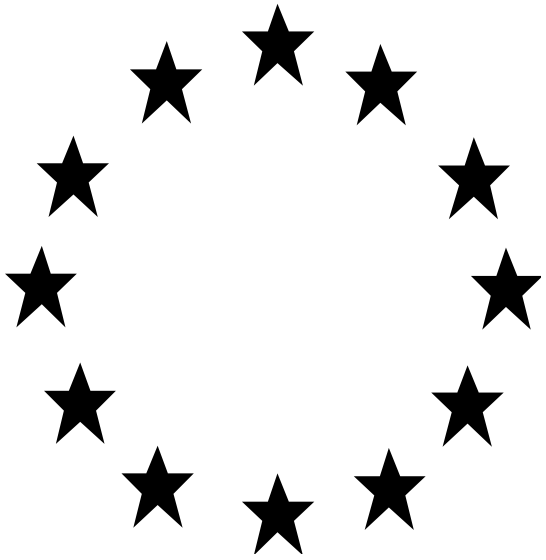

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Spain for the existing active substance

RAPESEED OIL

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, part 5, B.9

September 2008

ANNEX B

Rapeseed oil

B - 9: ECOTOXICOLOGY

General Remarks by the Notifier

Since the formulation NEU 1160 I contains 4% emulgator, which is not of toxicological interest, and 96% Rapeseed oil, it is assumed that the results of studies with NEU 1160 I will not differ from studies conducted with Rapeseed oil. Thus, the results of NEU 1160 I may be extrapolated to Rapeseed oil.

The formulation NEU 1161 I contains 90% Rapeseed oil and 2% Pyrethrum. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil.

B.9 Ecotoxicology

Rapeseed oil is a dietary vegetable oil derived from seeds of Brassica napus. Dietary lipids are processed by known metabolic pathways within the body and contribute to normal physiological functions. They are utilized as a carbon and energy source.

Rapeseed oil is used as a contact acaricide and/or insecticide in formulations for the use against spider mites, mealy bugs and scales in ornamentals and orchards and/or for the suppression of winter eggs of spider mites in orchards and woody ornamentals. It is intended to be used for greenhouse ornamentals with 3 applications (glass house, indoor) per growing season, or in orchards and woody ornamentals in the field (1 application) at the start of the vegetation period. Application rates are dependent on the height of the plants.

Rapeseed oil suffocates insects and mites by blocking the spiracles. In addition, the oil also blocks the body pores, which are used by the mite to take in moisture in order to maintain the levels of water in the body. Since this mode of action is mechanical rather that chemical, insect resistance or tolerance to oils is not expected.

The formulated product NEU 1160 I is an emulsifiable concentrate [EC] containing 883 g/L of rapeseed oil. The formulation NEU 1160 I contains 96% Rapeseed oil and 4% emulgator, which is not of toxicological relevance (please refer to MSDS in Doc J, confidential information). Several ecotoxicological studies (acute earthworms and soil microorganism toxicity studies, and not target plants) have been performed with a different formulation NEU 1161 I which contain 90 % Rapeseed oil and 2 % Pyrethrum extract. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil.

B.9.1 Effects on birds (IIA 8.1; IIIA 10.1)

B.9.1.1 Acute oral toxicity

ACTIVE SUBSTANCE

No acute bird toxicity studies have been submitted.

PLANT PROTECTION PRODUCT

No acute bird toxicity studies have been submitted.

B.9.1.2 Short-term toxicity. Avian dietary (5-day test)

ACTIVE SUBSTANCE

No short-term bird toxicity studies have been submitted.

PLANT PROTECTION PRODUCT

No short-term bird toxicity studies have been submitted.

Notifier justification: Since fatty acids are naturally contributing to the feed of birds, Rapeseed oil is considered not to present any risk to birds from feeding.

B.9.1.3 Subchronic and reproductive toxicity to birds

ACTIVE SUBSTANCE

No short-term bird toxicity studies have been submitted.

PLANT PROTECTION PRODUCT

No short-term bird toxicity studies have been submitted.

B.9.1.4 Supervised cage or field trials

Supervised cage or field trials were not conducted.

B.9.1.5 Acceptance of bait, granules or treated seeds by birds (palatability test)

Acceptance test with baits, granules or treated seeds have not been conducted for this product. This information is not relevant because NEU 1160 I will not be used as bait, granules, or treated seeds.

B.9.1.6 Effects of secondary poisoning (Annex IIIA, point 10.1.4)

Notifier justification: Pesticides with a high BCF may accumulate in fish tissue following an exposure for a prolonged period of time and may constitute a risk for humans and predators feeding on contaminated fish. Studies on the bioconcentration potential of the active substance in fish were not performed since Rapeseed oil degrades rapidly in water (DT50 between 3 and 9 days based on laboratory studies on soil, see fate section).

Therefore, Rapeseed oil from use of NEU 1160 I will not bioconcentrate significantly in fish and thus secondary poisoning is unlikely to occur.

RMS opinion: Based estimated D50 in water it is logical to assume a low potential of bioconcentration for Rapeseed oil. Not further information is needed.

B.9.1.7 Summary of data for avian toxicity

No avian toxicity studies have been submitted with the active substance or with the formulated NEU 1160 I.

Notifier justification: Since fatty acids are naturally contributing to the feed of birds, Rapeseed oil is considered not to present any risk to birds from feeding.

Because Rapeseed oil degrades rapidly in water it is expected that Rapeseed oil from use of NEU 1160 I will not bioconcentrate significantly in fish, and thus secondary poisoning for birds eating contaminated food is unlikely to occur.

B.9.1.8 Risk assessment for birds

RMS comments: Rapeseed oil is a natural oil which is also used as a food commodity. It is a mixture of esters (triglycerides) of different fatty acids. It is known that dietary lipids are processed by known metabolic pathways within the body and contribute to normal physiological functions. They are utilized as a carbon and energy source.

Assuming that:

1) fatty acids are naturally contributing to the feed of birds,
2) the mode of action of rapeseed oil is mechanical rather than chemical,
3) secondary poisoning for birds eating contaminated food is unlikely to occur and
4) low rat acute toxicity is showed (LD50 > 1794.1 mg a.i./kg b.w)

Therefore low risk it is expected to birds for formulated products containing rapeseed oil.

Not further information is needed.

B.9.2 Effects on aquatic organisms (IIA 8.2; IIIA 10.2)

B.9.2.1 Acute toxicity to fish (IIA 8.2.1; IIIA 10.2.1)

ACTIVE SUBSTANCE

Heintze, A. (2000a): Rep. No. 99505/01-AAOm.

The objective of this study was to determine the acute toxicity of Rapessed oil (Rüböl/Rapsöl, technical rapessed oil, Lot/batch EK 3162299) in rainbow trout (Oncorhynchus mykiss) (Teleostei, Salmonidae) (size 5 ± 1 cm) under semi-static conditions during 96 h of exposure. The test was performed according to OECD No. 203 and EC-Method C.1 (92/69/EC) guidelines and it was conducted under GLP.
Deviations: No deviation of the guideline was observed. No indication of purity and stability of the compound are described. Analytical verification indicates Rapeseed oil was always < 80% nominal. The study is valid.

During acclimation period fish were held under testing conditions in 300 L containers with continuous renewal of water (5-10% per day) and permanent aeration of water for more than 20 days. Feeding was stopped 24 h before initiation of the test.Temperature:15.7-16.8°C ± 1°C and Photoperiod:16 hours daily.

Test design: Range finding-test and main test were performed with groups of 10 fish in each treatment. In the range finding test the fish were exposed to rapeseed oil at nominal concentrations of 0, 0.001, 0.01, 0.1, 1.0, 10 and 100 mg/L under static conditions during 96 h. In the main test the fish were exposed to rapeseed oil at nominal concentrations of 0, 2.2, 4.8, 10.6, 23.4, 51.5, 113.4 and 249.4 mg/L under semi-static conditions (24h renewal) over a period of 96 hours. The test was performed without replication and without a reference substance. A loading rate of max. 4 g fish/L was maintained.

Analytical controls were performed for three representative concentration levels. The Rüböl/Rapsöl content was based on the determination of triglyceride by an enzymatic optical method. Samples were taken out of the centre of the water column in 24 hour intervals (prior to and after renewal of test medium) from initiation to termination of the test. Measurements of temperature, pH-value and dissolved oxygen concentration were performed in 24 hour intervals, prior to and after renewal of water.

Observations: Fish were observed at 3, 6, 24, 48, 72 and 96 hours after initiation of the test.

Findings: The results for each concentration level are shown in Table 9.2.1-1. In the range finding-test 40% fish were dead after a period of 96 h at 100 mg/L.

In the main test, no mortalities, abnormalities or behavioural changes were found in comparison with the control group at and below a nominal concentration of 249.4 mg/L. Not 96 h LC50 could be determined and the higher concentration tested represents the NOEC.

Table 9.2.1-1: Acute toxicity of Rapeseed oil to rainbow trout.

Main test								
Time [h]	**Nominal concentration of Rapeseed oil [mg/L]**							
	Control	**2.2**	**4.8**	**10.6**	**23.4**	**51.5**	**113.4**	**249.4**
	Mortality [%]							
3	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0

Range finding test							
Time [h]	**Nominal concentration of Rapeseed oil [mg/L]**						
	Control	**0.001**	**0.01**	**0.1**	**1**	**10**	**100**
	Mortality [%]						
3	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0
48	0	0	0	0	0	20	20
72	0	0	0	0	0	20	20
96	0	0	0	0	0	30	40

The results of analytical control measurements during the main test are given in Table 9.2.1-2. Low concentrations were determined. The circumstance that only low concentrations of test substance could be determined shortly after preparation of the test medium indicates the phase separation. The aged test solutions indicate no significant bacterial processes of turning the triglyceride to free glycerol and fatty acids, which would lead to higher results in the aged test media or visual bacteria on the surface. There is no emulsifier or solvent vehicle allowed for toxicity testing to keep dispersions stable over more than 5 min.

Table 9.2.1-2: Analytical control measurements of Rapeseed oil in rainbow trout acute toxicity test.

Rapeseed oil	Nominal concentration in mg/L		
Time [h]	10.6	58.3	249.4
	Actual concentration in % of nominal		
0	n.d.	12.0	n.d.
24 aged	n.d.	29.2	2.9
24 fresh	n.d.	29.0	4.5
48 aged	74.5	12.3	2.6
48 fresh	n.d.	14.2	n.d.
72 aged	n.d.	29.2	2.9
72 fresh	n.d.	58.0	4.0
96	74.5	12.3	2.6

n.d. below detection limit (6 mg/L)

RMS comments: The analytical measurements indicate technical difficulties to maintain Rapeseed oil dissolved in the test media. Because in the study the measured concentrations are < 80% of the nominal, toxicity values will be presented as mean measured concentrations. This is in agreement with SANCO/3268/2001. The RMS have been calculated the mean measure concentration at the high concentration tested (see table 9.2.1-2) given a value > 7.48 mg/L (around 3% of nominal, considering for this calculations that samples with n.d. = 6 mg/L).

Conclusion: As no mortality occurred in the main test, not 96 h LC50 could be determined for rainbow trout but it can be estimated that the 96 h LC50 is above the maximum concentration in water of rapeseed oil. The NOEC was observed at a nominal concentration of the maximum concentration in water of the test substance Rapeseed oil.

Based on data from this study Notifier proposes to use for risk assessment a 96 h LC50 > 249.4 mg/L. RMS disagrees with notifier because analytical concentrations are always less that 80%, and propose a 96 h LC50 > 7.48 mg/L (around 3% of maximum concentration tested, see table 9.2.1-2) for risk assessment.

PLANT PROTECTION PRODUCT

Not data have been submitted.

B.9.2.1.1 Warm water fish species

Not specific studies were conducted on warm water species due to the lack of acute toxicity to rainbow trout. Moreover, rapeseed oil is practically insoluble in water and thus is, fish are very unlikely to be contaminated.

Not further information is required.

B. 9.2.1.2 Acute toxicity of metabolites to fish species

Not specific studies were conducted. Notifier indicates that Rapeseed oil is mainly degraded by β-oxidation to fatty acids with lower molecular weight and finally to carbon dioxide. Therefore it is not expected to found metabolites with ecotoxicological relevance.

Not further information is needed.

B. 9.2.2 Chronic toxicity to fish (IIA,8.2.2)

B.9.2.2.1 Chronic toxicity (28 day exposure) to juvenile fish growth and behaviour

ACTIVE SUBSTANCE

No chronic fish toxicity studies have been submitted.

PLANT PROTECTION PRODUCT

No chronic fish toxicity studies have been submitted.

B.9.2.2.2 Fish early stage toxicity test

Notifier justification: No specific studies were conducted on fish early life stage toxicity due to the lack of acute toxicity to cold water fish species (refer to Annex IIA, point 8.2.1.1). Moreover, Rapeseed oil is practically insoluble in water and thus, fish are unlikely to be contaminated.

Not further information is required.

B.9.2.2.3 Fish full life cycle test

Not additional information was provided by the notifier.

The notifier assumes that long-term exposure of fish to Rapeseed oil is not indicated due to the rapid degradation of Rapeseed oil in surface water.

RMS opinion: In the frame work of directive 91/414/EEC chronic toxicity data on fish is always required if DT50 in water is > 2 days or there is more than 1 application.

In this case, the Rapeseed oil BCF and the DT50 in water was not investigated. However, due to the low solubility on water of rapeseed oil, the low acute toxicity of rapeseed oil on rainbow trout , and the estimated low potential of bioconcentration of rapeseed oil prolonged exposure to rapeseed oil and accumulation of the compound in fish are not expected.

No further information is required.

B.9.2.3 Bioconcentration in fish (IIA, point 8.2.3.a)

Not studies have been conducted.

Notifier justification: Rapeseed oil is practically insoluble in water and therefore it is very unlikely that fish get in contact with Rapeseed oil through ingestion. For this reason studies on the bioconcentration potential of the Rapeseed oil in fish were not performed.

RMS opinion: In the frame work of directive 91/414/EEC fish bioconcentration study is always required if logPow > 3 and DT90 (w/s) > 10 days or if > 1 application.

In this case, the Rapeseed oil Log Pow = 23.2908 (obtained by calculation) and the DT90 in water was not investigated. However, due to the low solubility on water of rapeseed oil, the low acute toxicity of rapeseed oil on rainbow trout, and the estimated low potential of bioconcentration of rapeseed oil prolonged exposure to rapeseed oil and accumulation of the compound in fish are not expected.

B.9.2.4 Acute toxicity to aquatic invertebrates

B.9.2.4.1 Acute toxicity to Daphnia

ACTIVE SUBSTANCE

Heintze, A. (2000b): Rep. No. 99505/01-AADm

The objective of this study was to determine the acute toxicity of Rapessed oil (Rüböl/Rapsöl, technical rapessed oil, Lot/batch EK 3162299) on first instar daphnids Daphnia magna (Cladocera: Daphniidae) under semi-static conditions during 48 h of exposure. The test was performed according to EEC-Directive C.2, Daphnia Immobilisation Test guideline and it was conducted under GLP:

Deviations: No deviation of the guideline was observed. No indication of purity and stability of the compound are described. Not acclimation period stated. The study is valid.

Daphnids were feed with Scenedesmus subspicatus at least three times a week. Temperature:19.5 – 20.2 °C. Photoperiod:16 hours of illumination and 8 h of darkness. pH:7.69 – 7.97. Dissolved oxygen: > 60 % of air saturation (approx. 6.0 mg O2/L). For the test, freshly hatched daphnids of an age between 6 and 24 h were used.

Test design: the test was performed in four 100 mL glass beakers for each test substance concentration and the controls, containing 5 daphnids each and filled with 50 mL test medium. The concentrations to be tested were made up with test substance and M4 medium. One control with test medium and two concentrations of the reference compound potassium dichromate were also tested.

Range finding-test and main test were performed with groups of 5 daphnids in each treatment. In the range finding test daphnids were exposed to rapeseed oil at nominal concentrations of 0, 0.001, 0.01, 0.1, 1.0, 10 and 100 mg/L under semi static conditions during 48 h. Following a preliminary range-finding test (static design), Daphnia magna were exposed to eleven nominal test concentrations 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, 51.2, 102.4 and 204.8 mg Rapeseed oil/L (serial dilution factor of 2.0) for 48 hours under semi-static test conditions with a renewal after 24 h. Four replicates of each concentration group were tested with 5 daphnids per replicate. One control with test medium and two concentrations of the reference substance potassium-dichromate (0.9 mg/L and 1.9 mg/L) were also tested.

Analytical controls were performed for three representative concentration levels. The Rüböl/Rapsöl content was based on the determination of triglyceride by an enzymatic optical method. Samples were taken out of the centre of the water column in 24 hour intervals (prior to and after renewal of test medium) from initiation to termination of the test. Measurements of temperature, pH-value and dissolved oxygen concentration were performed in 24 hour intervals, prior to and after renewal of water.

Observations: After 24 h and 48 h the immobilised daphnids were counted. All daphnids were not able to swim within 15 seconds after gentle agitation of the test vessel were considered to be immobilised. The test temperature and the pH as well as the oxygen saturation rate of the test solutions were measured at representative concentration at 0, 24 and 48 h.

Findings: The immobilisation in the main test was between 0% at 0.2 mg/L after 48 h of test duration and 100% at 204.8 mg/L. See Table 9.2.4.1-1 for details.

Table 9.2.4.1-1: Immobilisation data in % of Daphnia magna exposed to Rapeseed oil.

Concentration (mg Rapeseed oil /L)	Immobilized daphnids (%)	
	24 hours	48 hours
0.200	0	0
0.400	0	5
0.800	0	30
1.60	0	35
3.20	0	55
6.40	0	65
12.8	0	70
25.6	0	75
51.2	0	75
102.4	0	80
204.8	20	100
ref.* 0.9	5	35
ref. * 1.9	50	100

Ref. = K2Cr2O7. * the resting daphnids were fixed on surface but still alive

The results of analytical control measurements during the main test are given in Table 9.2.4.1-2. Low concentrations were determined (range: 2.4-10.4 % of nominal for the highest concentration tested). The circumstance that only low concentrations of test substance could be determined shortly after preparation of the test medium indicates the phase separation. The aged test solutions indicate no significant bacterial processes of turning the triglyceride to free glycerol and fatty acids, which would lead to higher results in the aged test media or visual bacteria on the surface.

The toxicological response seemed to be only and effect of the fixation of the daphnids to the surface. Therefore the nominal concentrations could be taken for the statistical evaluation of the toxicological response.

Table 9.2.4.1-2: Analytical concentrations of Rapeseed oil during the main test in percent of nominal concentration on Daphnia magna acute toxicity test.

Rapeseed oil	Nominal concentration in mg/L		
	12.8	51.2	204.8
Time [h]	Actual concentration in % of nominal		
0	n.d.	n.d.	4.5
24 aged	n.d.	n.d.	n.d.
24 fresh	50.8	19.9	10.4
48	n.d.	n.d.	n.d.

n.d. below detection limit (6 mg/L)

At concentrations of 0.8 mg/L and above, the daphnids were fixed to the surface by oil spots and oil films. After 24 h nearly all of them were re-mobilised by the exchange of the test medium but were fixed again to the surface. Due to the distribution of data the statistical evaluation was performed by probit analysis using nominal concentrations. The EC50 (48 h) was calculated to be 4.5 mg Rapeseed oil/L, the NOEC was determined to be 0.2 mg Rapeseed oil/L.

Conclusion: At concentrations of 0.8 mg/L and above, the daphnids were fixed to the surface by oil spots and oil films. Because the compound is not water soluble and has water phase separation the toxicity parameters will be calculated using nominal concentrations besides that analytical verification measurement indicates concentrations < 80%.

Based on nominal concentrations the EC50 (48h) was determined to be 4.5 mg a.i./L (95% confidence limits: 2.6 – 7.9 mg a.i./L) using probit analysis. The NOEC was determined to be 0.2 mg a.i./L.

PLANT PROTECTION PRODUCT

Hertl, J. (2002). Rep. No. 12742220.

The objective of this study was to determine the acute toxicity of NEU 1160 I (active ingredient Rüböl/Rapsöl > 90%, Lot/batch 015031) on first instar daphnids Daphnia magna (Cladocera: Daphniidae) under static conditions during 48 h of exposure. The test was performed according to Directive 92/69/EEC, C.2 1992 and OECD No. 202, Part I, 1984 and it was conducted under GLP.
Deviations: No indication of stability by the sponsor. The test item was stored between 1 and 11°C. This deviation will not have a negative outcome in the study. The study is valid.

The acclimation was done for 6 hours under test conditions. Temperature: 21 C. Photoperiod:16 hours of illumination and 8 h of darkness. For the test, freshly hatched daphnids of an age between 6 and 24 h were used.

Test design: the test was performed in four 50 mL glass beakers for each test substance concentration and the controls, containing 10 daphnids each and filled with 50 mL test medium. The concentrations to be tested were made up with test substance and reconstituted water. One control with reconstituted water and two concentrations of the reference compound potassium dichromate were also tested.
Following a preliminary range-finding study, daphnids (20 daphnids/concentration; divided into two groups of ten animals) were exposed to a control and the test material at measured concentrations of 4.8, 7.3, 18.2, 38.4 and 101.4 mg/L for 48 hours under static test conditions. The control group was maintained under identical conditions but not exposed to the test material.

Analytical controls were performed for all test concentration. The NEU 1160 I content was based on the determination of triglyceride by photometrical method (determination Triglyceride GPO-PAP test

kit). Duplicate samples from the freshly prepared test media of all test concentrations and the control were taken at the start of the test and at the end of the test.

Observations: Immobilisation and any adverse reactions to exposure were recorded after 24 and 48 hours and analytical determinations of test item content were conducted from all test concentrations. Temperature, pH-value and oxygen concentration of the test solutions were measured after 0 and 48 hours.

Findings: Immobilisation data from the exposure of Daphnia magna to NEU 1160 I are given in table 9.2.4.1-3. A low toxicity of NEU 1160 I on Daphnia magna is observed, not negative effects are observed up to the highest concentration tested (101.4 mg/L). At the lowest test concentration of 4.8 mg/L one Daphnia was immobile and some of the Daphnia were trapped at the water surface. However, none of the trapped test animals was immobile.

Table 9.2.4.1-3: Percent of immobilized Daphnia magna exposed to NEU 1160 I (20 animals/concentration)

Mean measured Concentration (mg/L)	24 h	48 h
Control	0	0
4.8*	0	0
7.3	0	0
18.2	0	0
38.4	0	0
101.4	0	0

* Some daphnids were trapped at the surface of the test media

The results of analytical control measurements during the main test are given in Table 9.2.4.1-3. NEU 1160I was not well soluble in test water and the concentration decreased during the test period of 48 hours. Therefore all reported results are related to mean measured concentration. Therefore the measured concentrations could be taken for the statistical evaluation of the toxicological response.

Conclusion: The 48-hour EC50 for NEU 1160I to Daphnia magna was clearly higher than 101 mg test item/L (> 96.72 mg rapeseed oil/L). This value could not be quantified due to the absence of toxicity of NEU 1160 I up to this test concentration. The NOEC might even be higher than this concentration, but concentrations in excess of 101 mg product/L have not been tested.

B.9.2.4.2 Acute toxicity for aquatic insects

No studies on aquatic insects, crustaceans and gastropods were conducted.

According to Sanco/3268/2001 it is not necessary to perform the acute tests since application of Rapeseed oil will not take place directly at or in the vicinity of surface waters.

Moreover, Rapeseed oil is practically insoluble in water and thus, aquatic insects, crustaceans and gastropods are unlikely to be contaminated.

Not further information is needed.

B.9.2.5 Chronic toxicity to aquatic invertebrates

B.9.2.5.1 Chronic toxicity to Daphnia magna

Not information has been submitted.

Notifier justification: Long-term exposure of Rapeseed oil to aquatic invertebrates is not indicated due to the rapid degradation in surface water (extrapolated from soil degradation DT50 between 3 and 9 days).

RMS opinion: In the frame of directive 91/414/EEC and following the recommendations of SANCO guidance in aquatic ecotoxicology a chronic daphnid test is always required if DT50 (water) > 2 days or if there is more than 1 application.

In this case the Rapeseed oil DT50 in water was not investigated, but extrapolated values from soil indicated a value between 3 and 9 days. According to the GAP, in some cases more that 1 application is recommended but it is for indoor uses. Therefore, due to the low solubility on water of rapeseed oil, the low acute toxicity of rapeseed oil on Daphnia magna , the mechanical mode of action and the estimated low potential of bioconcentration of rapeseed oil prolonged exposure to rapeseed oil and accumulation of the compound in aquatic invertebrates are not expected.

Not further information is needed.

B.9.2.5.2 Chronic toxicity to aquatic insects

Not information has been submitted.

Notifier justification: No chronic studies on aquatic insects and molluscs are necessary, since Rapeseed oil will not be applied directly on or in the vicinity of surface waters.

B.9.2.5.3 Aquatic field testing

Not data have been submitted.

No further information is required.

B.9.2.6 Effects on algal growth

ACTIVE SUBSTANCE

Dengler, D. (2000): Rep. No. 99505/01-AASs

The objective of this study was to determinate the toxic effect of Rapeseed oil (Rüböl/Rapsöl, technical rapessed oil, Lot/batch EK 3162299) on the single cell green alga Desmodesmus subspicatus (3 days algal culture) in a static 96 h toxicity test. The study was performed according to OECD 201 EEC Directive C.3 (92/69/EC) and under GLP. The study is valid.

Deviations: Not indications of the purity of rapeseed oil. At the higher concentration levels, the pH dropped below the initial values. This may be due to degradation products of rapeseed oil, caused by bacterial growth, which was observed under microscope. These deviations are not considered to have a negative impact on the outcome of the study.

RMS comments: The content of Rüböl/Rapsöl in water was below 80% during the test. According with SANCO guidance of aquatic ecotoxicology analytical concentrations could be used for the statistical evaluation of the toxicological response when measurement are < 80% of nominal. The RMS proposes to estimate the concentration basis on the analytical data showed on table 9.2.6.2. The toxicological endpoints selected for the risk assessment will be based on estimated analytical measurement (50% of nominal).

The algae are grown semi-continuously in the laboratory in aerated liquid cultures under permanent illumination. Temperature: 23-24.5 °C. Lighting: from the top (OSRAM Powerstar, HQI-T, 400 W) approx. 8000 lux.

Study design: A range finding test was performed with concentrations of 0, 0.01, 0.1, 1.0, 10 and 100 mg Rapeseed oil/L. The test flasks were inoculated with cells from semi-static liquid cultures to an initial cell density of 104 cells/mL. Like in the range-finding test, cells from a semi-static liquid stock culture were used for the main test. The cell density was adjusted to an initial concentration of 104 cells/mL in each test vessel. The main test was performed with 6 concentrations: 0, 10, 18.0, 32.4, 58.3,

105.0 and 189 mg/L test substance (geometric factor of 1.8) under static conditions over a period of 72 hours. Each concentration was tested in 3 parallel cultures. The control was tested in 6 parallel cultures. Analytical measurement. The determination of active ingredient from water samples was performed by an optical enzymatic method of triglyceride determination. The concentration course of Rapeseed oil was tested in test at three concentration levels and blank at initiation of the test. Additional sampling was performed after 24 h, 48 h and at termination of the test.

Observations: After 24, 48, and 72 hours, the cell growth was determined by counting. The pH was measured at the beginning and at the end of the test. The ErC50 values were calculated by log-linear regression analysis (probit).

Findings: The effects of Rapeseed oil on Desmodesmus subspicatus are presented in Table 9.2.6-1 and 9.2.6-3. The percentage inhibition of the cell growth based on the area under the growth curve and the average specific growth rate calculated for the main test at 72 h are given in Table 9.2.6-1. Based on nominal values inhibitory effects were observed from 32.4 to 189.0 mg/L after 72 h for the biomass and from 58.3 to 189.0 for the growth rate. The EC50 (growth rate) value was determined at 287.4 mg/L. This is more than the highest concentration (189 mg/L) tested in this study, and therefore will be not selected as endpoint for the risk assessment.

Table 9.2.6-1: Effects of Rapeseed oil on Desmodesmus subspicatus after a 72-hour exposure based on nominal concentrations.

Nominal concentrations (mg/L)	Average cell numbers/mL x 10-4*				0-72 h	
	0 hours	24 hours	48 hours	72 hours	(%) Biomass Inhibition	(%) Growth Rate Inhibition
0	1.00	10.16	55.47	202.08	0.0	0.0
10.0	1.00	7.81	45.83	208.85	5.2	-0.7
18.0	1.00	7.29	48.44	181.77	12.2	1.9
32.4	1.00	5.21	36.98	159.38	27.3*	4.4
58.3	1.00	7.29	46.87	108.85	35.4*	11.7*
105.0	1.00	2.08	36.98	63.02	58.5*	22.6*
189.0	1.00	3.65	25.52	32.29	73.9*	37.2*

* Algal counts are divided by 10000. At the start, the cell density was adjusted to 104 cells/mL.
* significance difference to control ($p < 0.05$)

Analytical concentrations of Rapeseed oil during the test are summarized in Table 9.2.6-2. The content of Rüböl/Rapsöl in water was below 80% during the test. The raising concentration in the lowest test concentration might be a result of the algae themselves. Algae show the tendency to give aminoacids and glycerol to media to control the osmotic pressure in their environment. The content of Rüböl/Rapsöl in the test substance was below 80%. Therefore, according with SANCO guidance of aquatic ecotoxicology the analytical concentrations could be used for the statistical evaluation of the

toxicological response. The RMS proposed to estimated the analytical concentration using a factor of 50% at 72 h based on the data presented on table 9.2.6-2.

Table 9.2.6-2: Analytical concentrations during the main test in percent of nominal concentration.

Rapeseed oil	**Nominal concentration in mg/L**		
Time [h]	**10**	**58.3**	**189**
	Actual concentration in % of nominal		
0	108.0	28.8	20.6
24	189.0	32.6	34.3
48	177.0	31.2	57.1
72	450.0	44.1	46.8

n.d. below detection limit (6 mg/L)

Conclusion: Based on nominal concentrations the EC50 (biomass) value was determined at 82.2 mg Rapeseed oil/L (measured = 41.1 mg/L) for Desmodesmus subspicatus. The NOEC for growth rate was 32.4 mg Rapeseed oil/L (measured = 16.2 mg/L) and for biomass 18.0 mg Rapeseed oil/L (measured = 9.0 mg/L).

For the risk assessment the toxicological values using analytical estimated data will be used.

Table 9.2.6-3: Determination of the toxicological endpoints based on nominal and analytical values. Analytical values are estimated by RMS using data from Table 9.2.6-2 (50% of nominal at 72 h) .

72 hour EC Value	**Nominal Rüböl/ Rapsöl [mg/L]**	**Analytical Rüböl/ Rapsöl [mg/L]**
EbC10 (biomass)	15.2	7.6
EbC50 (biomass)	82.2	41.1
LOEbC (biomass)	32.4	16.2
NOEbC (biomass)	18.0	9
ErC10 (growth rate)	53.0	26.5
ErC50 (growth rate)	287.4	143.7
LOErC (growth rate)	58.3	29.15
NOErC (growth rate)	32.4	16.2

Further studies on a second algal species are not required since the active substance is not a herbicide.

B.9.2.7 Effects on sediment dwelling organisms

Not information has been submitted.

Notifier justification: accumulation of Rapeseed oil in the sediment is unlikely, due to the rapid degradation in nature. Assuming a considerable inhibition of the adsorption of Rapeseed oil to sediment by the emulsifier under use conditions, it can be concluded that significantly lower concentrations are

reached under more realistic circumstances than predicted by standard simulation runs (refer to fate section).

Furthermore effects of Rapeseed oil on sediment dwelling organisms are not expected since daphnids and non target arthropods were not affected when exposed to Rapeseed oil under use conditions.

B.9.2.8 Microcosm or mesocosm study (IIIA, point 10.2.2)

Not further information is needed.

Notifier justification: NEU 1160 I is intended for use in orchards and ornamentals. Ecologically relevant habitats for aquatic organisms will therefore not be exposed. In view of various findings on aquatic organisms, rapid degradation of Rapeseed oil and TER values presented and discussed in this chapter (B.9.2.10), such testing is not need.

RMS opinion: no further information is needed.

B.9.2.9 Summary of toxicity data on aquatic organisms

The more sensitive toxicity endpoints showed in table 9.2.9-1 are considered for risk assessment of Rapeseed oil on aquatic organisms. There are some discrepancies between toxicity endpoints selected by the notifier and the RMS (see Table 9.2.9-1) that can be explained for the low solubility of rapeseed oil on water, and the technical problems associated with the analytical verification of the rapeseed oil in the toxicity tests.

All the ecotoxicity studies have been conducted with analytical verifications of the compound tested, and in all of them the analytical concentrations were always less that 80%.

The notifier proposes to choose the toxicity parameters selecting nominal concentrations because of the lower solubility of the rapeseed oil in water and the water phase separation. However, RMS disagrees and proposes to use the toxicity parameters using measured (or estimated) concentrations. This is in agreement with SANCO guidance on aquatic ecotoxicology because analytical concentrations were always less that 80% of nominal values in all ecotoxicity tests performed.

For daphnids, notifier proposes to use toxicity data based on the formulated product (see table 9.2.9-1), however toxicity data with the technical substance indicates that it is more toxic that the formulated. Therefore, RMS proposes to use data base on the technical product as a conservative approach.

For algae, notifier selected the toxicity endpoint bases on ErC50 (72h) (based on growth rate) , however this endpoint is less sensitive that EbC50 (72h, based on biomass). Therefore RMS proposes to use the more sensitive endpoint (EbC50).

Table 9.2.9-1: Summary of Rapeseed oil toxicity data on aquatic organisms.

Test organism	Reference	Test substance	Endpoint	Toxicity (mg a.i./L) Notifier	Toxicity (mg a.i./L) RMS
Active substance					
Fish- Acute Toxicity					
Oncorhynchus mykiss	Heintze, A. (2000a): Rep. No. 99505/01-AAOm.	Rüböl/Rapsöl, Lot/batch EK 3162299	LC50 (96 h)	> 249.4	> 7.48 (3% of nominal)
Aquatic invertebrates –Acute Toxicity					
Daphnia magna	Heintze, A. (2000b): Rep. No. 99505/01-AADm	Rüböl/Rapsöl, Lot/batch EK 3162299	EC50 (48h)	4.5	4.5 Selected for risk assessment
Green algae-					
Desmodesmus subspicatus	Dengler, D. (2000): Rep. No. 99505/01-AASs	Rüböl/Rapsöl, Lot/batch EK 3162299	EbC50 (72h) ErC50 (72h)	82.2 287.4	41.1 143.7
Plant protection product : NEU 1160 I					
Aquatic invertebrates-Acute toxicity					
Daphnia magna	Hertl, J. (2002). Rep. No. 12742220.	NEU 1160 I, 90% Rüböl/Rapsöl, Lot/batch 015031	EC50 (48h)	> 96.72	Not selected for risk assessment

Notifier indicates that it is possible to extrapolate from the risk assessment presented on the acute toxicity of Rapeseed oil to daphnids and no further data are provided on the acute effects of Rapeseed oil on aquatic insect, crustacean and gastropod mollusc species. Long-term exposure of aquatic insect, crustacean and gastropod mollusc species to Rapeseed oil is not indicated due to the rapid degradation of Rapeseed oil in surface water.

B.9.2.10 Aquatic risk assessment

Aquatic organisms may be exposed to NEU 1160 I by emissions from treated fields. The provided studies and data permit an assessment of acute risk following exposure to under practical conditions. No chronic or bioaccumulation studies have been submitted by the notifier. Not main Rapeseed oil metabolites that have the potential to reach surface waters are identified and therefore not toxicity database is available.

The acute toxicity of the active substance and one formulated product, NEU 1160 I, have been established in several studies for fish, Daphnia and green algae (see Table 9.2.9-1). A comparison of the results from studies with the active substance and the formulation product for aquatic invertebrates

indicates that the toxicity of Rapseed oil is higher that the formulated product NEU 1160 I (see Table 9.2.9-1), therefore using the toxicity data based on testing with the active substance will provide a conservative estimate of the risk of Rapseed oil to aquatic organisms.

However, it is clear that formulated product NEU 1160 I is less toxic that the technical product on daphnids, and this can be considered in the risk assessment. This is in agreement with the technical composition of the formulation since the formulation contains an emulgator which reduces the mechanical effect of Rapeseed oil on daphnids.

For the characterization of Rapeseed oil ecotoxicological profile on several aquatic organisms the same batch of the technical substance has been used (see Table 9.2.9-1). The complete technical specifications of the different batches used in these ecotoxicological studies are not provided by the notifier but because are identical batches the comparability is granted. As conclusion, based on the assumption above exposed all the technical substances used in the ecotoxicological profile of Rapeseed oil are comparable and the (see below) aquatic risk assessment is adequate.

For prediction of the concentration of Rapeseed oil in surface waters we refer to Fate section.

First-tier risk assessment: FOCUS step 1

Acute risk for aquatic organisms

Taking into account the more sensitive species in the reported acute ecotoxicity data: the aquatic invertebrate Daphnia magna (48h-EC50 = 4.5 mg a.i./L, Notifier 96.72 mg a.i./L) is the most sensitive standard test species tested follow of the vertebrate fish Oncorhynchus mykiss (96h-LC50 > 7.48 mg a.i./L, Notifier > 249.4 mg a.i./L) and the green algae (72h-EbC50 = 41.1 mg a.i./L, Notifier 72h-ErC50 = 287.4 mg/L). Applying an assessment factor of 10 (for algae) or 100 (to vertebrates and invertebrates) to the lowest toxicity data, the trigger value according to the EU Uniform principles results for aquatic primary producers in a first-tier acceptable concentration of 4.11 mg a.i./L, for aquatic invertebrates of 0.045 mg a.i./L and for aquatic vertebrate of 0.0748 mg a.i./L.

The short-term first-tier data mentioned above indicate that primary producers are potentially at risk at exposure concentrations higher than 4.11 mg a.i./L. Potential risks for aquatic invertebrates might occur at concentrations higher than 0.045 mg a.i./L and for aquatic vertebrates at concentrations higher than 0.0748 mg a.i/L.

Acute toxicity exposure ratios (TERa) were calculated using toxicity data for Rapeseed oil technical (see table 9.2.9-1) and the PEC values calculated by the notifier (B.9.7). Results are shown in the table 9.2.10-1.

Table 9.2.10-1: Acute TER (TERa) values for aquatic organism exposed to Rapeseed oil. FOCUS step 1 scenario.

Organism	Toxicity (mg a.i./L)		PECsw actual concentrations (mg a.i. /L)	TERa	Trigger value EU 91/414/EEC
Pome/stone (early). Field application					
Aquatic vertebrate: fish Oncorhynchus mykiss	Notifier	> 249.4	2.580	> 96.6	100
	RMS	> 7.48	2.580	> 2.89	100
Aquatic crustacean Daphnia magna	Notifier	> 96.72	2.580	> 37.49	100
	RMS	4.5	2.580	1.74	100
Algae Desmodesmus subspicatus	Notifier	287.4	2.580	111	10
	RMS	41.4	2.580	16	10
Ornamentals (leafy veg.). Field application					
Aquatic vertebrate: fish Oncorhynchus mykiss	Notifier	> 249.4	0.570	> 437	100
	RMS	> 7.48	0.570	> 13	100
Aquatic crustacean Daphnia magna	Notifier	> 96.72	0.570	> 169	100
	RMS	4.5	0.570	8	100
Algae Desmodesmus subspicatus	Notifier	287.4	0.570	504	10
	RMS	41.4	0.570	72.6	10
Ornamentals (plant height > 125 cm (glass house use)					
Aquatic vertebrate: fish Oncorhynchus mykiss	Notifier	> 249.4	0.02842	> 8775	100
	RMS	> 7.48	0.02842	> 263	100
Aquatic crustacean Daphnia magna	Notifier	> 96.72	0.02842	> 3403	100
	RMS	4.5	0.02842	158	100
Algae Desmodesmus subspicatus	Notifier	287.4	0.02842	10112	10
	RMS	41.4	0.02842	492	10

The TER values for acute toxicity to aquatic algae clearly meet the acceptability criteria according to Annex VI of the EU-directive 91/414/EEC (TERa ≥ 10) for all crops. Therefore it can be concluded that no short-term adverse effects on aquatic algae are to be expected from the use of NEU 1160 I according to the proposed use pattern.

According with FOCUS step 1, the acute TER values were slightly below the trigger of 100 for aquatic vertebrates and invertebrates. Thus, NEU 1160 I may be a hazard for aquatic vertebrates and invertebrates. A refinement is calculated below for aquatic vertebrates and invertebrates.

Refinement tier risk assessment: FOCUS step 2

Acute toxicity exposure ratios (TERa) were calculated using toxicity data for Rapeseed oil technical (see table 9.2.9-1) and the PEC values calculated by the notifier using FOCUS step 2 scenarios for North and South of Europe (B.9.7). Results are shown in the table 9.2.10-2.

Table 9.2.10-2: Acute TER (TERa) values for aquatic organism exposed to Rapeseed oil. FOCUS step 2 scenario for North and south of Europe.

Organism		Toxicity (mg a.i./L)	PECsw actual concentrations (mg a.i. /L)	TERa	Trigger value EU 91/414/EEC
Pome/stone (early). Field application					
Aquatic vertebrate: fish Oncorhynchus mykiss	Notifier	> 249.4	2.580	> 96.6	100
	RMS	> 7.48	2.580	> 2.89	100
Aquatic crustacean Daphnia magna	Notifier	> 96.72	2.580	> 37.49	100
	RMS	4.5	2.580	1.74	100
Ornamentals (leafy veg.). Field application					
Aquatic vertebrate: fish Oncorhynchus mykiss	Notifier	> 249.4	0.567	> 437	100
	RMS	> 7.48	0.567	> 13	100
Aquatic crustacean Daphnia magna	Notifier	> 96.72	0.567	> 169	100
	RMS	4.5	0.567	8	100

According with FOCUS step 2, the TER values were slightly below the trigger of 100. Thus, NEU 1160 I may be a hazard for aquatic vertebrates and invertebrates. A refinement is calculated below for aquatic vertebrates and invertebrates using FOCUS step 3 scenarios.

Refinement tier risk assessment: FOCUS step 3

Acute toxicity exposure ratios (TERa) were calculated using toxicity data for Rapeseed oil technical (see table 9.2.9-1) and the PEC values calculated by the notifier using FOCUS step 3 scenarios. Results are shown in table 9.2.10-3.

Table 9.2.10-3: Acute toxicity/exposure ratios (TER) for fish and daphnids after use of Rapeseed oil (field use) using FOCUS step 3 scenarios.

Crop	Step	Scenario	Water body	TER (100) RMS	Drift (%)	PECsw (µg a.i./L) global max. act. conc.	TER (100) Notifier
Aquatic vertebrates: fish							
Pome/stone (early)	3	D3	Ditch	>8.62	23.599	867.773	> 287.40
	3	D4	Pond	>170	4.730	43.961	> 5673.21
	3	D4	Stream	>8.8	25.899	842.798	> 295.92
	3	D5	Pond	>170	4.730	43.949	> 5674.76
	3	D5	Stream	>8.9	25.899	839.754	> 296.99
	3	R1	Pond	>170	4.730	43.953	> 5674.24
	3	R1	Stream	>10.7	25.899	693.076	> 359.84
	3	R2	Stream	>8	25.899	934.169	> 266.97
	3	R3	Stream	>7.5	25.899	1001.670	> 248.98
	3	R4	Stream	>289	25.899	693.248	> 359.75
Ornamentals (leafy veg.)	3	D6	Ditch	>55.75	5.173	134.161	>1858.9
	3	R1	Pond	>1937	0.612	3.861	>64594
	3	R1	Stream	>76.51	5.152	97.76	>2551
	3	R2	Stream	>56	5.152	132.058	>1888
	3	R3	Stream	>52.8	5.152	141.543	>1762
	3	R4	Stream	>76.53	5.152	97.728	>2551
Aquatic invertebrates: daphnids							
Pome/stone	3	D3	Ditch	>5.2	23.599	867.773	> 111.46

Crop	Step	Scenario	Water body	TER (100) RMS	Drift (%)	PECsw (µg a.i./L) global max. act. conc.	TER (100) Notifier
(early)	3	D4	Pond	>102	4.730	43.961	> 2200.30
	3	D4	Stream	>5	25.899	842.798	> 114.77
	3	D5	Pond	>102	4.730	43.949	> 2200.91
	3	D5	Stream	>5.35	25.899	839.754	> 115.18
	3	R1	Pond	>102	4.730	43.953	> 2200.71
	3	R1	Stream	>6.49	25.899	693.076	>139.56
	3	R2	Stream	>4.8	25.899	934.169	> 103.54
	3	R3	Stream	>4.5	25.899	1001.670	> 96.57
	3	R4	Stream	>6.5	25.899	693.248	> 139.53
Ornamentals (leafy veg.)	3	D6	Ditch	>33	5.173	134.161	> 720.98
	3	R1	Pond	>1165	0.612	3.861	> 25052.50
	3	R1	Stream	>46	5.152	97.76	> 989.44
	3	R2	Stream	>34	5.152	132.058	> 732.46
	3	R3	Stream	>32	5.152	141.543	> 683.38
	3	R4	Stream	>47	5.152	97.728	> 989.76

Notifier calculations: According with FOCUS step 3, the acute TER values for aquatic vertebrates and invertebrates were above trigger of 100. Thus, NEU 1160 I does not impose an acute risk to fish and daphnids when applied according to good agricultural practice. The detected endpoint used by the notifier is from formulated product and also is more a No Observed Effect Concentration (NOEC) and not an EC50 value due to the absence of mortality. The realistic TER value would be considerably higher if an EC50 would have been determined. Risk mitigation measurements are not required.

Notifier indicates that it is possible to extrapolate from the risk assessment presented on the acute toxicity of Rapeseed oil to daphnids and no further data are provided on the acute effects of Rapeseed oil on aquatic insect, crustacean and gastropod mollusc species.

RMS opinion: In the case of daphnids, based on the studies submitted by the notifier it is clear that formulated product NEU 1160 I is less toxic that the technical product on daphnids, and this should be considered in the risk assessment. No toxicity data have been presented for fish with the formulated product.The differences in toxicity between formulated product and technical product are in agreement with the technical composition of the formulation since the formulation contains an emulgator which reduces the mechanical effect of Rapeseed oil on daphnids. However, for the risk assessment the data from the active substance, as a worst case, has to be selected according to 91/441/EEC. Therefore, calculations performed with FOCUS step 3 (RMS calculations, Table 9.2.10-3) shown that acute TER values for aquatic vertebrates and invertebrates were below the trigger of 100. Thus, NEU 1160 I may be a hazard for aquatic vertebrates and invertebrates. A further refinement using PECtwa is calculated below.

Refinement tier risk assessment: FOCUS step 3 using 4d-PECtwa and 2d-PECtwa

According to SANCO/3268/2001 "the first stage of the acute and chronic risk assessments should be based on the initial/maximum PEC values", these calculations have been performed (see Table 9.2.10-2 and 9.2.10-3). The guidance continue… "If the chronic TERs calculated using the initial/maximum PEC are below the relevant triggers, it may be appropriate to refine the risk assessment using PECtwa values if an unrealistic exposure regime prevailed in the relevant toxicity test". In this particular case of Rapeseed oil (low solubility in water, measured concentration < 80% of nominal), RMS thinks that will be appropriate for an acute refinement risk assessment to compare the mean measured concentration with an appropriate PECtwa (4 days for fish, and 2 days for daphnids).

Acute toxicity exposure ratios (TERa) were calculated using toxicity data for Rapeseed oil technical (see table 9.2.9-1, based on measured concentrations) and the PECtwa values calculated by the notifier using

Crop	Step	Scenario	Water body	Drift (%)	PECtsw (µg a.i./L)	TER (100)
Aquatic vertebrates: fish (LC50 >7.48 mg a.s/L)						
Pome/stone (early)	3	D3	Ditch	23.599	128.739	> 58
	3	D4	Pond	4.730	23.524	> 318
	3	D4	Stream	25.899	12.958	> 577
	3	D5	Pond	4.730	22.372	> 334
	3	D5	Stream	25.899	7.654	> 977
	3	R1	Pond	4.730	22.472	> 332
	3	R1	Stream	25.899	27.289	> 274
	3	R2	Stream	25.899	18.442	> 405
	3	R3	Stream	25.899	65.893	> 113
	3	R4	Stream	25.899	27.456	> 272
Ornamentals (leafy veg.)	3	D6	Ditch	5.173	10.5	> 712
	3	R1	Pond	0.612	2.046	> 3655
	3	R1	Stream	5.152	3.704	> 2019
	3	R2	Stream	5.152	2.578	> 2901
	3	R3	Stream	5.152	8.893	> 841
	3	R4	Stream	5.152	3.678	> 2131
Aquatic invertebrates: daphnids (LC50 = 4.5 mg a.s./L)						
Pome/stone (early)	3	D3	Ditch	23.599	231.764	19
	3	D4	Pond	4.730	30.043	149
	3	D4	Stream	25.899	25.829	174
	3	D5	Pond	4.730	29.243	154
	3	D5	Stream	25.899	15.283	294
	3	R1	Pond	4.730	29.319	153
	3	R1	Stream	25.899	54.174	83
	3	R2	Stream	25.899	36.75	122
	3	R3	Stream	25.899	128.812	35
	3	R4	Stream	25.899	54.5	82
Ornamentals (leafy veg.)	3	D6	Ditch	5.173	20.232	222
	3	R1	Pond	0.612	2.654	1698
	3	R1	Stream	5.152	7.363	611
	3	R2	Stream	5.152	5.141	875
	3	R3	Stream	5.152	17.468	258
	3	R4	Stream	5.152	7.312	615

FOCUS step 3 scenarios. Results are shown in table 9.2.10-4.

Table 9.2.10-4: Acute toxicity/exposure ratios (TER) for fish and daphnids after use of Rapeseed oil (field use) using FOCUS step 3 scenarios. The following PECtwa have been used: 4d-PECtwa (fish) and 2d-PECtwa (daphnids).

Crop	Step	Scenario	Water body	Drift (%)	PECtsw (µg a.i./L)	TER (100)
Aquatic vertebrates: fish (LC50 >7.48 mg a.s/L)						
Pome/stone (early)	3	D3	Ditch	23.599	128.739	> 58
	3	D4	Pond	4.730	23.524	> 318
	3	D4	Stream	25.899	12.958	> 577
	3	D5	Pond	4.730	22.372	> 334
	3	D5	Stream	25.899	7.654	> 977
	3	R1	Pond	4.730	22.472	> 332
	3	R1	Stream	25.899	27.289	> 274
	3	R2	Stream	25.899	18.442	> 405
	3	R3	Stream	25.899	65.893	> 113
	3	R4	Stream	25.899	27.456	> 272
Ornamentals (leafy veg.)	3	D6	Ditch	5.173	10.5	> 712
	3	R1	Pond	0.612	2.046	> 3655
	3	R1	Stream	5.152	3.704	> 2019
	3	R2	Stream	5.152	2.578	> 2901
	3	R3	Stream	5.152	8.893	> 841
	3	R4	Stream	5.152	3.678	> 2131
Aquatic invertebrates: daphnids (LC50 = 4.5 mg a.s./L)						
Pome/stone (early)	3	D3	Ditch	23.599	231.764	19
	3	D4	Pond	4.730	30.043	149
	3	D4	Stream	25.899	25.829	174
	3	D5	Pond	4.730	29.243	154
	3	D5	Stream	25.899	15.283	294
	3	R1	Pond	4.730	29.319	153
	3	R1	Stream	25.899	54.174	83
	3	R2	Stream	25.899	36.75	122
	3	R3	Stream	25.899	128.812	35
	3	R4	Stream	25.899	54.5	82
Ornamentals (leafy veg.)	3	D6	Ditch	5.173	20.232	222
	3	R1	Pond	0.612	2.654	1698
	3	R1	Stream	5.152	7.363	611
	3	R2	Stream	5.152	5.141	875
	3	R3	Stream	5.152	17.468	258
	3	R4	Stream	5.152	7.312	615

The acute TER values for aquatic vertebrates and invertebrates were above the trigger of 100 for majority of FOCUS scenarios, however in some scenarios TER values are below the trigger of 100 (see table 9.2.10-4). Thus, NEU 1160 I may be a hazard for aquatic vertebrates and invertebrates in some cases. Risk mitigation measures can be defined at Member sate level to protect aquatic vertebrates and invertebrates after NEU 1160 I application.

Chronic risk for aquatic organisms

Not chronic toxicity data was submitted by the notifier.

For one of the proposed uses of NEU 1160 I (on glass house, applied indoor), and that the product has low tendency to bioaccumulate (see point B.9.2.3) the chronic exposure of aquatic organisms to NEU 1160 I is very unlikely.

However for the field uses, the rapeseed oil could be transported to water from the intended use site by emissions from treated fields. For an initial risk assessment, the notifier assumed that long term exposure of NEU 1160 I to aquatic vertebrates and invertebrates is not indicated due to the rapid degradation of Rapeseed oil in surface water. Long-term exposure of aquatic insect, crustacean and gastropod mollusc species to Rapeseed oil is not indicated due to the rapid degradation of Rapeseed oil in surface water.

Besides that not long term toxicity database is available to calculate a long-term risk assessment, with the information available aquatic vertebrates and invertebrates could be not at risk after NEU 1160 I for the following reasons:

* Low solubility of Rapeseed oil in water
* Mode of action mechanical rather than chemical
* Relatively short DT50 in water (estimated from soil data DT50 = 3-9 days)
* Low potential for bioaccumulation
* In field only 1 application is proposed
* Dietary lipids are processed by known metabolic pathways within the body and contribute to normal physiological functions. They are utilized as a carbon and energy source.

Thus, it is expected that NEU 1160 I does not impose a long-term risk to aquatic organisms when applied according to good agricultural practice.

Sediment dwelling organisms

Accumulation of Rapeseed oil in the sediment is unlikely, due to the rapid degradation in nature. Assuming a considerable inhibition of the adsorption of Rapeseed oil to sediment by the emulsifier

under use conditions, it can be concluded that significantly lower concentrations are reached under more realistic circumstances than predicted by standard simulation runs (refer to Section 5, point 9.7.2). Furthermore, based on toxicity data with the formulation NEU 1160 I on daphnids (48 EC50 > 96.72 mg a.i./L) effects of Rapeseed oil on sediment dwelling organisms are not expected. Not further information is required.

Overall conclusion

Rapeseed oil is used as a contact acaricide and/or insecticide in formulations for the use against spider mites, mealy bugs and scales in ornamentals and orchards and/or for the suppression of winter eggs of spider mites in orchards and woody ornamentals. It is intended to be used for greenhouse ornamentals with 3 applications (glass house, indoor) per growing season, or in orchards and woody ornamentals in the field (1 application) at the start of the vegetation period. Application rates are dependent on the height of the plants.

Rapeseed oil suffocates insects and mites by blocking the spiracles. In addition, the oil also blocks the body pores, which are used by the mite to take in moisture in order to maintain the levels of water in the body. Since this mode of action is mechanical rather that chemical, insect resistance or tolerance to oils is not expected.

It is clear that Rapeseed oil has a poor solubility in water suggesting: 1) that exposure in the relevant toxicity tests can be unrealistic, and 2) lower concentrations can be reached under more realistic circumstances that the predicted for standard simulations (FOCUSsw). In addition, data from toxicity test on daphnids, performed with the formulated product, suggests that low toxicity with the formulation can be expected under more realistic conditions. Therefore, risk assessment performed with toxicity database from technical substance is a conservative approach.

In a first tier risk assessment, the TERa values calculated in the framework of Directive 91/414/EEC (using notifier PECsw calculations), indicates that negligible short- risk to algae species can be expected after NEU 1160 I use if it is applied according with Good Agriculture Practices.

In a refined risk assessment, using FOCUS step 2 and step 3, the TERa values calculated for aquatic vertebrates and invertebrates taking the more conservative approach proposed by RMS show that aquatic vertebrates and invertebrates can be at risk after NEU 1160 I application in field. Risk mitigation measures are needed. However, calculations performed by the notifier indicate that negligible acute risk can be expected for aquatic vertebrates and invertebrates after NEU 1160 I application. These differences can be explained for the difficulty to maintain nominal concentrations through the exposure period in the toxicity tests, as consequence of the low solubility in water of Rapeseed oil, and the water phase separation. Thus, notifier calculations are based on nominal concentrations and RMS are based on measured (estimated) concentrations.

Not long term toxicity database is available to calculate a long-term risk assessment, but according with the information available aquatic vertebrates and invertebrates could be not at risk after NEU 1160 I for the following reasons:

* Low solubility of Rapeseed oil in water
* Mode of action mechanical rather than chemical
* Relatively short DT50 in water (estimated from soil data DT50 = 3-9 days)
* Low potential for bioaccumulation
* In field only 1 application is proposed
* Dietary lipids are processed by known metabolic pathways within the body and contribute to normal physiological functions. They are utilized as a carbon and energy source.

Thus, it is expected that NEU 1160 I does not impose a long-term risk to aquatic organisms when applied according to good agricultural practice.

Not relevant metabolites in water have been identified.

Sediment dwelling organisms: accumulation of Rapeseed oil in the sediment is unlikely, due to the rapid degradation in nature (DT50 = 3-9 days, in soil). Assuming a considerable inhibition of the adsorption of Rapeseed oil to sediment by the emulsifier under use conditions, suggests that significantly lower concentrations are reached under more realistic circumstances than predicted by standard simulation runs (refer to Fate section). Furthermore, based on toxicity data with the formulation NEU 1160 I on daphnids (48 EC50 > 96.72 mg a.i./L) effects of Rapeseed oil on sediment dwelling organisms are not expected. No further information is required.

Conclusion: In a first tier risk assessment, the TERa values calculated in the framework of Directive 91/414/EEC (using notifier global maximum PECsw calculations, FOCUS step 1), indicates that negligible short- risk to algae species can be expected after NEU 1160 I use if it is applied according with Good Agriculture Practices.

Taking into account the more conservative approach proposed by RMS it is clear that aquatic vertebrates and invertebrates can be at short-term risk after NEU 1160 I application in field in some scenarios. In order to be conservative RMS proposes that mitigations measures can be defined to Member state level to protect aquatic vertebrates and invertebrates after NEU 1160 I application.

It is expected that NEU 1160 I does not impose a long-term risk to aquatic organisms and sediment dwelling organisms when applied according to good agricultural practice.

B.9.3 Effects on other terrestrial vertebrates (IIIA 10.3)

B.9.3.1 Toxicological data for mammals

The values summarized on the table 9.3.1-1 came from studies from toxicity section (chapter B5). These values were selected because of its ecotoxicological relevance in accordance with the requirements of Annex III, point 10.3 of directive 91/414/EEC.

Table 9.3.1-1: Ecotoxicological relevant endpoints for terrestrial vertebrates other than birds

Organism	Study type/ Duration	Test-substance	Dossier file No.	Ecotoxicological endpoint
Rat	acute, oral	NEU 1161 I Lot/batch: 190/97	IIIA, 7.1.1/01	LD50 > 1794.1 mg Rapeseed oil/kg b.w

The oral LD50 value of NEU 1161 I in rats was established as exceeding 2000 mg product/kg body weight. This corresponds to an LD50 of > 1794.1 mg Rapeseed oil/kg b.w., since NEU 1161 I contains 825.3 g Rapeseed oil/L and has a density of 0.920 kg/L.

Rat acute toxicity data of formulation product containing Rapeseed oil shows a LD50 > 1794 mg a.i./kg bw suggesting low toxicity for mammals. Not acute toxicity data with the formulated product NEU 1160 I are submitted by the notifier. Instead the acute toxicity with the formulation, NEU 1161 I was reported (see Table 9.3.1-1). According with the notifier, NEU 1161 I contains 90% Rapeseed oil and 2% Pyrethrum. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil. RMS agrees with notifier comments and it is logical to assume that NEU 1161 I will be more toxic that NEU 1160 I.

As shown in the table 9.3.1-1 the acute LD50 value of formulated product (NEU 1161 I) indicates moderately low toxicity for mammals, suggesting potentially low risk to mammals even if they get in contact with the substance by accident.

Not long-term toxicity data for Rapeseed oil is required because the expected low bioaccumulation potential and the low acute toxicity observed in rats.

B.9.3.2 Risk assessment to mammals

Formulated products containing the technical substance Rapeseed oil has relatively low acute oral toxicity to the rat with LD50 > 1794 mg a.i./kg bw.

Not acute toxicity data with the formulated product NEU 1160 I are submitted by the notifier. Instead the acute toxicity with the formulation NEU 1161 I was reported. According with the notifier, NEU 1161 I contains 90% Rapeseed oil and 2% Pyrethrum. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil. RMS agrees with notifier comments and it is logical to assume that NEU 1161 I will be more toxic that NEU 1160 I.

Notifier indicates that long-term exposure of Rapeseed oil to mammals is not indicated due to the rapid microbial degradation of Rapeseed oil in soil (refer to Annex III A, point 9.1) and plants.

RMS comments: Rapeseed oil is a natural oil which is also used as a food commodity. It is a mixture of esters (triglycerides) of different fatty acids. It is known that dietary lipids are processed by known metabolic pathways within the body and contribute to normal physiological functions. They are utilized as a carbon and energy source.

Assuming that:

1) fatty acids are naturally contributing to the feed of birds,
2) the mode of action of rapeseed oil is mechanical rather than chemical,
3) secondary poisoning for mammals eating contaminated food is unlikely to occur and
4) low rat acute toxicity is showed (LD50 > 1794.1 mg a.i./kg b.w)

Therefore it is expected low risk to wild mammals for formulated products containing rapeseed oil.

Not further information is needed.
Conclusion: negligible risk to wild mammals species can be expected after NEU 1160 I use if it is applied according with Good Agriculture Practices.

B.9.4 Effects on bees (IIA 8.3.1; IIIA 10.4)

B.9.4.1 Acute and oral contact toxicity to bees

Studies describing the toxicity of Rapeseed oil to bees have been not submitted by the Notifier. Rapeseed oil will be applied before sprout, when there is no bee activity therefore no more information is needed.

B.9.4.1.1 Bee brood feeding test

Rapeseed oil is not and insect regulator, therefore a bee brood feeding test is not required.

B.9.4.1.2 Cage test

No information concerning this point was submitted and it is not needed.

B.9.4.1.3 Field test

No information concerning this point was submitted and it is not needed.

B.9.4.1.4 Risk assessment for bees

It is not relevant to calculate the risk of Rapessed oil to bees because preparations containing Rapeseed oil will be applied before sprout, when there is no bee activity therefore no more information is needed.

Therefore negligible risk to bees species can be expected after NEU 1160 I use if it is applied according with Good Agriculture Practices.

B.9.5 Effects on non-target terrestrial arthropod species (IIA 8.3.2; IIIA 10.5)

B.9.5.1 Effects on non-target terrestrial arthropods using artificial substrates

B.9.5.1.1 Parasitoid (e.g. *Aphidius rhopalosiphi*)

No specific studies for Rapeseed oil or formulated product using artificial substrates were conducted.

B.9.5.1.2 Predatory mites (e.g. *Typhlodromus pyri*)

No specific studies for Rapeseed oil or formulated products using artificial substrates were conducted.

B.9.5.1.3 Ground dwelling predatory species

No specific studies for Rapeseed oil or formulated product were conducted.

B.9.5.1.4 Foliage dwelling predatory species

No studies with Rapeseed oil or formulated product were performed considering the rapid microbial degradation of fatty acids in the environment.

B.9.5.2 Effects on non-target terrestrial arthropods in extended laboratory/semi field test

B.9.5.2.1 Parasitoid (e.g. *Aphidius rhopalosiphi*)

No specific studies with Rapeseed oil technical product were conducted

PLANT PROTECTION PRODUCT

Fussell, S. (2002). Rep. No. NEU-02-2.

The aim of this study was to determine the direct toxic effects of fresh residues of NEU 1160 I (batch 207050, 96% rapeseed oil) on adults of the aprasitoid, *Aphidius rhopalosiphi*, under extended laboratory test conditions, with wasps being confined over treated barley seedlings. The study was conducted based on the draft guideline of Mead-Briggs *et al* (under preparation). The study was performed following GLP. The study is valid.

Deviations: No deviations.

Test conditions: The emergence chambers were stored in controlled environment rooms maintained at 19-22 °C and 44-96 % relative humidity (RH), with a 16 h photoperiod.

Study design: The effects of NEU 1160 I on the parasitic wasp, *Aphidius rhopalosiphi*, was evaluated at 5 rates under extended conditions over a period of 48 h. NEU 1160 I was applied to seedling barley at 100, 60, 30, 10 and 1 L product/ha (400 L spray solution/ha). Also included in this test was a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 10 mL product/ha. Five female wasps were confined over each pot, with six replicates (a total of 30 wasps) prepared for each treatment. Also included in this test was a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate). Five female wasps were confined over each pot, with six replicates (a total of 30 wasps) prepared for each treatment.

Observations: The behaviour of the wasps was assessed during the first 3 h after treatment and wasps survival was assessed over a period of 48 h. Fecundity assessments were carried out for the control and the three highest treatment rates of the test item in which >50% of the test insects were still alive at 48 h. After 10 days the number of aphid mummies that developed was recorded.

Findings: The results of the definitive bioassay are summarised in Table 9.5.2.1-1.

Table 9.5.2.1-1: Effects of NEU 1160 I on the mortality and fecundity of Aphidius rhopalosiphi

Test item	**NEU 1160 I**		
Test species	***Aphidius rhopalosiphi***		
Exposure	**Barley seedlings**		
	% wasps on treated plant during mortality assessments	Mortality at 48 h (%)	Mean number of mummies per female 1
Control	62	0	33.0
Application rate			% reduction in reproductive performance relative to control 2
NEU 1160 I			
100 L prod./ha	57	50	-
60 L prod./ha	69	25	56***
30 L prod./ha	61	7	6
10 L prod./ha	59	0	-22
1 L prod./ha	62	0	-
Perfekthion			
10 mL prod./ha	63	97	-

1 Based on data for females found alive at end of 24-h oviposition period.
2 A negative value indicates an increase in reproduction, relative to the control. The results for the individual treatments were compared by ANOVA and asterisks indicate treatments that differed significantly from the control (*** $P < 0.001$).

Conclusion: Under extended laboratory test conditions, the 48-h LR50 for NEU 1160 I with respect to the parasitoid *Aphidius rhopalosiphi* was calculated to be 100 L product/ha (95% confidence limits = 78 and 167 L product/ha). The residues of all treatment rates were not found to be significantly repellent to the test insects. The fecundity of wasps exposed to residues at treatment rates of up 30 L product/ha was not significantly affected.

B.9.5.2.2 Predatory mites (e.g. *Typhlodromus pyri*)

ACTIVE SUBSTANCE

No specific studies with Rapeseed oil were conducted.

PLANT PROTECTION PRODUCT

Taruza, S. (2002). Rep No. NEU-02-3

The aim of this study was to determine the LR50 (median lethal rate) for NEU 1160 I (containing 96% w/w of Rapessed oil, batch number 207050, purity 95.49%) based on mortality of juvenile (protonymphs less than 24 h old) mites *Typhlodromus pyri* over a 7-day exposure period. In addition a check was made for significant sub-lethal treatment effects on the fecundity of the mites. The study was conducted according to Blümel et al. (2000): Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. The study was performed following GLP. The study is valid.

Deviations: 1. Twenty-one mites were added to one replicate arena in the dose rate group 3.3 L product/ha instead of 20. 2. In the range-finding test the ambient temperature was 19-21°C, due to a malfunction of the test room's air-conditioning unit. 3. Prior to the test the culture was maintained at a temperature of 24-29°C. These deviations are considered that did not affect the outcome of the test or the integrity of the study.

Test conditions: For the bioassays, the arenas were set up in a controlled environment room under 16h photoperiod. In the definitive test, the ambient conditions were 23-26C and 68-87% relative humidity (RH).

Study design: NEU 1160 I was evaluated under extended laboratory conditions to the predatory mite, *Typhlodromus pyri* (Acari: Phytoseiidae), at 5 application rates, equivalent to 30, 10, 3.3, 1.0 and 0.33 L product/ha. Each replicate arena contained 20 mites. There were three replicate arenas (i.e. 60 mites) per treatment. Also included in this test were a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 30 mL product/ha (nominally 12 g a.i./ha). Treatments were applied at a volume rate equivalent to 200 L spray solution/ha to excised apple leaves.

Observations: Mortality of the mites was assessed at 7 DAT and the fecundity of the surviving mites was assessed between 7 and 14 DAT. An assessment of the condition of the mites was made under a binocular microscope at approximately 24 h and 7 days after treatment (DAT). They were recorded as being: alive: still moving, dead: no sign of movement, stuck: embedded in the sticky barrier, drowned: dead on the filter paper, missing: not visible. Any dead and stuck mites were removed at the time of each assessment.

The fecundity assessments were made for the control treatment and for all treatment rates of the test item where corrected mortality was < 50%. For 7 days, the total egg production (number of eggs plus

live and dead juvenile stages) was recorded for each unit. Assessment of oviposition activities were carried out at 9, 12 and 14 DAT.

Findings: The results of the definitive bioassay are summarized in the table 9.5.2.2-1. The results for the 30 and 10 L product/ha treatment rates differed significantly from the control (ANOVA, $p < 0.01$), but those for the three lower treatment rates of NEU 1160 I did not differ significantly from.

Table 9.5.2.2-1: Effects of fresh residues of NEU 1160 I on Typhlodromus pyri.

Treatment	% mortality at 7 d	Corrected % mortality at 7 d	Mean number of eggs per female	% reduction in fecundity relative to control#
Control	13	-	8.6	-
NEU 1160 I				
30 L/ha	38	29	3.2**	63
10 L/ha	27	16	2.4**	72
3.3 L/ha	7	0	6.8	21
1.0 L/ha	13	0	9.0	-4
0.33 L/ha	8	0	10.5	-22
Perfekthion				
30 mL/ha	93***	92	~	~

Mortality data angularly transformed and analysed by one-way ANOVA. Asterisk indicate results for mean % mortality that differed significantly from the control (*** $P < 0.001$)
Fecundity data analysed by one-way ANOVA. Asterisks indicate results for mean egg production that differed significantly from control (** $P < 0.01$)
A negative value indicates an increase in reproduction, relative to the control
~ No assessments made

Conclusion: Under extended laboratory conditions, the 7-day LR50 for NEU 1160 I was determined as being > 30 L product/ha. At treatment rates of 30 and 10 L product/ha, NEU 1160 I resulted in a significant reduction in mite fecundity, but treatment rates of 3.3 L product/ha and below did not have a statistically significant effect on fecundity.

B.9.5.2.3 Ground dwelling predatory species

No specific studies for Rapeseed oil were conducted.

B.9.5.2.4 Foliage dwelling predatory species

No studies were performed considering the rapid microbial degradation of fatty acids in the environment.

B.9.5.3 Effects on non-target terrestrial arthropods in semi-field tests

Not information concerning this point was submitted and it is not needed.

B.9.5.4 Field tests on arthropod species

Not information concerning this point was submitted and it is not needed.

B.9.5.5 Risk assessment for terrestrial arthropods other than bees

B.9.5.5.1 Summary of toxicity studies

Studies describing the toxicity of Rapeseed oil to terrestrial arthropods other than bees have been not submitted by the Notifier. However, two studies describing the toxicity of formulated product NEU 1160 I are submitted and summarized in table 9.5.1-1. In an extended laboratory study with *Typhlodromus pyri* the 7-day LR50 of NEU 1160 I was determined as being > 30 L product/ha, which is equivalent to 26.49 kg Rapeseed oil/ha, since the product contains 883 g Rapeseed oil/L.

In a further extended laboratory study with *Aphidius rhopalosiphi* the 48-h LR50 for NEU 1160 I was calculated to be 100 L product/ha, which is equivalent to 88.30 kg Rapeseed oil/ha, since the product contains 883 g Rapeseed oil/L.

Table 9.5.5-1: Summary of toxicity of NEU 1160 I to terrestrial arthropods other than bees.

Organism	Study type/ Duration	Test-substance	Report No.	Ecotoxicological endpoint (rapeseed oil)
Typhlodromus pyri	Extended laboratory study/7 days	NEU 1160 I Lot/batch: 207050	Rep No. NEU-02-3	7d-LR50 > 26.49 Kg /ha
Aphidius rhopalosiphi	Extended laboratory study/2 days	NEU 1160 I Lot/batch: 207050	Rep. No. NEU-02-2.	48h-LR50 > 88.30 Kg/ha

B.9.5.5.2 Hazard quotient calculations

To assess the risk to terrestrial arthropods following the use of NEU 1160 I, the hazard quotient (HQ), which is the ratio of the application rate (in kg a.i./ha) and acute toxicity (LR50 in kg a.i./ha), was determined.

The resulting HQ values are presented for the worst-case use (x 3 applications) for ornamentals (glass house) and for orchards (1x application, assuming 3 m crown height, 30 L product/ha) in field, in

Table 9.5.5.2-1. The scenario orchards covers the in field use of woody ornamentals (maximum application rate is 24 L product/ha).

Table 9.5.5.2-1: Predicted risk for terrestrial arthropods arising from the use of NEU 1160 I in ornamentals (glass house) and orchards (in field use).

Glass house: Predicted risk for terrestrial arthropods arising from the use of NEU 1160 I in ornamentals					
Test species	Endpoint	LD50 (kg a.i./ha)	Exposure scenario	Application rate (kg a.i./ha)	HQ
Typhlodromus pyri	LR50, 7 d, lab.	> 26.49	In - crop	70.64	< 2.66
			Off – crop1	4.87	< 0.18
Aphidius rhopalosiphi	LR50, 24 h, lab.	88.30	In - crop	70.64	0.80
			Off – crop1	4.87	0.05
In field: Predicted risk for terrestrial arthropods arising from the use of NEU 1160 I in orchards					
Test species	Endpoint	Result (kg a.i./ha)	Exposure scenario	Application rate (kg a.i./ha)	HQ
Typhlodromus pyri	LR50, 7 d, lab.	> 26.49	In - crop	26.49	< 1.00
			Off – crop2	7.73	< 0.29
Aphidius rhopalosiphi	LR50, 24 h, lab.	88.30	In - crop	26.49	0.30
			Off – crop2	7.73	0.09
1 Off crop exposure was considered at 3 m distance, assuming 6.9 % drift (vines, late)					
2 Off crop exposure was considered at 3 m distance, assuming 29.20 % drift (orchards, early)					

As indicated by hazard quotients upper the trigger value of 2, agreed on the ESCORT 2 workshop, March 2000 effects were observed for *Typhlodromus pyri* but not effects were observed for *Aphidius rhopalosiphi* after application of NEU 1160 I at the recommended field rate in the in-crop area of ornamentals in the glass house. Therefore risk mitigation measures are needed in order to protect predatory mites after products containing Rapeseed oil for ornamentals in glass house.

As indicated by hazard quotients upper the trigger value of 2, agreed on the ESCORT 2 workshop, March 2000 not effects were observed for *Typhlodromus pyri* and for *Aphidius rhopalosiphi* in-crop after application of NEU 1160 I at the field rate in orchards (application rate of 30 L product/ha, 3m crown height, 26.49 kg a.i./ha). This scenario covers the in field use of NEU 1160 I in woody ornamentals (maximum application rate 24 L product/ha).

In order to protect predatory mite populations risk mitigation measures are required at Member state level for the intended use of NEU 1160 I for ornamentals in the glass house.

Notifier opinion: The calculated HQ value overestimates the effect of NEU 1160 I on *Typhlodromus pyri* because an LR50 value could not be determined due to low mortality. The realistic HQ value for ornamentals in glass houses would be considerably higher if an LD50 would have been determined. Therefore no risk mitigation measures are required.

RMS opinion: RMS agrees with notifier that a LD50 for *Typhlodromus pyri* has not been calculated, but data from the extended laboratory test shows that significant effects were observed on *Typhlodromus pyri* at 30 and 10 L product/ha (these are 3 and 8 times lower that the maximum application rate), on percentage of mortality and fecundity. This is indicated by hazard quotients upper the trigger value of 2, agreed on the ESCORT 2 workshop, March 2000. Therefore risk mitigation measures are needed in order to protect predatory mites after products containing Rapeseed oil for ornamentals in glass house.

Conclusion: risk to predatory mites can be expected after NEU 1160 I, for the use proposed in glass house (3 applications). Thus in order to protect predatory mite populations risk mitigation measures are required at Member state level for the intended use of NEU 1160 I for ornamentals in the glass house.

A negligible risk to terrestrial arthropods other than bees can be expected after in field use (1x application) of NEU 1160 I in orchards (assuming maximum application rate 30 L product ha, 3 m crown height, 26.49 kg a.i./ha) and in woody ornamentals if it is applied according with Good Agriculture Practices.

B.9.6 Effects on earthworms

B.9.6.1 Acute toxicity to earthworms

ACTIVE SUBSTANCE

No specific studies for Rapeseed oil were conducted.

PLANT PROTECTION PRODUCT

Wachter, S. (1998a), Rep. No. 98028/01-NLEf

The objective of this study was to determinate the acute effects of NEU 1161 I (Lot/batch #: 190/97; Purity: 4.59 g/L Pyrethrins; 825.3 g/L Rapeseed oil) by dermal and alimentary uptake to adult (with clitelium, 300 to 600 mg) earthworms of mixed population of *Eisenia foetida foetida*. The test followed OECD 207 (1984) guideline and it was conducted under GLP. The study is valid.

Deviations: The stability of the substance is not stated.

The acclimatization period was conducted over 24 h in moist artificial soil (without the test substance) at the same environmental conditions that the main test. Temperature 20°C ± 2°C, lighting: continuous artificial light (approx. 400 – 800 lux), soil: water content: initial: 35% and pH: initial: 6.0-6.3, at termination: 6.2-6.3.

Study design: A range-finding test was conducted where earthworms were exposed to five concentration between 1 and 1000 mg NEU 1161 I/kg artificial soil. The main test was performed with concentrations at 100, 178, 316, 562 and 1000 mg/kg artificial soil (4 replicates per treatment group, 10 individuals per test unit) over a period of 14 days. A toxic standard at concentrations of 10, 18, 32, 56 and 100 mg 2-chloroacetamide/kg soil dry weight was also tested. The control group was running in parallel (4 replicates).

Observations: Any mortalities of the earthworms were recorded after 7 and 14 days of exposure to the test substance. Earthworm mortalities occurring after exposure to the test substance were calculated as percentage. Additionally the body weight of the earthworms at the beginning of the test and after 14 days of exposure was determined.

Findings: The LC50 of 2-chloroacetamide was found to be between 18 and 32 mg/kg artificial soil, which confirms the validity of the test.

The effects of NEU 1161 I are presented in Table 9.6.1-1. After 14 days of exposure to NEU 1161 I the average weight was between 90.1% and 96.3% of the initial weight for the treated group. The average weight of the control organisms was 90.0% compared with the initial weight.

Table 9.6.1-1: Toxicity of NEU 1161 I on mixed populations of *Eisenia foetida foetida* and *Eisenia foetida andrei* after 14 days of exposure.

Concentration [mg/kg soil dry weight]	**Control**	**100**	**178**	**316**	**562**	**1000**
Mortality [%] DAA 14	0	0	0	0	0	0
Mean Weight Change [%] DAA 14	90.0	90.5	90.1	90.8	93.5	96.3

DAA = Days after application

No significant difference between the body weights compared with the control were recorded at all test substance concentrations.

Conclusion: Since the Rapeseed oil Log Pow is higher than 2 (= 23.2908, obtained by calculation), the endpoint LC50 should be divided by 2. The median lethal concentration 14d-LC50 of NEU 1161 I to *Eisenia foetida* is shown to be greater than 1000 mg/kg artificial soil. Therefore the LC50correc > 500 mg product/kg artificial soil (14d-LC50corre > 448.95 mg a.i./kg dry weight soil).

B.9.6.2 Sublethal effects on earthworms

Notifier justification: No studies were performed considering the rapid microbial degradation of fatty acids in soil, mainly by ß-oxidation.

No further information is required.

B.9.6.3 Field tests (effects on earthworms)

No studies were submitted and non further information is required.

B.9.6.4 Residue content of earthworms

Notifier justification: Studies on the sublethal effects on earthworms are not required due to the rapid degradation of Rapeseed oil in soil.

RMS opinion: No studies were submitted and non further information is required.

B.9.6.5 Summary of effects on earthworms

The acute toxicity of formulated product NEU 1161 I containing Rapeseed oil have been investigated on earthworms (*Eisenia foetida*). Not information about the acute toxicity whit the active substance Rapeseed oil has been submitted.

RMS comments: Not acute toxicity data with the formulated product NEU 1160 I are submitted by the notifier. Instead the acute toxicity with the formulation, NEU 1161 I was reported (see Table 9.6.5-1). According with the notifier, NEU 1161 I contains 90% Rapeseed oil and 2% Pyrethrum. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil. RMS agrees with notifier comments and it is logical to assume that NEU 1161 I will be more toxic that NEU 1160 I.

The long-term effects of Rapeseed oil have not been investigated on earthworms, and not information has been submitted by the notifier.

A summary of the acute toxicity of Rapeseed oil on earthworms has been summarized on Table 9.6.5-1. Rapeseed oil has a moderate low acute toxicity to earthworms, thus the 14d-LC50correc. > 500 mg NEU 1161 I/kg dry weight soil.

Table 9.6.5-1: Ecotoxicological relevant endpoints for earthworms (*Eisenia foetida*) exposed to NEU 1161 I.

Organism	Study type/ Duration	Test-substance	Reference	Ecotoxicological endpoint mg NEU 1161 I/kg dry weight soil
Earthworms: *Eisenia foetida*	Short-term: 14 days	NEU 1161 I Lot/batch #: 190/97; Purity: 4.59	Wachter, S. (1998a), Rep. No. 98028/01-NLEf	14d-LC50corre > 500 14d-LC50corre > 448.95 mg a.i./kg dry weight soil

		g/L Pyrethrins; 825.3 g/L Rapeseed oil		

B.9.6.6 Risk assessment on earthworms

According with Fate section Rapeseed oil has a high Koc value of 1 x 1010 (obtained by calculation) and is immobile in soils, and is likely to remain on the soil.

The initial and long-term PEC values of NEU 1160 I in soil are reported in Fate section. The model calculation shows that soil residues (actual predicted environmental concentration) in the worst case scenario ornamentals (glass house) at a max. rate of 70.64 kg a.i./ha immediately following the last of three applications will be 85.34 mg Rapeseed oil/kg soil (depth 5 cm, density 1.5 g/cm³, 25% interception by plants).

Acute toxicity exposure ratio for earthworms

Based on the results of the acute toxicity test summarized in B.9.5.3 the LC50 of NEU 1161 I to *Eisenia foetida* determined after 14 days exposure was shown to be > 500 mg product/kg artificial soil, which is equivalent to 448.95 mg Rapeseed oil/kg artificial soil, resulting from a density of 0.920 kg product/L and a content of 825.3 g a.i./L.

To assess the acute risk occurring from the application of NEU 1161 I, the acute toxicity/exposure ratio TERa was calculated, which is the ratio of the LC50 (mg a.i./kg d.w.) and the initial PEC (mg a.i./kg d.w.). The resulting TERa values are presented for the worst-case use (x 3 applications) for ornamentals (glass house) and for orchards (in field, 1x application, assuming 3 m crown height, 30 L product/ha) in Table 9.6.6-1. The scenario orchards covers the in field use of woody ornamentals (maximum application rate is 24 L product/ha).

Table 9.6.6-1: Acute toxicity/exposure ratios for earthworms in ornamentals (glass house) and in field use (ornamentals and orchards).

Glass house: Acute toxicity/exposure ratios for earthworms in ornamentals					
Appl. Rate (kg a.i./ha)	Test species	LC50 (mg a.i./kg d.w.)	Exposure scenario	Initial PEC related to soil depth (mg a.i./kg d.w.)	TER_A
Ornamentals 70.64	Eisenia foetida	> 448.95	In-crop (25% interception)	85.34	> 5.26
			Off-crop1	5.89	> 76.22
In field: Acute toxicity/exposure ratios for earthworms in orchards (pome/stone) and ornamentals					
Appl. Rate (kg a.i./ha)	Test species	LC50 (mg a.i./kg d.w.)	Exposure scenario	Initial PEC related to soil depth (mg a.i./kg d.w.)	TER_A
Orchards 26.49	Eisenia foetida	> 448.95	50% reaching soil	17.66	> 25.42
Ornamentals 21.19	Eisenia foetida	> 448.95	50% reaching soil	21.19	> 21.18

1 Off crop exposure was considered at 3 m distance, assuming 6.9 % drift (vines, late)

The calculated TERa values for Rapeseed oil show values above the trigger of 10 for orchards and ornamentals (in field use) and below the trigger of 10 in ornamentals (glass house). Thus, NEU 1161 I does not impose an acute risk to earthworms for the uses proposes in field and does impose an acute risk to earthworms for one of the uses proposes (ornamentals, glass-house).

Therefore, risk mitigation measures are needed for the use of earthworms in ornamentals (glass house application).

Long-term toxicity exposure ratio for earthworms

Notifier justification: No specific studies were conducted due to the rapid degradation of Rapeseed oil in soil.

RMS comments: Not long term toxicity database is available to calculate a long-term risk assessment, but according with the information available earthworms could be not at risk after NEU 1160 I for the following reasons:

* Relatively short DT50 in soil (estimated from laboratory data DT50 = 3-9 days)
* Low potential for bioaccumulation
* In field only 1 application is proposed

* Dietary lipids are processed by known metabolic pathways within the body and contribute to normal physiological functions. They are utilized as a carbon and energy source.

Thus, it is expected that NEU 1160 I does not impose a long-term risk to earthworms when applied according to good agricultural practice.

Overall conclusion

Acute risk to earthworms can be expected after NEU 1160 I application for the intended use in glass house (3 applications). Thus, in order to protect earthworm populations risk mitigation measures are required at Member state level (for the intended use of NEU 1160 I for ornamentals in glass house).

A negligible risk to earthworms can be expected after in field use (1x application) of NEU 1160 I in orchards (assuming maximum application rate 30 L product ha, 3 m crown height, 26.49 kg a.i./ha) and in woody ornamentals if it is applied according with Good Agriculture Practices.

Chronic risk from the Rapeseed oil use can not be expected on earthworms because of rapid degradation of Rapeseed oil in soil and the low bioaccumulation potential.

With the information available a safe use of NEU 1160 I is granted for in field use of NEU 1160 I (1 application, maximum application rate 30L product/ha) to earthworms and/or other soil macro-organisms species. However, a potential acute risk is identified for use of NEU 1160 I in ornamentals (glass house, 3 applications). Therefore, risk mitigation measures are needed for ornamentals in glass house in order to protect earthworms in soil.

B.9.7 Effects on other soil non-target macro-organisms (IIIA 10.6.2)

Notifier justification: Based on the risk assessment on earthworms, no studies on other soil non-target macro organisms are regarded necessary.

RMS comments: no studies were provided and are not needed

B.9.8 Effects on soil non-target micro-organisms (IIA 8.5; IIIA 10.7)

B.9.8.1 Effects on soil microbial activity

ACTIVE SUBSTANCE

No specific studies for Rapeseed oil were conducted.

PLANT PROTECTION PRODUCT

Wachter, S. (1998b), Rep. No. 98028/01-AB

The objective of this study was investigated the possible effects of NEU 1161 I (Lot/ batch 190/97, Purity: 4.59 g/L Pyrethrins and 825.3 g/L Rapeseed oil) on soil microbial activity using two soil types: sandy soil (BBA type 2.3) and sandy loam soil (see table 9.8.1-1 for soil characteristics). The effects of NEU 1161 I on the soil microflora were measured in a test on nitrogen turnover after addition of ground lucern and on short term respiration after enriching with glucose. The study was conducted following BBA-Guideline for the Official Testing of Pesticides, Part VI, 1-1, 2nd edition, (1990). The study was performed under GLP. The study is valid.

Table 9.8.1-1: Parameters of the soils used to test the effects of NEU 1161 I.

Soil type		**Sand BBA 2.3**	**Sandy loam**
Dry weight (%)		91.8	74.3
pH		7.1	7.5
Org. C (%)		0.87	2.82
Humus (%)		Approx. 1.74	Approx. 5.64
% microbial biomass (mg C/100 g dry weight), calculated from respiration activity		66.43	212.92
NH4+-N (mg/100 g dry weight)		0.26	0.50
NO3--N (mg/100 g dry weight)		1.69	4.78
NO2--N (mg/100 g dry weight)		*	*
Total N (mg/kg dry weight)		700	2400
MWC** (mL H2O/100 g soil dry weight)		41.4	64.7
Specific gravity (g/L)		1295	1070
Clay (%) < 2 µm		3.9	7.0
Silt (%)	Fine ≥ 2 – 6 µm	3.6	6.1
	Middle ≥ 6 – 20 µm	6.8	24.5
	Coarse ≥ 20 – 63 µm	19.0	46.1
Sand (%)≥ 63 – 2000 µm		66.7	16.3

* = below quantitation limit
(soil 1: 0.056 mg/100 g dry weight; soil 2: 0.111 mg/100 g dry weight)
** = maximum water holding capacity

Deviations: The test was not performed according to guideline OCDE 216/217. Two concentrations have been tested, but these do not cover 5x the maximum PECmaxim reached in soil. The certificate analysis of the test substance was not provided. These deviations are not considered to have a negative outcome in the results and the study is valid for risk assessment.

Study design: Two soil types were tested: sandy soil (BBA type 2.3) and sandy loam soil (see table 9.8.1-1 for soil characteristics). The water content was adjusted to 40% (soil 1) and 60 % (soil 2) of its maximum water holding capacity. 3 x approx. 800 g soil for each study group were filled into 1000 mL glass bottles. For the nitrogen turnover test the samples were mixed homogeneously with lucerne meal (0.5% of the soil dry weight). For the short term respiration test the amount of glucose for an optimum respiration rate during the first 12 hours of measurement was determined prior to the application. Four study groups with 3 replicates were performed: 1. untreated soil (control); 2. soil treated with 12 L NEU 1161 I/ha (1x); 3. soil treated with 120 L NEU 1161 I/ha (10x); 4. soil treated with reference substance 20 L Herbogil liquide/ha (250 g Dinoterb/L, 5x). The samples were stored at 20 ± 2 °C in the dark. About every 7 days, the moisture loss was determined by reweighing and readjusted by adding water.

Observations: the content of ammonium-N, nitrate-N and nitrite-N was determined prior to and at 3 hours, 14 days, 28 days, 56 days (only soil type 1) and 90 days (only soil type 1) after the application of the test substance. 3 hours, 14 days and 28 days after the application, samples were taken, and the oxygen uptake was measured immediately after the addition of glucose. At each day of sampling soil dry weight and pH (not after 14 days) was determined.

Analytical procedures: Soil nitrification was determined by measuring the NH4+, NO3 and NO2- contents of aqueous soil elutions by means of calibrated ion sensitive electrodes and the Orion expandable Ionanalyser, Model EA 940. Nitrogen concentrations were then calculated from the measured values.

For the test assays in the respirometer, 100 g of soil, each, were transferred into the reaction vessels and were mixed with the quantity of glucose to achieve an optimum short term respiration rate previously determined. The incubation period was at least 20 h at 20 ± 2°C. The quantity of oxygen consumed during the measurement period was read from recording lines within the first 12 hours, and the CO2 produced per hour per 100 g dry substance was calculated. The principle of the Sapromat B12 respirometer is the electrolytical supply of oxygen, consumed by microbial activities. CO2 produced during short term respiration was stoichiometrically calculated from the consumed O2, thereby 1 mg of respired O2 refers to 1.375 mg CO2. The values were calculated as the mean of 3 replicates.

Findings: The short term respiration rate in the treatment groups was not significantly different from control over the 28 d incubation period. The reference substance had inhibition effects of 30.9% (soil 1) and 38.5% (soil 2) after 28 days. The results of the short term respiration are shown in table 9.8.1-1.

Table 9.8.1-1: Short term respiration test, % deviation from control.

Soil 1			
Time	**NEU 1161 I, 12 L/ha**	**NEU 1161 I, 120 L/ha**	**Reference**
3 h	12.62	2.84	41.64
14 d	15.33	10.80	43.55
28 d	3.25	-6.91	30.89
Soil 2			
3 h	6.00	-5.81	-22.67
14 d	-2.70	4.47	17.12
28 d	4.28	-4.78	38.45

Negative values mean stimulating effects

The results of the nitrogen turnover are shown in Table 9.8.1-2 and Table 9.8.1-3.

In soil type 1, the nitrogen turnover was prolonged to 90 days. After 90 days incubation period the deviation to the control was les that 25%, indicating a potential for recovery. Not significant differences can be observed at 1x and at 10x treatments after 90 days of incubation.

In soil type 2, there were no distinct effects of the test substance on the nitrogen turnover activities of soil microorganisms in comparison to the control after 28 days. The ammonium and nitrate contents, expressed at Nmin in the treatment groups were not significantly different from the control at soil type 2 within the 28 days incubation period.

Table 9.8.1-2: Effects of NEU 1161 I on soil nitrogen turnover. Data are presented as deviation from the control (soil 1) in percent.

Time	**3 h**	**14 d**	**28 d**	**56 d**	**90 d**
NEU 1161 I 12 L/ha (1x)					
NH4+-N	-3.85	19.05	6.67	-	-
NO3-N + NO2-N	-4.73	34.12	19.93	16.03	22.77
Nmin	-4.62	32.76	19.31	16.03	22.77
NO2- -N	-	-	-	-	-
NEU 1161 I 120 L/ha (10x)					
NH4+-N	0.00	-9.52	-6.67	-	-
NO3-N + NO2-N	-13.02	56.40	55.88	27.27	24.75
Nmin	-11.28	50.43	52.96	27.27	24.75
NO2- -N	-	-	-	-	-
Reference					
NH4+-N	-15.38	28.57	6.67	-	-
NO3-N + NO2-N	-24.85	-64.93	-42.48	-17.94	-15.84
Nmin	-23.59	-56.47	-40.19	-15.73	-15.84
NO2- -N	-	-	-	-	-

- = not calculated
negative values mean stimulating effects (Deviation from the control)

Table 9.8.1-3: Effects of NEU 1161 I on soil nitrogen turnover. Data are presented as deviation from the control (soil 2) in percent.

Time	3 h	14 d	28 d
NEU 1161 I 12 L/ha			
NH4+-N	8.00	-52.63	0.00
NO3-N + NO2-N	-1.26	11.80	1.82
Nmin	-0.38	9.91	1.77
NO2- -N	-	-	0.00
NEU 1161 I 120 L/ha			
NH4+-N	6.00	-52.63	-4.76
NO3-N + NO2-N	-1.88	19.62	9.75
Nmin	-1.14	17.49	9.37
NO2- -N	-	-	-16.67
Reference			
NH4+-N	-8.00	-31.58	-38.10
NO3-N + NO2-N	11.30	-30.78	-38.62
Nmin	9.47	-30.80	-38.61
NO2- -N	-	-	-75.00

- = not calculated
negative values mean stimulating effects (Deviation from the control)

The reference group (with a formulation of dinoterb) was also tested and demonstrates the normal sensitivity of the soil microflora.

The concentrations used in the test cover the maximum PEC expected (see table 9.8.1-4), therefore the results of the test can be used for assessing the risk or NEU 1161 I to soil microorganisms.

Table 9.8.1-4: Conversion of test concentration used in the study Rep. No. 98028/01-AB to the PEC values.

Wachter, S. (1998b), Rep. No. 98028/01-AB			Propose uses or NEU 1160 I according to GAP		
Application rate used	**Crop**	**PECinitial expected (mg a.s./kg)**	**Crop**	**Use**	**Maximum PECinitial (mg a.s./kg)**
12 L/ha	orchards	9.936	ornamental	Glass house	85.34
	ornamental	6.624	orchards	Field	17.66
120 L/ha	orchards	99.36	ornamental	Field	21.19
	ornamental	66.24			

Conclusion: The impact on short term respiration activities can be assessed as negligible even at a dosage rate of 120 L/ha of NEU 1161 I (PECini= 99.36 mg a.s./kg), since the deviation effects were within ± 15% compared with the control over 28 days for soil type 1 and 2.

The impact on soil nitrogen turnover can be assessed as negligible even at a dosage rate of 120 L/ha of NEU 1161 I, since the deviation effects were within ± 15% compared with the control over 28 days for soil type 2. However the nitrogen turnover in soil type 1 should be identified as group III because deviation effects were within ± 55% compared with the control over 28 days and showing a recovery at 90 days (< 25% effect)

B.9.8.2 Rates of recovery following treatment

Notifier justification: Further studies are not required since the active substance is not a soil sterilant.

RMS comments: not information was submitted and it is not required.

B.9.8.3 Effects on organic matter breakdown

Notifier justification: Not required since the DT90f values in soil are < 365 days.

RMS opinion: No studies were submitted and not further information is required.

B.9.8.4 Risk assessment on soil non-target micro-organisms

RMS comments: Not soil toxicity data with the formulated product NEU 1160 I are submitted by the notifier. Instead the toxicity with the formulation, NEU 1161 I was reported. According with the notifier, NEU 1161 I contains 90% Rapeseed oil and 2% Pyrethrum. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil. RMS agrees with notifier comments and it is logical to assume that NEU 1161 I will be more toxic that NEU 1160 I.

Negligible risk can be expected after use of NEU 1160 I on soil microflora because the effects on short term respiration rate are < 25% after 28 days for two soil types at dosage rate higher that those proposed in the GAP.

Also, negligible risk can be expected after use of NEU 1160 I on soil microflora because the effects on nitrogen turnover are < 25% after 28 days for one soil type, and for the other soil the time-course show recovery at 90 days (< 25% effect after 90 days).

Notifier comments: Following the study results (Rep. No. 98028/01) considerable side effects on the soil non-target micro organisms after use of NEU 1160 I are not expected. Any specific measures to reduce possible risks are not required.

Conclusion: negligible risk to soil microorganism can be expected after NEU 1160 I use if it is applied according with Good Agriculture Practices and the recommended use pattern.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6)

No data have been submitted.

B.9.10 Effects on biological methods for sewage treatment (IIA 8.7)

No data have been submitted.

B. 9.11 Effects on terrestrial vascular plants

Notifier justification: No studies required due to the rapid microbial degradation of Rapeseed oil.

RMS comments: not further information is required.

B.9.12 Effects on non-target plants

B.9.12.1 Effects on non-target terrestrial plants

Notifier justification: Studies on non-target terrestrial plants are not required since NEU 1160 I is not a herbicide and Rapeseed oil is rapidly degraded.

B.9.12.2 Seed germination

Notifier justification: Studies on seed germination are not required since NEU 1160 I is not applied on bare soil.

RMS comments: not further information is required.

B.9.12.3 Vegetative vigour

ACTIVE SUBSTANCE

No studies have been submitted

PLANT PROTECTION PRODUCT

Spatz, B. (2001). Rep. No. 11841087

The purpose of this study was to determine the effects of the NEU 1161 I (batch number 026061, 4.19 g/L pyrethrins, 825.3 g/L rapessed oil, purity 90%) on the vegetative vigour of six non-target plant species (*Raphanus sativus, Cucumis sativus, Vicia faba, Lycopersicon esculentum, Allium cepa, Avena sativa*) at 2 to 4 leaf stage representing six plant families over a period of 21 days. The study was designed to comply with the following method: OECD Guideline for the Testing of Chemicals,

Proposal for Updating Guideline 208, Draft Document July 2000. The study was conducted under GLP. The study is valid because control plants showed normal growth throughout the test. There was not mortality of control plants.

Deviations: None

Environmental conditions: Soil: LUFA 2.3 (sandy loam), all particles under 0.2 cm, 1.32± 0.1% organic matter; pH 6.5 ± 0.1. Temperature: During exposure: mean day: 24°C, mean night: 18°C, Lighting:16 h light:8 h dark, mean light intensity: 10791 lux., Humidity:During exposure: mean day: 68%, mean night: 90%

Experimental design: *Raphanus sativus, Cucumis sativus, Vicia faba, Lycopersicon esculentum, Allium cepa* and *Avena sativa* were exposed to 30 L NEU 1161 I/ha in 200 L tap water/ha using a laboratory-spraying equipment and were observed for the following 21 days. In total 42 plants for the dicotyledoneae species (3 seeds per pot and species, 14 pots per species) and 40 plants for the monocotyledonae species (5 seeds per pot and species and 8 pots per species) per species and treatment group were tested. The samples were stored deep frozen until analysis.

Observations: Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were made on day 7, 14 and 21. Plant fresh weight, number of plants died and growth stages were determined at day 21. The plants of one pot represented one replicate.

Fresh weight data were tested for normality by using Kolmogorof-Smirnov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted Bartlett Test was used for all data with $n > 10$ and Cochran Test for data with $n < 10$. Afterwards Student -t Test was used to compare test item and control.

Findings: NEU 1161 I did not cause any statistically significant effect on fresh weight in any of the tested species. Mortality did not occur. Phytotoxicity was only observable in Vicia faba (< 2%) but was considered as a normal process which occurs in older leaves. Consequently, there is no evidence for an interaction between NEU 1161 I and the plant species tested. A summary of the results are shown in table 9.1.2.3-1.

Table 9.1.2.3-1: Effects of NEU 1161 I on the vegetative vigour test.

Days after application		Mortality (%)	Fresh weight (g)	Effect (%)	Statistics	Phytotoxicity (%)			Growth stage (BBCH Code)	
		21				7	14	21	appl. day	21
Species	**Treatment group**									
R. sativus	control	0.0	8.84	0.0		0.0	0.0	0.0	13-14	42-49
	30 L/ha	0.0	8.33	-5.7	n.s.	0.0	0.5	0.0		42-49
C. sativus	control	0.0	13.15	0.0		0.0	0.0	0.0	12	13-51
	30 L/ha	0.0	12.56	-4.5	n.s.	0.0	0.0	0.0		13-61
V. faba	control	0.0	33.57	0.0		0.0	0.1	1.0	12	18-19
	30 L/ha	0.0	31.93	-4.9	n.s.	0.0	0.8	1.5		17-19
L. esculentum	control	0.0	15.45	0.0		0.0	0.0	0.0	13-14	13-17
	30 L/ha	0.0	18.10	17.1	n.s.	0.0	0.0	0.0		13-17
A. cepa	control	0.0	5.08	0.0		0.0	0.0	0.0	12	13-14
	30 L/ha	0.0	4.68	-7.8	n.s.	0.0	0.0	0.0		12-14
A. sativa	control	0.0	9.92	0.0		0.0	0.0	0.0	12	15-23
	30 L/ha	0.0	9.34	-5.8	n.s.	0.0	0.0	0.0		14-23

n.s. not significant
- negatice values represent reduction compared to control; the results represent rounded values calculated on the exact raw data

Conclusion: NEU 1161 I did not cause any statistically significant effect on fresh weight of any test species. Mortality did not occur. Phytotoxicity was only observable in Vicia faba and Raphanus sativus (< 2%) but was considered as a normal process which occurs in older leaves. For all species tested the NOEC and LOEC was > 30 L NEU 1161 I/ha. Consequently, there is no evidence for an interaction between NEU 1161 I and the plant species tested.

B.9.12.4 Risk assessment for terrestrial plants

Not toxicity data with the formulated product NEU 1160 I on non-target plants are submitted by the notifier. Instead the toxicity with the formulation, NEU 1161 I was reported. According with the notifier, NEU 1161 I contains 90% Rapeseed oil and 2% Pyrethrum. As Pyrethrum is the more toxic ingredient of NEU 1161 I it can be concluded that the toxicity of Rapeseed oil is much lower. Thus, the results of NEU 1161 I may be extrapolated to Rapeseed oil. RMS agrees with notifier comments and it is logical to assume that NEU 1161 I will be more toxic that NEU 1160 I.

The toxic effects of the NEU 1161 I on the vegetative vigour of six non-target plant species (*Raphanus sativus, Cucumis sativus, Vicia faba, Lycopersicon esculentum, Allium cepa, Avena sativa*) have been investigated. NEU 1161 I did not cause any statistically significant effect on fresh weight in any of the tested species. Mortality did not occur. Phytotoxicity was only observable in Vicia faba (< 2%) but was considered as a normal process which occurs in older leaves. Consequently, there is no evidence for an interaction between NEU 1161 I and the plant species tested.

Notifier justification: A quantitative risk assessment for terrestrial plants is not presented here since no ER50 values could be determined. It can however be concluded from the results that there is no risk for terrestrial plants after application of NEU 1160 I since there are no data indicating more than 50% phytotoxic effects at an application rate of 24.76 kg Rapeseed oil/ha, assuming that 1 L NEU1161 I is equivalent to 825.3 g Rapeseed oil.

RMS comments: Taking into account the information available we can conclude that there is not risk for terrestrial plants after one application rate of NEU 1160 I (= 24.76 kg Rapeseed oil/ha), since not phytotoxic effects had been detected at this application.

B.9.12.5 Seedling emergence

Notifier justification: Studies on seedling emergence are not required since NEU 1160 I is not applied on bare soil and Rapeseed oil degrades rapidly on soil.

RMS comments: not further information is needed.

B.9.12.6 Terrestrial field testing

Notifier justification: No terrestrial field testing required due to the results obtained in B.9.12. 4.

RMS comments: not further information is needed.

B.9.12.7 Effects on non-target aquatic plants

Not information have been submitted and it is not needed.

B.9.12.8 Aquatic plant growth-Lemna

B.9.12.9 Aquatic field testing

Notifier justification: NEU 1160 I is used as an insecticide, therefore no tests on higher aquatic plants are required. Moreover, NEU 1160 I will not be applied directly at or in the vicinity of surface waters.

RMS comments: Not further information is needed.

B.9.13 References relied on

Annex point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data Protec-tion Claimed yes/no	Owner
Annex II Data and Information					
IIA 8.2.1.1/01	Heintze, A.	2000a	Acute toxicity testing of Rüböl/Rapsöl in rainbow trout (Oncorhynchus mykiss) (Teleostei, Salmonidae) AG GAB Biotech/IFU, D-75223 Niefern-Öschelbronn W. Neudorff GmbH KG Report-no. 99505/01-AAOm GLP: yes published: no	yes	NEU
IIA 8.3.1.1/01	Heintze, A.	2000b	Assessment of Toxic Effects of Rüböl / Rapsöl on Daphnia magna using the 48 h Acute Immobilisation Test AG GAB Biotech/IFU, D-75223 Niefern-Öschelbronn W. Neudorff GmbH KG Report-no. 99505/01-AADm GLP: yes published: no	yes	NEU
IIA 8.4/01	Dengler, D.	2000	Testing of Toxic Effects of Rüböl / Rapsöl on the Single Cell Green Alga Scenedesmus subspicatus AG GAB Biotech/IFU, D-75223 Niefern-Öschelbronn W. Neudorff GmbH KG Report-no. 99505/01-AASs GLP: yes published: no	yes	NEU
Annex III Data and Information					
IIIA 10.2.2.2/01	Hertl, J	2002	ACUTE TOXICITY OF NEU 1160I TO DAHNIA MAGNA IN A 48-HOUR IMMOBILIZATION TEST Institute fur Biologische Analytik und Consulting IBACON GmbH, 31860 Emmerthal W. Neudorff GmbH KG Report-no. 12742220 GLP: yes Published: no	yes	NEU
IIIA 10.5.2/01	Taruza, S	2002	A rate-response extended laboratory test to determine the effects of nEU 1160I on the pREDATORY MITE, tYPHLODROMUS PYRI (ACARI: PHYTOSEIIDAE) Mambo-Tox Ltd. Biomedical Sciences Building, Southampton SO16 7PX W. Neudorff GmbH KG Report-no. NEU-02-03 GLP: yes published: no		

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