higher than the spray drift rate at 1 m (i.e. 120 g a.s./ha), generative endpoints, such as seed production, seed weight, germination capacity and seed viability, were not affected. Reproduction of the exposed species was therefore not at risk.

Furthermore, the use of modern technology for the reduction of drift (for example low pressure nozzles, anti-drift nozzles, air-assistance spraying systems) is recommended and will help decrease the potential toxicity from sprayed product to non-target plants on field margins.

**Based on this assessment, a TER trigger ≥1 according to the Terrestrial Guidance Document is achieved without taking drift reduction measures or buffer zones into account. Good spray practices will also minimise exposure of non-target plants (and crops) to spray drift.**

### IIIA 10.8.1.1 Seed germination

This is not an EC data requirement/not required by Regulation 1107/2009/EC.

### IIIA 10.8.1.2 Vegetative vigour

Three greenhouse studies investigating the potential effects of glyphosate on vegetative vigour of non-target terrestrial plants have been performed. Further details of the studies are presented in Table 10.8.1-1. Summaries are provided in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.12.

A vegetative vigour study was also conducted on the lead formulation MON 52276 and is summarised in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.12/05.

### IIIA 10.8.1.3 Seedling emergence

A study on seed germination with a 500 WG formulation was conducted and is presented in the Glyphosate EU Dossier, Annex IIA, Document M-II, Section 6, Point IIA 8.12. No adverse effects on 12 plant species were observed at the maximum rate tested, 4.48 kg a.s./ha.



[3] Zwerger, P. and Pestemer, W. (2000). Testing the phytotoxic effects of herbicides on higher terrestrial non-target plants using life-cycle test. *Z. PflKrankh. PflSchutz. Sonderh.* 17: 711-718.

### IIIA 10.8.1.4 Terrestrial field testing

The TER values for glyphosate were greater than the criterion of Terrestrial Guidance Document [viii] of 1 for species sensitivity distributions (Point IIIA 10.8.1 in this document).

Therefore, field-testing using the formulated product is not necessary. A summary of TER values for glyphosate is presented in Point IIIA 10.8.1.

## IIIA 10.8.2 Effects on non-target aquatic plants

A summary of the effects of MON 52276, glyphosate acid, glyphosate IPA-salt and its metabolites on aquatic macrophytes is provided in Table 10.8.2-1.

**Table 10.8.2-1: Summary of toxicity of glyphosate acid, glyphosate IPA-salt, the metabolites AMPA and HMPA and MON 52276 to aquatic macrophytes**

| Test species | Test substance | Test duration | NOEC [mg/L] | $EC_{50}$ [mg/L] | Effect parameter | Reference |
|---|---|---|---|---|---|---|
| *Lemna gibba G3* | Glyphosate acid | 14-day semi-static | 6.0 | 20 | Dry weight | AII 8.6/01 [redacted]5662/[redacted] *et al.*, 1996 |
| | | | 3.0 | 12 | Frond number | |
| *Lemna gibba G3* | Glyphosate acid | 14-day static | 9.0 | 25.5 | Frond number | 1092-02-1100-5 [redacted]1987 |
| *Myriophyllum aquaticum* | Glyphosate acid | 14-day exposure + 7 day recovery | 5.0 | 279 | shoot length, relative increase | AII 8.6/04 [redacted]-015/4-80/[redacted] 2012a |
| | | | <5.0 | 276 | shoot length, growth rate | |
| | | | <5.0 | 22.3 | fresh weight, relative increase | |
| | | | <5.0 | 23.4 | fresh weight, growth rate | |
| *Lemna gibba G3* | Glyphosate IPA-salt | 14-day static | 25 | 62.59 | Dry weight | AII 8.6/02 980909[redacted] 1999 |
| | | | 25 | 53.56 | Frond number | |
| *Lemna gibba G3* | Glyphosate IPA-salt | 7-day semi-static | 11.7 | 46.2 | Dry weight | IIA 8.6/03 [redacted]-1873 [redacted], 2002 |
| | | | 11.7 | 25.5 | Frond number | |
| | | | 10.7 | 42.6 | Growth rate | |
| *Lemna gibba G3* | MON 52276 | 7-day semi-static | 5.9 | 36.50 | Dry weight | IIA 10.8.2.1/01 [redacted]-2002-051 [redacted], 2002 |
| | | | 5.9 | 20.57 | Frond number | |
| | | | 5.9 | 46.35 | Growth rate | |
| *Myriophyllum aquaticum* | MON 52276 | 14-day exposure + 7 day recovery | 5.16 | 13.4 | Shoot length, relative increase | AII 10.8.2.1/02 [redacted]-016/4-80/[redacted] 2012b |
| | | | 5.16 | 42.8 | Shoot length, growth rate | |
| | | | < 0.30 | 4.44 | Fresh weight, relative increase | |
| | | | < 0.30 | 10.3 | Fresh weight, growth rate | |
| *Myriophyllum aquaticum* | AMPA | 14-day exposure + 7 day recovery | 14.3 | 103 | Shoot length, relative increase | AII 8.6/05 [redacted]-022/4-80/A [redacted] 2012c |
| | | | 14.3 | >94.6 | Shoot length, growth rate | |
| | | | 14.3 | 70.8 | Fresh weight, relative increase | |
| | | | 14.3 | 97.3 | Fresh weight, growth rate | |
| *Lemna gibba G3* | HMPA | 14-day | 123 | >123 | Dry weight | AII 8.6/06 139A-397 [redacted] *et al.* 2011 |
| | | | 123 | >123 | Frond number | |

**Values in bold: Confirmed EU endpoints (SANCO/6511/VI/99-final, or EU Review Monograph)**

**Assessment of risk to aquatic plant growth**

On the basis of the worst-case toxicity values and the relevant worst case $PEC_{sw}$ values (see section 10.2), toxicity exposure ratios for exposure of *Lemna gibba* and *Myriophyllum aquaticum* were calculated (Table 10.8.2-2).

**Table 10.8.2-2: Long-term TER values for *Lemna gibba* and *Myriophyllum aquaticum* for glyphosate acid and metabolites AMPA and HMPA based on FOCUS Step 1 $PEC_{SW}$ values**

| Test organism | Test substance | $EC_{50}$ [µg a.s./L] | FOCUS Step 1 Max $PEC_{sw}$ [µg a.s./L] | $TER_{LT}$ | Trigger value |
|---|---|---|---|---|---|
| *Lemna gibba G3* | Glyphosate acid | 12,000 | 101.233 | 119 | 10 |
| *Myriophyllum aquaticum* | Glyphosate acid | 12,300 | 101.233 | 122 | |
| *Lemna gibba G3* | Glyphosate IPA salt | 53,560 | 101.233 | 529 | |
| *Lemna gibba G3* | MON 52276 | 20,570 | 101.233 | 203 | |
| *Myriophyllum aquaticum* | MON 52276 | 4449 | 101.233 | 43.9 | |
| *Myriophyllum aquaticum* | AMPA | 70,800 | 40.978 | 1728 | |
| *Lemna gibba G3* | HMPA | >123,000 | 6.710 | >18331 | |

The $TER_{LT}$ values for glyphosate acid, glyphosate IPA-salt, AMPA and HMPA exposure to aquatic macrophytes all exceed the Annex VI trigger of 10, indicating that glyphosate acid, IPA salt, AMPA and HMPA pose a low long-term risk to aquatic macrophytes following application according to the proposed uses of MON 52276.

## IIIA 10.8.2.1 Aquatic plant growth

| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| AIII 10.8.2.1/01 | [redacted] | 2002 | Assessment of toxic effects of MON 52276 on aquatic plants using the duckweed *Lemna gibba* [redacted]<br>Report No: [redacted]-2002-051<br>Date: 2002-11-18<br>GLP: yes<br>not published |
| **Guideline:** | | | OECD 221 (draft of October 2000) |
| **Deviations from OECD 221:** | | | None |
| **Dates of experimental work:** | | | 2002-05-24 to 2002-06-15 |



**Executive Summary**

The effects on the growth of the aquatic plant *Lemna gibba* G3 exposed to MON 52276 (30.9% w/w glyphosate acid) were determined in a seven-day semi-static study. For the main test, three replicates of 12 fronds in AAP Medium for *Lemna gibba* were exposed in glass beakers under continuous illumination to nominal MON 52276 concentrations of 0 (control), 0.9, 2.4, 6.8, 19.1, 53.6 and 150 mg/L. Renewal of the test media was performed on day 3 and 5 after test initiation. Direct counts of number of fronds were conducted on day 3, 5 and 7. Observations of changes in plant development, frond size, appearance, necrosis or other abnormalities were also performed at those times. The effect on biomass production was evaluated by determining the final dry weights of the plants. The growth

rate inhibition was determined by counting the number of fronds produced for each test concentration and control group. The effect on biomass production was evaluated by determining the final dry weights of the plants. Samples from all the test concentrations were collected for analysis of glyphosate by HPLC on Days 0, 3, 5 and 7.

Significant inhibitory effects of MON 52276 were observed at 53.6 and 150 mg/L (43%) for frond numbers, growth rate and biomass increase.

The $EC_{50}$ for frond number, biomass and growth rates based on frond number and biomass for MON 52276 was determined to be 66.58, 118.16 and >150 mg MON 52276/L, respectively. The overall NOEC was determined to be 19.1 mg MON 52276/L. The validity criteria according to guideline OECD 221 are fulfilled.

**Based on nominal concentrations, the $EC_{50}$ for frond count of *Lemna gibba* exposed to MON 52276 under semi-static test conditions for 7 days was 66.58 mg/L (95% confidence limits of 56.30 and 79.66 mg MON 52276/L), equivalent to 20.57 mg a.e./L. Since the percentage inhibition compared to control was only 43% at the highest MON 52276 concentrations tested, the $E_rC_{50}$ was estimated to be > 150 mg MON 52276/L, equivalent to 46.35 mg a.e./L. Based on nominal concentrations, the $E_bC_{50}$ was 118.16 mg MON 52276/L (95% confidence limits of 91.37 and 171.37mg MON 52276/L), equivalent to 36.51 mg a.e./L. The no-observed-effect-concentration (NOEC) was 19.1 mg MON 52276/L, equivalent to 5.90 mg a.e./L.**



## I. MATERIALS AND METHODS

### A. MATERIALS

**1. Test material:**

Test item:: MON 52276
Description: Light amber-brown liquid formulation
Lot/Batch #: A16120410
Purity: 30.9% glyphosate acid equivalent, as 41.5% isopropylamine salt of glyphosate

**2. Vehicle and/or positive control:** none

**3. Test organism:**

Species: Young *Lemna gibba* G3, 2 – 5 fronds
Source: [redacted] Germany

**4. Environmental conditions**:

Temperature: 22 - 25 °C
Light intensity: Continuous illumination, 7000 lux
pH: 7.49 – 9.42 (adjusted to 7.5)
Conductivity: not stated
Hardness: Not stated

## B: STUDY DESIGN AND METHODS

**1. Experimental treatments:** On the basis of the results of a range finding test, the definitive test was performed with six concentration levels, 0.9, 2.4, 6.8, 19.1, 53.6 and 150 mg MON 52276/L, with 3 replicates per test concentration. Three control replicates (without test substance) were tested under the same conditions. Colonies consisting of 2-5 fronds totalling 12 fronds per replicate were added to each replicate test chamber. The plants were placed in 100 mL test vessels containing 50 mL 20X-AAP test media. The pH of the test medium was adjusted at each test media renewal to 7.5, to avoid extreme pH values. The test was conducted under a 7-day static-renewal test conditions. The renewal of the test media was performed on day 3 and 5 after test initiation.

**2. Observations:**

Biological data: Observations were made on the number and the condition of the fronds on days 3, 5 and 7. The growth rate inhibition was determined by counting the number of fronds produced for each test concentration and control group. The effect on biomass production was evaluated by determining the final dry weights of the plants.

Physical data: pH and temperature of the test vessels were measured on days 0, 3, 5 and 7. Samples from all the test concentrations were collected for analysis of glyphosate by HPLC on Days 0, 3, 5 and 7.

**3. Statistical calculations:** The 7-day $EC_{50}$ value for frond counts and growth rates based on frond counts and biomass were determined by calculation of statistical significance using one-way analysis of variance (ANOVA) and Dunnett's test ($\alpha = 0.05$).



# II. RESULTS AND DISCUSSION

## A. FINDINGS

Analytical data: The mean measured glyphosate concentrations were 0.8, 2.3, 6.9, 21.4, 56.3 and 156 mg/L (82.9, 94.5, 101, 112, 105 and 104 % of nominal concentrations, respectively). Results were based on nominal MON 52276 concentrations.

| Endpoint | Frond number [mg/L] | Growth rate based on frond number [mg/L] | Biomass [mg/L] |
|---|---|---|---|
| $EC_{50}$ ( 7 days) | 66.58 (56.30 – 79.66) | >150 | 118.16 (91.37 – 171.37) |
| NOEC ( 7 days) | 19.1 | 19.1 | 19.1 |

## B. OBSERVATIONS

Observations: Significant inhibitory effects were observed at 2.4 and 6.8 mg/L for frond numbers and growth rates, and at 6.8 mg/L for biomass. However, these effects were not dose-related and were considered to be due to a reduced uptake of nutrients following a root decay caused by a bacterial infection. Additional dose-related significant inhibitory effects were observed at 53.6 and 150.0 mg/L for frond numbers, growth rates and biomass increase.

**Table 10.8.2.1-1: Toxicity of MON 52276 to *Lemna gibba* under semi-static conditions**

| MON 52276 concentration (mg/L) [1] | Mean frond number [2] | | | Mean dry weight (mg) [3] | Average specific growth rate (μ) | Mean biomass increase (based on dry weight) |
|---|---|---|---|---|---|---|
| | Day 3 | Day 5 | Day 7 | Day 7 | 0 - 7 days | 0 - 7 days |
| 0 (control) | 44 | 120 | 270 | 32.4 | 0.444 | 31.0 |
| 0.9 | 45 | 116 | 234 | 28.5 | 0.4233 | 27.2 |
| 2.4 | 43 | 100 | 204 | 27.8 | 0.4010 | 26.5 |
| 6.8 | 40 | 98 | 193 | 26.3 | 0.3961 | 25.0 |
| 19.1 | 49 | 119 | 242 | 28.3 | 0.4284 | 27.0 |
| 53.6 | 39 | 84 | 157 | 24.6 | 0.3668 | 23.3 |
| 150.0 | 27 | 48 | 71 | 14.1 | 0.2533 | 12.8 |

[1] Nominal values.
[2] Initial mean frond number: 12
[3] Initial mean dry weight: 1.3 mg

The doubling time of frond numbers in the control was less than 2.5 days (37.4 hours). The validity criteria according to the current guideline OECD 221 are therefore fulfilled.

## III. CONCLUSION

**Based on nominal concentrations, the $EC_{50}$ for frond count of *Lemna gibba* exposed to MON 52276 under semi-static test conditions for 7 days was 66.58 mg/L (95% confidence limits of 56.30 and 79.66 mg MON 52276/L), equivalent to 20.57 mg a.e./L. Since the percentage inhibition compared to control was only 43% at the highest MON 52276 concentrations tested, the $E_rC_{50}$ was estimated to be > 150 mg MON 52276/L, equivalent to 46.35 mg a.e./L. Based on nominal concentrations, the $E_bC_{50}$ was 118.16 mg MON 52276/L (95% confidence limits of 91.37 and 171.37mg MON 52276/L), equivalent to 36.51 mg a.e./L. The no-observed-effect-concentration (NOEC) was 19.1 mg MON 52276/L, equivalent to 5.90 mg a.e./L.**



| Annex point | Author(s) | Year | Study title |
|---|---|---|---|
| AIII 10.8.2.1/02 | [redacted] | 2012 | Effect of MON52276 (Glyphosate formulation) on the Growth of *Myriophyllum aquaticum* in the Presence of Sediment, with a subsequent Recovery Period.<br>[redacted]<br>Germany<br>Report No: [redacted]016/4-80[redacted]<br>Date: 2012-02-24<br>GLP: yes<br>not published |

**Guideline:** Maltby, L., et al. (2008): Aquatic Macrophyte Risk Assessment for Pesticides, SETAC AMRAP

**Deviations to Guideline** Specific criteria for macrophyte growth tests have not been set yet.

**Dates of experimental work:** 2010-10-28 to 2010-11-18

**Executive Summary**

The toxicity of MON52276 on growth of *Myriophyllum aquaticum* was evaluated in a 14 day static toxicity test, with subsequent 7 day recovery test, performed at concentrations of 0.78, 3.91, 19.6, 97.8, 489 and 2445 mg MON52276/L, equivalent to 0.24, 1.2, 6.0, 30, 150 and 750 mg glyphosate acid equivalents/L. A negative control (Smart & Bako medium) was prepared in parallel.

Two sets of vessels (exposure and recovery set) were prepared, with each set comprising three replicates for each test concentration and six replicates for the controls. Test vessels were 2-L beakers, each containing five individual plants potted in individual pots containing artificial sediment. Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 3, 7, 10 and 14 days and after 21 days (recovery vessels). At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants. At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root.

Test media were analysed for Glyphosate acid content at test start and end of exposure and recovery periods. The measured concentrations ranged from 83.9-145% of nominal. Glyphosate was not detected in the control group.

Result showed a significant inhibition of fresh weight of 20.7% at the lowest test concentration of 0.3 mg glyphosate acid equivalents/L. Shoot length increase and growth rate were unaffected at this concentration. Relative to the control group, at the highest treatment rate (723 mg test item/L) there was 93.8% growth inhibition based on fresh weights, shoot length increase was inhibited by 94.1% and growth rate by 90.2%. The recovery period demonstrated that *Myriophyllum aquaticum* pre-exposed to up to 26.80 mg MON52276/L were able to recover to control levels of growth, in untreated culture medium within 7 days of transfer.

The study fulfilled the validity criteria of achieving at least 50% increase in control plant growth in terms of length within 7 days of test initiation. The test was therefore considered to be valid.

**MON52276 significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days at a mean measured concentration of <0.3 mg glyphosate acid equivalents/L. Shoot length was inhibited at or above mean measured concentrations of 5.16 mg glyphosate acid equivalents/L. The 14-d $EC_{50}$ value for fresh weight inhibition was 4.4 mg glyphosate/L and for shoot length it was 13.44 mg glyphosate acid equivalents/L. *Myriophyllum aquaticum* pre-exposed for 14 day to up to 26.80 mg glyphosate acid equivalents/L were able to recover in untreated culture medium after a 7 day recovery period.**



## I. MATERIALS AND METHODS

### A. MATERIALS

**1. Test material:**

| | |
|---|---|
| Test item: | Glyphosate SL formulation (MON52276) |
| Description: | Clear, yellow, viscous liquid |
| Lot/Batch #: | A9K0106104 |
| Purity: | 358.8 ± 4.0 g glyphosate acid equivalent/L (30.68% w/w) |

**2. Vehicle and/or positive control:** None

**3. Test organism:**

Species: *Myriophyllum aquaticum*

Source: [redacted] Germany

**4. Environmental conditions**:

Growth medium: Smart & Bako medium

Artificial sediment: 4-5% peat
20% kaolin clay
75-76% quartz sand
$CaCO_3$ (if needed to adjust pH to 7.0 ± 0.5)
Based on artificial soil used in OECD guideline 219
Moistening of sediment up to 30% with deionised water or nutrient medium (ammonium chloride and sodium phosphate)

Temperature: 20.0 °C

Photoperiod: 16 h light

Light intensity 7295-7518 lux

pH: Values recorded at test start and end (in brackets) of 14 day exposure period:
Controls = 7.97 (8.78-8.82)
0.3 mg/L = 8.25 (8.82)
1.1 mg/L = 8.01 (8.82)
5.16 mg/L = 8.15 (8.82)
26.8 mg/L = 7.79 (8.81-8.82)
145 mg/L = 7.26 (6.11-8.82)
723 mg/L = 5.86 (6.09-6.82)
Values at start and end of 7 day recovery period:
Recovery period start = 6.0 – 9.2
Recovery period end = 8.3 – 9.8

Oxygen saturation Values recorded at test start and end (in brackets) of 14 day exposure period:
Controls = 96% (102-108%)
0.3 mg/L = 90% (107-108%)
1.1 mg/L = 96% (107-111%)
5.16 mg/L = 91% (114-132%)
26.8 mg/L = 95% (100-104%)
145 mg/L = 90% (116-122%)
723 mg/L = 96% (4-9%)
Values at start and end of 7 day recovery period:
Controls = 103-110% (99-109%)
0.3 mg/L not included in the recovery period
1.1 mg/L = 108-114% (103-110%)
5.16 mg/L = 111-113% (115-121%)
26.8 mg/L = 123-130% (123-126%)
145 mg/L = 127-137% (104-143%)

723 mg/L = 6-33% (107-111%)

## B: STUDY DESIGN AND METHODS

**1. Experimental treatments:** The toxicity test on *Myriophyllum aquaticum* was performed with six concentration levels of 0.24, 1.2, 6.0, 30, 150 and 750 mg glyphosate/L, equivalent to 0.78, 3.91, 19.6, 97.8, 489 and 2445 mg MON52276/L, with 3 replicates per test concentration. Six control replicates (without test substance) were tested under the same conditions as the test groups. Two sets of vessels (exposure and recovery) were prepared at the start of the test.

The plants were planted in small plastic plant pots into sediment and placed in glass beakers (test vessels), containing 2 L Smart & Bako medium. The test was conducted under static conditions. Five plants were added to each test and control replicate.

After 14 days exposure another set of *Myriophyllum aquaticum* replicates, exposed to the same concentration levels, were transferred into freshly prepared test medium without test item to determine the potential recovery after an exposure event.

**2. Observations:** Plant length, fresh weight, dry weight and root length were determined in all vessels. Plant length was recorded at test start and after 5, 8, 11 and 14 days. At test start and test end, fresh weight of each plant was determined. Dry weight was determined at test initiation using 25 additional plants and at test end on the tested plants (dried at 105 °C for 24 h). At the end of the test all plants were harvested and the root length was assessed semi-quantitatively in terms of length of the main root. Temperature in the test chamber was recorded continuously. Oxygen content, pH and light intensity was recorded at test start and after 14 days.

Analytical control measurements of the actual concentration of the glyphosate acid were performed by means of LC/MS-MS analysis at test start, after 14 (after exposure phase) and 21 days (after recovery phase).

**3. Statistical calculations:** The $EC_{10}$, $EC_{20}$ and $EC_{50}$ and its 95% confidence interval were calculated by probit analysis modified for continuous data. The NOEC values were determined by calculation of statistical significance using one-way analysis of variance (ANOVA), followed by Williams' t-test, Dunnett's t-test or Welch's t-test ($p = 0.05$).



## II. RESULTS AND DISCUSSION

## A. FINDINGS

Analytical data: Analytical control measurements of the actual concentration of the glyphosate were performed at test start, after 14 and 21 days (after recovery phase). The measured concentrations ranged from 83.9-145% of nominal. Therefore the test was evaluated using the geometric mean measured concentrations.

The $EC_{50}$ and NOEC values after 14 day growth inhibition test are given below based on geometric mean measured concentrations.

| Endpoint | Glyphosate Acid [mg/L] | |
|---|---|---|
| | 14 Day $EC_{50}$ (CI*) | 14 Day NOEC |
| Shoot length/relative increase | 13.44 (7.72 – 23.74) | 5.16 |
| Shoot length/growth rate | 42.79 (24.74 – 76.48) | 5.16 |
| Fresh weight/relative increase | **4.44** (2.28 – 8.51) | < 0.30 |
| Fresh weight/ growth rate | 10.33 (5.59 – 19.21) | < 0.30 |
| Dry weight/relative increase | n.d. | 145 |
| Dry weight/ growth rate | 143.34 (10.06 – n.d.) | 145 |
| Root length/relative increase | 5.84 (4.65 – 7.37) | 1.10 |
| Root length/growth rate | 46.50 (34.75 – 62.52) | 1.10 |

* CI = 95% confidence interval
n.d.: not determined due to mathematical reasons or inappropriate data; highlighted value indicates most sensitive measured parameter

The $EC_{50}$ and NOEC values after 7 day recovery period are given below based on geometric mean measured concentrations.

| Endpoint | Glyphosate Acid [mg/L] | |
|---|---|---|
| | 7 Day Recovery $EC_{50}$ | 7 Day Recovery NOEC |
| Shoot length/relative increase | n.d. | 26.80 |
| Shoot length/growth rate | n.d. | 26.80 |
| Fresh weight/relative increase | n.d. | ≥723 |
| Fresh weight/ growth rate | n.d. | ≥723 |
| Dry weight/relative increase | n.d. | ≥723 |
| Dry weight/ growth rate | n.d. | ≥723 |
| Root length/relative increase | n.d. | ≥723 |
| Root length/growth rate | n.d. | ≥723 |

n.d.: not determined due to mathematical reasons or inappropriate data



## B. OBSERVATIONS

There was a concentration dependent effect on growth, root length, fresh and dry weight of *Myriophyllum aquaticum.* Growth was significantly reduced at 5.16 mg glyphosate/L, fresh weight at <0.3 mg glyphosate/L, dry weight at 145 mg glyphosate/L and root length at 1.10 mg glyphosate acid equivalents/L during the 14 day exposure test. In the subsequent recovery test it was shown that *Myriophyllum aquaticum*, pre-exposed to up to 26.80 mg glyphosate acid equivalents/L were able to recover to control levels of growth in untreated culture medium within 7 days of the exposure period.

**Table 10.8.2.1-2: Percentage of inhibition of *Myriophyllum aquaticum* exposed for 14 days to MON52276**

| Test parameters | Glyphosate Acid [mg/L] (mean measured) | | | | | |
|---|---|---|---|---|---|---|
| | **0.3** | **1.1** | **5.12** | **26.8** | **145** | **723** |
| Inhibition of shoot length increase (%) | -3.5 | 5.1 | 30.5 | 74.1 | 70.3 | 94.1 |
| Inhibition of shoot length growth rate (%) | -2.6 | 2.0 | 17.5 | 58.1 | 53.6 | 88.3 |
| Inhibition of fresh weight increase (%) | 20.7 | 19.2 | 61.2 | 80.1 | 77.6 | 93.8 |
| Inhibition of fresh weight growth rate (%) | 14.6 | 13.3 | 49.4 | 70.9 | 67.8 | 90.2 |
| Inhibition of dry weight increase (%) | 14.7 | 18.2 | 34.3 | 15.8 | -6.9 | 106.6 |
| Inhibition of dry weight growth rate (%) | 11.1 | 14.4 | 29.6 | 19.6 | -4.7 | 112.3 |
| Inhibition of root length increase (%) | -6.8 | -3.9 | 52.0 | 82.9 | 94.5 | 98.3 |
| Inhibition of root length growth rate (%) | -1.7 | 0.9 | 18.3 | 43.9 | 66.7 | 86.8 |

For *Myriophyllum aquaticum,* plant fresh weight measurements are relevant for risk assessment as lower variability is associated with individual plant measurement compared to procedure used for dry weights which attracts a greater variability - with all plants pooled according to treatment and then compared to dry weights established at test start using a separate set of plants. Furthermore, root length measurements are considered semi-quantitatively, as only the length of the longest roots have been measured. The number of side roots and total number have not been determined given the practical constraints associated with the sediment *Myriophyllum* test design. Effects on roots are considered to be reflected in fresh weight measurements.

The study fulfils the validity criteria as stated in the study plan which follows the criteria established by the AMRAP working group, with an increase of biomass (shoot length) in controls was > 50 %, indicating that continuous growth was supported throughout the test duration. Furthermore, constant maintenance of temperature (20 ± 2 °C) was also achieved.



## III. CONCLUSION

**MON52276 significantly inhibited the fresh weight of *Myriophyllum aquaticum* after 14 days at a mean measured concentration of <0.3 mg glyphosate acid equivalents/L. Shoot length was inhibited at or above mean measured concentrations of 5.16 mg glyphosate acid equivalents/L. The 14-d $EC_{50}$ value for fresh weight inhibition was 4.4 mg glyphosate acid equivalents/L and for shoot length it was 13.44 mg glyphosate acid equivalents/L. *Myriophyllum aquaticum* pre-exposed for 14 days to up to 26.80 mg glyphosate acid equivalents/L were able to recover in untreated culture medium after a 7 day recovery period.**

## IIIA 10.8.2.2 Aquatic field testing

No aquatic field testing was required due to the low acute toxicity demonstrated with MON 52276, glyphosate acid, glyphosate-IPA salt and its major aquatic metabolites AMPA and HMPA.

## IIIA 10.9 Effects on other non-target organisms (flora and fauna) believed to be at risk

Tests on other non-target species are not required.

### IIIA 10.9.1 Summary of available data from preliminary tests used to assess biological activity and dose range finding, which may provide information on other non-target species (flora and fauna)

See IIIA 10.9.

### IIIA 10.9.2 A critical assessment as to the relevance of the preliminary test data to potential impact on non-target species

See IIIA 10.9.

## IIIA 10.10 Other/special studies

There are no additional European requirements for formulated products.

### IIIA 10.10.1 Other/special studies – laboratory studies

No additional studies were required.

### IIIA 10.10.2 Other/special studies – field studies

No other studies have been conducted and no additional studies are deemed necessary.

## IIIA 10.11 Summary and evaluation of points IIIA 9 and IIIA 10.1 to 10.10, together with a detailed and critical assessment of the data

MON 52276 is a liquid soluble concentrate containing 360 g glyphosate acid/litre. The product is used to control emerged annual, perennial and biennial weeds. It is applied at a maximum single application rate of 2.88 g a.s./ha, equivalent to 8 L product/ha, with a maximum annual application rate of 4.32 g a.s./ha, equivalent to 12 L product/ha in any 12 month period across all use categories. MON 52276 is typically applied to emerged weeds before planting of crops, post-planting/pre-emergence of crops, during crop maturity of cereals and oilseed rape and along tree lines and around the base of trunks of stone fruits, pome fruits, olives, vines, citrus and tree nuts. MON 52276 is applied post-emergence to the crops, using tractor mounted or knapsack, ground-directed broadcast sprayers and volumes ranging between 100 and 400 L/ha.

Risk assessment was conducted for field use on annual weeds growing on bare soil (pre-planting and pre-emergence of crops), cereals, oilseed rape, orchards and vines.

Based on the results of the current risk assessment, it has been demonstrated that the supported uses of MON 52276 do not cause unacceptable effects on any of the species tested (aquatics, birds, mammals, bees, NTA, soil macro- and micro fauna and non-target plants).

### IIIA 10.11.1 Predicted distribution and fate in the environment and the time courses involved

Initial concentrations, maximum and minimum plateau concentrations, and actual and time weighted average concentrations of glyphosate and AMPA in soil were calculated for a single maximum application rate of 4320 g glyphosate acid/ha. ModelMaker™ (version 4.0) was used to calculate concentrations in soil as a function of time for glyphosate and its metabolite AMPA. Accumulation potential was investigated for no-tillage and tillage systems, *i.e.* for respective soil depths of 5 and 20 cm. The $PEC_s$ calculations for glyphosate and AMPA and were based on their respective longest half-lives derived from field dissipation studies.

Predicted environmental concentrations in groundwater were calculated for glyphosate and its major soil metabolite AMPA for a range of uses in various crops in the EU. The exposure assessment was based on a representative use pattern derived from the joint representative GAP. Depending on the crop, two- or three-consecutive applications (respective intervals as defined in the joint representative GAP) at rates ranging from 720 to 2880 g glyphosate acid/ha were evaluated. In order to cover a wide range of uses, the representative FOCUS crop-scenarios were chosen so as to ensure that all FOCUS groundwater scenarios are considered for representative uses chosen for modelling. The assessment was performed using the leaching models FOCUS PEARL 4.4.4 and FOCUS PELMO 4.4.3.

Predicted environmental concentrations in surface water ($PEC_{sw}$) and sediment ($PEC_{sed}$) were calculated for the active substance glyphosate and its metabolites AMPA and HMPA in aquatic systems. The simulations were performed for a number of glyphosate uses on various crops in the EU reflecting the representative joint GAP using the current versions of FOCUS STEPS 1-2 (version 2.1) for Step 1 and 2 and FOCUS SWASH (version 3.1) for Step 3. Step 3 calculations were carried out to provide more realistic estimates of the $PEC_{sw}$ and $PEC_{sed}$ for the active substance only. For the metabolite HMPA; $PEC_{sw}$ and $PEC_{sed}$ were calculated based on Step 1 and 2 results of the parent compound glyphosate assuming 10% maximum occurrence for HMPA and accounting for the molar mass difference. Depending on the crop and simulation model used, single and multi applications at rates up to 4320 g glyphosate acid /ha were considered in Steps 1 to 3. In order to cover a wide range of uses, representative FOCUS crop scenarios were chosen. Several application scenarios were considered for the following representative crops: winter and spring cereals, potatoes and pome/stone

fruit. Both single and multiple application scenarios representative for all intended uses were taken into account.

**$PEC_{soil}$**

The initial $PEC_s$ values for glyphosate and AMPA at 5 cm depth were 5.76 and 1.48 mg/kg, respectively. Maximum plateau $PEC_s$ values of glyphosate and AMPA were calculated to be 8.07 and 5.35 mg/kg for no-tillage systems. For systems with tillage, the respective values were predicted to be 2.02 and 1.34 mg/kg.

**$PEC_{gw}$**

The predicted environmental concentrations in groundwater ($PEC_{gw}$) of glyphosate and its metabolite AMPA were calculated to be < 0.001 µg/L for all crops and scenarios.

**$PEC_{sw}$ and $PEC_{sed}$**

The overall maximum $PEC_{sw}$ value of glyphosate at Steps 1, 2 and 3 was 101.2, 39.0 and 17.7 µg/L, respectively. The overall maximum $PEC_{sw}$ value of AMPA at Step 1 and 2 was 41.0 and 16.9 µg/L. The overall maximum $PEC_{sw}$ value of HMPA at Step 1 and 3 was 6.7 and 2.6 µg/L.

## IIIA 10.11.2 Non-target species at risk and extent of potential exposure

**Birds**

The risk assessment for birds was carried out according to the ***EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)***.

The acute and long-term risks of MON 52276 to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with glyphosate and its metabolites according to the proposed use patterns.

Risk of secondary poisoning assessment is not required, as glyphosate acid has a log $P_{OW}$ value of <-3.2 and its metabolite AMPA has a log $P_{OW}$ of -5.18. It was therefore not necessary to consider the risk from secondary poisoning further. Therefore, based on the low log $P_{OW}$ values the risk from bioaccumulation to fish-eating and earthworm-eating birds is negligible.

The TER values (Screening step or Tier 1) calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk, indicating that the risk to birds is acceptable following use of MON 52276 according to the proposed use pattern.

**Terrestrial vertebrates (other than birds)**

The risk assessment for mammals was carried out according to the ***EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).***

The acute and long-term risks of MON 52276 to mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with glyphosate acid and its metabolites according to the proposed use pattern.

Risk of secondary poisoning assessment is not required, as glyphosate acid has a log $P_{OW}$ value of <-3.2 and its metabolite AMPA has a log $P_{OW}$ of -5.18. It was therefore not necessary to consider the risk from secondary poisoning further. Therefore, based on the low log $P_{OW}$ values the risk from bioaccumulation to fish-eating and earthworm-eating mammals is negligible.

The TER values (Screening step or Tier 1 calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk, indicating that the risk to mammals is acceptable following use of MON 52276 according to the proposed use pattern.

**Aquatic species**

The risk assessment for aquatic organisms was carried out according to the ***EU Guidance Document on Aquatic Ecotoxicology (2002).***

The acute and long-term risks of glyphosate acid, its salts and metabolites AMPA and HMPA to aquatic organisms were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with glyphosate acid, its salts and metabolites AMPA and HMPA, and predicted environmental concentrations in surface water ($PEC_{sw}$) following applications according to the proposed use pattern. $PEC_{sw}$ used for the risk assessment are provided in ***Part B, Section 5, Point 9.7*** and details on the calculation method and used parameters are given in that section.

The TER values calculated for all crop scenarios exceed the trigger values of 100 for acute risk and 10 for long-term risk, indicating that the risk is acceptable following use of MON 52276 according to the proposed use pattern.

**Bees**

The risk assessment for bees was carried out according to the ***EU Guidance Document on Terrestrial Ecotoxicology (2002)*** **and** ***EPPO 2010.***

All the hazard quotients are substantially less than 50, indicating that the active substance and the formulation pose a low risk to bees. Furthermore, no effect on bee brood was observed. Therefore, a low risk to bees is expected from the application of MON 52276.

**Other non-target arthropods**

The risk assessment for non-target arthropods was conducted according to the recommendations of ***ESCORT 2 guidance document (Candolfi et al, 2001)*** and the ***EU Guidance Document on Terrestrial Ecotoxicology.***

To assess the effects of MON 52276 on terrestrial non-target arthropods other than bees, six species of different arthropod taxa (*Typhlodromus pyri*, *Aphidius rhopalosiphi*, *Chrysoperla carnea*, *Aleochara bilineata*, *Poecilus cupreus*, and *Pardosa spp.*) were exposed to MON 52276. The results of these studies indicate that there would be low risk to foliar and soil dwelling organisms for the proposed uses of 2 MON 52276.

**Earthworms**

The risk assessments follow the ***EU Guidance Document on Terrestrial Ecotoxicology (SANCO 10329/2002).***

The potential acute and long-term risks of MON 52276 to earthworms were evaluated in an initial Tier 1 assessment based on comparison of PECsoil values and relevant ecotoxicological endpoints. This assessment showed low acute and long-term risk to earthworms at all proposed treatment rates.

**Effects on other soil non-target macro-organisms**

The risk assessments follow the ***EU Guidance Document on Terrestrial Ecotoxicology (SANCO 10329/2002).***

The potential long-term risks to other non-target macro-organisms were also evaluated for *Folsomia candida* and *Hypoaspis aculeifer*. This assessment showed low long-term risk to earthworms at all proposed treatment rates.

**Effects on soil non-target microorganisms**

The risk assessment follows the ***EU Guidance Document on Terrestrial Ecotoxicology (SANCO 10329/2002).***

The risk assessment showed acceptable risk for soil micro-organisms. Therefore, the use of MON 52276 according to the proposed use pattern can be considered not to result in any unacceptable adverse effects for soil micro-organisms.

**Organic matter breakdown**

No tests are required considering the persistence trigger in accordance with the EU Guidance Document SANCO/10329/2002 rev 2, 2002. The field $DT_{90}$ is lower than 365 days for glyphosate, its salts and soil metabolite AMPA indicating that there will be no long-term exposure or accumulation of residues.

**Non-Target Plants**

The risk assessment for non-target plants was conducted according to the recommendations of ***EU Guidance Document on Terrestrial Ecotoxicology (SANCO 10329/2002).***

Non-target plant testing with glyphosate and glyphosate formulations evaluating potential effects following pre-emergent (soil) exposure and post-emergent (foliar) exposure indicated that the compound demonstrated no activity in the seedling emergence study. For vegetative vigour, the effects of glyphosate acid and MON 52276 on vegetative vigour of a range of terrestrial non-target plants has been assessed in four glasshouse studies on non-target plants.

The effect endpoints used in the terrestrial non-target plant risk assessment (*i.e.* $ER_{50}$ for the 17 plant species tested for vegetative vigour) were re-evaluated to construct a species sensitivity distribution from which an $HC_5$ was obtained. According to the Guidance Document on Terrestrial Ecotoxicology SANCO/10329/2002 rev.2 (final), 17 October 2002, the risk for terrestrial plants is assumed to be acceptable if the $ER/EC_{50}$ for less than 5% of the species is below the highest predicted exposure level. As this is the case for terrestrial non-target plants, for the refined risk assessment the TER values considering the $HC_5$ are compared to a trigger of 1. Based on this assessment, a TER trigger of 1 according to the Terrestrial Guidance Document is achieved. Thus, no unacceptable risk to non-target terrestrial plants is to be expected for the use of MON 52276.



## IIIA 10.11.3 Short and long term risk for non-target species, populations, communities and processes

There are no additional European requirements for formulated products.

- The $TER_A$ and $TER_{LT}$ derived for proposed uses of MON 52276 indicate low risk to birds.
- The $TER_A$ and $TER_{LT}$ for the proposed uses of MON 52276 indicate low acute and chronic risk to aquatic organisms.
- The $TER_A$ and $TER_{LT}$ for the proposed uses of MON 52276 indicate low risk to mammals.
- The $Q_{ho}$ and $Q_{hc}$ are < 50. Therefore, MON 52276 will be of low risk to honey bees.

- No significant adverse effects on ground or foliar dwelling beneficial arthropods are likely from use of MON 52276.

- The $TER_A$ and $TER_{LT}$ value exceeds the relevant Annex VI decision making criteria of 10 and 5, respectively, for earthworms. Therefore, it can be concluded that MON 52276, glyphosate acid, glyphosate IPA-salt, AMPA and HMPA will be of low risk to earthworms.

- MON 52276, glyphosate acid, glyphosate IPA-salt, and their metabolites, are predicted to be safe to soil microflora when used according to Good Agricultural Practices.

- MON 52276 poses minimal risk to non-target plants (NTPs) growing adjacent to the application site. As for all herbicides good spray practice should minimise the exposure of non-target plants (and crops) to spray drift.

### IIIA 10.11.4 Risk of fish kills and fatalities in large vertebrates or terrestrial predators

There are no additional European requirements for formulated products.

### IIIA 10.11.5 Precautions necessary to avoid or minimize contamination of the environment and for the protection of non-target species

Use of MON 52276 at the proposed label rates and according to good agricultural practice, poses low risk to all non-target species.

## APPENDIX I: CALCULATION OF THE 21-DAY TIME-WEIGHTED-AVERAGE (TWA) FOR GLYPHOSATE IN GRASS FOLIAGE USED IN THE MAMMALIAN RISK ASSESSMENT

The methodology used to calculate the TWA for glyphosate in grass foliage for the long-term mammalian risk assessment follows the procedure described in the Guidance Document on Terrestrial Ecotoxicology (1). With the approach outlined in the Guidance Document on Terrestrial Ecotoxicology, residues are assumed to follow the standard pattern of first order exponential decline. The decline of glyphosate residue in grass was characterised using data from 22 residue trials each of which had a Day 0 value. Of these 22 trials, 18 of the trials were from 4 separate Monsanto reports (references 2 - 5) and 4 trials were from 2 separate Cheminova reports (references 6 and 7). MON 2139 and a comparable Cheminova formulation were used in these grass residue trials. The grass residue values from the Cheminova trials were taken directly from the Glyphosate Monograph (reference 8); the Cheminova reports themselves were not reviewed.

The dissipation of glyphosate in grass was estimated using the standard first order dissipation model:

$$C_t = C_i \times e^{-kt} \quad (1)$$

where $k$ is the first order rate constant, $C_i$ is the initial residue concentration, and $C_t$ is the residue concentration at time $t$. Residue half life time ($DT_{50}$) in days for these grass land trials was calculated with equation 2.

$$DT_{50} = \frac{-\ln 0.5}{k} \quad (2)$$

In each Monsanto report, residual glyphosate in mg/kg dry matter in grass was normalised to 1 kg a.s./ha and these values were plotted against time in days. For the Monsanto residue trials, many of the later sampling intervals were taken after plant desiccation. Therefore, for the purpose of accurately characterising glyphosate dissipation kinetics in grass, the glyphosate residues in mg/kg normalised to 100% dry matter content were used to eliminate the effect of sample weight losses during desiccation (Table II-1). However, since the final sampling day in the Cheminova trials was on Day 5, when grass desiccation was negligible, correction for moisture content was not necessary (Table II-2).

The dissipation of glyphosate was modelled with equation 1 using nonlinear regression (9). For 20 of the 22 trials, the standard first-order dissipation model provided an adequate fit for glyphosate dissipation ($R^2 > 0.8$). The standard first-order dissipation model inadequately fit one Monsanto trial and one Cheminova trial (coefficient of determination, $R^2 \leq 0.600$). For these two trials, the $DT_{50}$ was estimated by identifying the first day when a measured value had greater than 50% dissipation. Since the $DT_{50}$ was estimated in this fashion for these two trials, the glyphosate residues in Tables II-1 and II-2 are also expressed as a percentage of the initial concentration, which was set at 100% for Day 0 after treatment. **The average $DT_{50}$ for the 22 trials was 2.8 days.**

The linear first-order rate constant corresponding to a $DT_{50}$ of 2.8 days was calculated using equation 3:

$$k = \frac{-\ln 0.5}{DT_{50}} \quad (3)$$

which results in a rate constant $k$ of 0.2476 days$^{-1}$.

The 21-day time-weighted average (TWA) was calculated using equation 4:

$$TWA = \frac{(1 - e^{-kt})}{kt} \quad (4)$$

where *k* is the first order rate constant calculated using equation (3), and *t* is the window of time over which the TWA is calculated (i.e. 21 days). Using these parameters for *k*, and *t*, the 21-Day TWA is calculated to be **0.19** for the active substance glyphosate acid.

**Table I-1: Glyphosate residues in grass following a single treatment of Roundup® (MON 2139, SL/360). Source: Monsanto Field Residue Studies**

| Country, Year Trial, ID | App. Rate (kg a.s./ ha)[1] | NRG 100% of DM [2] | % of Day 0 a.s. residue | DAT[3] | $R^2$ | $k$ (days$^{-1}$) | $DT_{50}$ (days) | Glyphosate Monograph Reference; Monsanto Report No. |
|---|---|---|---|---|---|---|---|---|
| *Great Britain, 1981* | | | | | | | | |
| [redacted] 8125 | 1.08 | 101 | 100 | 1 h | 0.990 | 0.4106 | 1.7 | [redacted] 95-01242 [redacted] 30.080 |
| | | 27 | 26.7 | 3 | | | | |
| | | 12 | 11.9 | 7 | | | | |
| [redacted] 8125 | 2.88 | 67 | 100 | 1 h | 0.997 | 0.3251 | 2.1 | |
| | | 27 | 40.3 | 3 | | | | |
| | | 5 | 7.5 | 7 | | | | |
| [redacted] 30117 | 1.08 | 247 | 100 | 1 h | 0.997 | 0.9584 | 0.72 | |
| | | 14 | 5.7 | 3 | | | | |
| | | 8 | 3.2 | 7 | | | | |
| | | 7 | 2.8 | 9 | | | | |
| | | 6 | 2.4 | 10 | | | | |
| | | 3 | 1.2 | 14 | | | | |
| [redacted] 30117 | 2.88 | 130 | 100 | 1 h | 0.976 | 0.7063 | 0.98 | |
| | | 14 | 10.8 | 3 | | | | |
| | | 11 | 8.5 | 7 | | | | |
| | | 9 | 6.9 | 9 | | | | |
| | | 10 | 7 | 10 | | | | |
| | | 3 | 2.3 | 14 | | | | |
| [redacted] 30119 | 1.08 | 193 | 100.0 | 1 h | 0.809 | 0.1456 | 4.8 | |
| | | 175 | 90.7 | 4 | | | | |
| | | 38 | 19.7 | 9 | | | | |
| | | 9 | 4.7 | 11 | | | | |
| [redacted] 30119 | 2.88 | 161 | 100.0 | 1 h | 0.901 | 0.1550 | 4.5 | |
| | | 123 | 76.4 | 4 | | | | |
| | | 30 | 18.6 | 9 | | | | |
| | | 13 | 8.1 | 11 | | | | |
| *France, 1981* | | | | | | | | |
| 811 | 0.72 | 168 | 100.0 | 0 | 0.976 | 0.4576 | 1.5 | [redacted] 95-01245 [redacted] 30.082 |
| | | 9 | 5.4 | 5 | | | | |
| | | 23 | 13.7 | 8 | | | | |
| | | 5 | 3.0 | 12 | | | | |
| 811 | 1.08 | 134 | 100.0 | 0 | 0.950 | 0.3768 | 1.8 | |
| | | 9 | 6.7 | 5 | | | | |
| | | 27 | 20.1 | 8 | | | | |
| | | 5 | 3.7 | 12 | | | | |
| *The Netherlands, 1982* | | | | | | | | |
| NL 8207 | 1.44 | 682.0 | 100.0 | 0 | 0.998 | 0.4230 | 1.6 | [redacted] 95-01264 [redacted] 30.101 |
| | | 77.0 | 11.3 | 5 | | | | |
| | | 31.7 | 4.6 | 10 | | | | |
| *Denmark, 1981* | | | | | | | | |
| [redacted] -1981-0181Vi | 1.80 | 162.9 | 100 | 0 | 0.844 | 0.1415 | 4.9 | [redacted] 95-01273 [redacted] 30.132 |
| | | 36.0 | 22.1 | 7 | | | | |
| | | 52.6 | 32.3 | 14 | | | | |
| [redacted] 1981-0281Vi | 1.80 | 496.3 | 100 | 0 | 0.994 | 0.1537 | 4.5 | |
| | | 184.4 | 37.2 | 7 | | | | |
| | | 37.0 | 7.5 | 14 | | | | |
| [redacted] )-1981- | 1.80 | 437.9 | 100 | 0 | 0.961 | 0.2616 | 2.6 | |
| | | 51.2 | 11.7 | 7 | | | | |

| Country, Year Trial, ID | App. Rate (kg a.s./ ha)[1] | NRG 100% of DM [2] | % of Day 0 a.s. residue | DAT[3] | $R^2$ | $k$ ($days^{-1}$) | $DT_{50}$ (days) | Glyphosate Monograph Reference; Monsanto Report No. |
|---|---|---|---|---|---|---|---|---|
| 0981[redacted] | | 69.4 | 15.8 | 14 | | | | |
| [redacted] 1981-0481Vi | 1.80 | 190.7 | 100 | 0 | 0.937 | 0.1098 | 6.3 | |
| | | 69.0 | 36.2 | 7 | | | | |
| | | 59.0 | 30.9 | 14 | | | | |
| *Denmark, 1983* | | | | | | | | |
| [redacted] 9B | 1.44 | 158.9 | 100 | 0 | 0.995 | 0.9083 | 0.76 | [redacted]95-01273 [redacted]30.132 |
| | | 9.9 | 6.2 | 3 | | | | |
| | | 8.3 | 5.2 | 7 | | | | |
| | | 3.3 | 2.1 | 10 | | | | |
| | | 4.4 | 2.8 | 14 | | | | |
| [redacted] 1983 | 1.44 | 169.6 | 100 | 0 | 0.990 | 0.2853 | 2.4 | |
| | | 16.4 | 9.7 | 7 | | | | |
| | | 16.2 | 9.6 | 10 | | | | |
| | | 13.0 | 7.7 | 14 | | | | |
| [redacted] | 1.44 | 257.2 | 100 | 0 | * | * | $10^{4}$ | |
| | | 155.8 | 60.6 | 3 | | | | |
| | | 144.6 | 56.2 | 7 | | | | |
| | | 123.9 | 48.2 | 10 | | | | |
| | | 151.0 | 58.7 | 14 | | | | |
| [redacted] 1983 | 1.44 | 354.9 | 100 | 0 | 0.960 | 0.1718 | 4.0 | |
| | | 78.7 | 22.2 | 7 | | | | |
| | | 62.7 | 17.7 | 14 | | | | |
| | | 39.0 | 11.0 | 21 | | | | |
| [redacted] 1983 | 1.44 | 253.9 | 100 | 0 | 0.992 | 0.9014 | 0.77 | |
| | | 16.6 | 6.5 | 3 | | | | |
| | | 6.0 | 2.4 | 7 | | | | |
| | | 6.3 | 2.5 | 10 | | | | |
| | | 8.3 | 3.3 | 14 | | | | |

[1] a.s. = glyphosate acid.
[2] NRG 100% of DM = residual glyphosate mg/kg normalised to 1 kg a.s./ha and corrected to 100% dry matter content. Values taken directly from Monsanto reports.
[3] DAT = Days After Treatment.
[4] Estimated $DT_{50}$ value based on time when approximately 50% dissipation was reached.
* Did not fit standard 1st order dissipation model.

**Table I-2: Glyphosate residues in grass following a single treatment of CHE 3607 (SL/360). Source: Cheminova Field Residue Studies (cited in Glyphosate Monograph)**

| App. Rate (kg a.s. /ha) [1] | Residue (mg a.s./kg wet weight) | % of Day 0 a.s. Residue | DAT[2] | $R^2$ | $k$ (days$^{-1}$) | $DT_{50}$ (days) | Glyphosate Monograph Reference; Cheminova Report no. |
|---|---|---|---|---|---|---|---|
| *Great Britain, 1992* | | | | | | | |
| 2.16 | 237.6 | 100.0 | 4 h | 0.987 | 1.9629 | 0.35 | [redacted]95-01308<br>[redacted]-93/04572-01 |
| | 45 | 18.9 | 1 | | | | |
| | 19.6 | 8.2 | 3 | | | | |
| | 9.6 | 4.0 | 5 | | | | |
| 1.08 | 87.6 | 100.0 | 4 h | 0.937 | 2.0879 | 0.33 | |
| | 14.6 | 16.7 | 1 | | | | |
| | 14.3 | 16.3 | 3 | | | | |
| | 8.3 | 9.5 | 5 | | | | |
| 2.16 | 252.3 | 100.0 | 4 h | 0.951 | 0.4885 | 1.4 | [redacted]95-01312<br>[redacted]-93/13842-01 |
| | 131 | 51.9 | 1 | | | | |
| | 72.1 | 28.6 | 3 | | | | |
| | 36.8 | 14.6 | 5 | | | | |
| 1.08 | 90.4 | 100.0 | 4 h | * | * | 3 [3] | |
| | 142.8 | 158.0 | 1 | | | | |
| | 39.8 | 44.0 | 3 | | | | |
| | 17.3 | 19.1 | 5 | | | | |

[1] a.s. = glyphosate acid.
[2] DAT = Days After Treatment.
[3] Estimated $DT_{50}$ value based on time when approximately 50% dissipation was reached.
* Did not fit standard 1st order dissipation model.

## Appendix I References

1. Guidance Document on Risk assessment for Birds and Mammals Under Council Directive 91/414/EEC, September 2002, SANCO/4145/2000.
2. [redacted] 1982. Glyphosate residues in grass, hay and silage following preharvest application of Roundup® in the United Kingdom. [redacted] report number [redacted] 30.080, Annex II reference no. RIP95-01242.
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6. [redacted] 1994. Determination of residues of glyphosate in grass (grass and hay) – treatment with CHE 3607 – UK, season 1992. [redacted] Study plan [redacted]/1911/CN. [redacted] report no. [redacted]-93/04572-01, Annex II reference no. RIP95-01308. As cited in [8].[4]
7. [redacted] 1994. Determination of residues of glyphosate in grass (grass and silage) – treatment with CHE 3607 – UK, season 1992. [redacted] Study plan [redacted]/1912/CN. [redacted] report no. [redacted]-93/03842-01, Annex II reference no. RIP95-01312. As cited in [8].[4]
8. Glyphosate Monograph. 1998, Volume 3, B-6, Residue data, Table B.6.6.14-1.
9. Jandel Scientific. 2000. *SigmaPlot Scientific graphing system, Version 6.0.* San Rafeal, CA, USA.



[4] Note: Glyphosate residues in grass from this study were taken directly from the Glyphosate Monograph; the full Cheminova reports were not reviewed.

## APPENDIX II: CALCULATION OF THE 21-DAY TIME-WEIGHTED-AVERAGE CONCENTRATION OF GLYPHOSATE IN ARTHROPODS USED IN THE AVIAN RISK ASSESSMENT

The methodology used to calculate the TWA concentration of glyphosate in ground-dwelling arthropods for the long-term avian risk assessments follows the procedure described in the Guidance Document on Terrestrial Ecotoxicology (1). With the approach outlined in the Guidance Document on Terrestrial Ecotoxicology, residues are assumed to follow the standard pattern of first order exponential decline. The decline of glyphosate residues in ground-dwelling arthropods was determined from a field study conducted in an arable field following the spray application (nominally 2.88 kg a.s./ha) of the glyphosate formulation MON 52276 (2). The dissipation of glyphosate in arthropods was estimated using the standard first-order dissipation model (equation 1) using nonlinear regression (3):

$$C_t = C_i \times e^{-kt} \quad (1)$$

where $k$ is the first order rate constant, $C_i$ is the initial residue concentration (constrained to Day 0 mean concentration of 5.97 mg a.s./kg), and $C_t$ is the residue concentration at time $t$. Residue half life time ($DT_{50}$) in days for the study was calculated with equation 2.

$$DT_{50} = \frac{-\ln(0.5)}{k} \quad (2)$$

The mean residue concentrations of glyphosate (n=3, with the exception of DAT 3: n=2) were used to calculate the model estimates (Table II-1; illustration Figure II-1). The standard first-order dissipation model provided an adequate fit for glyphosate dissipation ($R^2 \geq 0.88$). The model estimates **a rate constant $k$ of 0.1250 days$^{-1}$ and a $DT_{50}$ value of 5.545 days**.
The 21-day time-weighted average (TWA) residue was calculated using equation 3:

$$TWA = \frac{(1 - e^{-kt})}{kt} \quad (3)$$

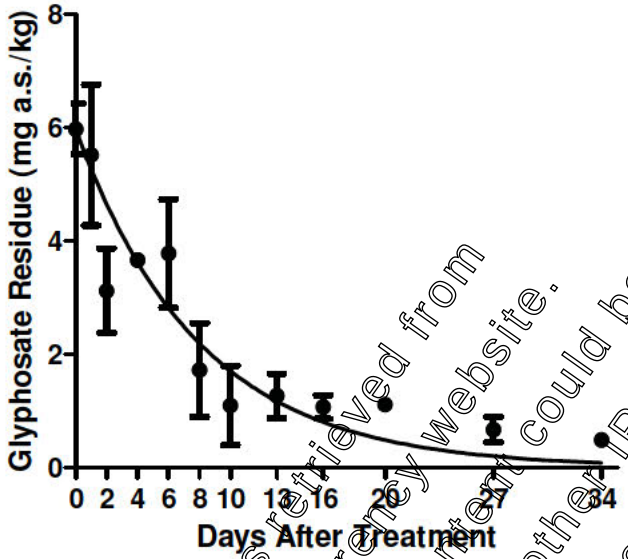
where $k$ is the first order rate constant calculated using equation (3), and $t$ is the window of time over which the TWA is calculated (i.e. 21 days). Using these parameters for $k$, and $t$, the 21-Day TWA is calculated to be **0.35** for the active substance glyphosate acid.

**Table II-1. Glyphosate residues in ground-dwelling arthropods following a single treatment of MON 52276 (2.88 kg a.s./ha application rate).**

| DAT [1] | Glyphosate Residue (mg a.s./kg) [2] | | | | |
|---|---|---|---|---|---|
| | StudyPlot 1 | StudyPlot 2 | StudyPlot 3 | Mean | SD |
| 0 | 5.22 | 5.92 | 6.78 | 5.97 | 0.78 |
| 1 | 7.98 | 4.57 | 4.00 | 5.52 | 2.15 |
| 2 | 3.48 | 1.69 | 4.19 | 3.12 | 1.29 |
| 4 | 3.78 | --- | 3.55 | 3.67 | 0.16 |
| 6 | 2.08 | 5.39 | 3.87 | 3.78 | 1.66 |
| 8 | 0.76 | 3.37 | 1.04 | 1.72 | 1.43 |
| 10 | 0.54 | 2.49 | 0.27 | 1.10 | 1.21 |
| 13 | 2.05 | 0.80 | 0.95 | 1.27 | 0.68 |
| 16 | 0.86 | 1.48 | 0.87 | 1.07 | 0.36 |
| 20 | 1.15 | 1.30 | 0.90 | 1.12 | 0.20 |
| 27 | 0.62 | 0.32 | 1.08 | 0.67 | 0.38 |
| 34 | 0.46 | 0.45 | 0.56 | 0.49 | 0.06 |

[1]DAT=days after treatment. Samples were collected over a 24-h period. For example, Day 0 samples were obtained for the sampling period which began immediately after application and terminated after 24 h.
[2]as glyphosate acid fresh weight

**Figure II-1. Glyphosate residues in ground-dwelling arthropods following a single treatment of MON 52276 (2.88 kg a.s./ha application rate).**






Appendix II References

1. Guidance Document on Risk assessment for Birds and Mammals under Council Directive 91/414/EEC, September 2002, SANCO/4145/2000.
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